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GENETICS AND BREEDING

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GROWTH OF LAMBS OF THE ILE-DE-FRANCE BREED FROM BIRTH TO WEANING AND FACTORS AFFECTING IT

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Abstract

The present study examines the growth of lambs of the Ile-de-France breed from birth to weaning (at 70 days old). The research involves 1662 lambs born in the period 2017-2018. The animals are bred under similar technological conditions in three farms located in Northern Bulgaria which are controlled by the Ile-de-France Breeders Association in Bulgaria (AILFB). It has been ascertained that the male lambs are born with an average live weight of 4.531 ± 0.042 kg, they reach 14.90 ± 0.078 kg at 30 days old, and 27.31 ± 0.155 at 70 days old, while the female lambs - 4.462 ± 0.043, 14.74 ± 0.085, and 27.56 ± 0.158, respectively. The gender is not a reliable source of variation regarding this parameter. The farm, year, season and month of birth, father and mother factors have a complex interrelationship but, as a whole, the farm and the father are the factors which influence significantly the live weight of lambs at birth and their growth until 70 days old. It has been established that it is possible the growth of lambs to be regulated by modelling the genetic and environmental factors influencing it.

Key words*: growth until weaning; lambs; Ile-de-France.*

INTRODUCTION

Ile de France breed is a specialized French meat breed. The breed formation process was started by Professor Auguste Yart of the National Veterinary College Maisons-Alfort, France in 1824.

The breed was acknowledged in 1920 and is given the name Ile-de-France. Currently, the breed is reared in 51 countries (INSEM OVIN, 2020) on all continents. According to data of France Génétique Elevage (2020), 210,000 sheep of the Ile-de-France breed are reared in France.

The first import of the Ile-de-France breed in Bulgaria was performed in 1968. The major purpose of the studies during the initial years was ascertainment of the acclimatization and productive characteristics of the breed and the opportunities for crossbreeding with the breeds reared in the country.

The Ile-de-France breed successfully adapts and has therefore been bred under extended reproduction for more than 50 years, being the major specialized meat breed in Bulgaria.

According to data of the Ile-de-France Breeders Association in Bulgaria (AILFB), in 2019 the livestock population was 6,853 full-bred animals and around 2,500 cross-breeds.

The population state, the animals growth and the factors influencing it regarding the Ile-de-France breed are studied by plenty of authors (Achkakanova et al., 2019; Dimitrov, 1991; Dimitrov et al., 2011; Ivanova et al, 2017; Metodiev et al., 2010; Raychev et al., 2010).

The possibility for crossbreeding with Bulgarian breeds and improvement of their meat qualities as well as the economic efficiency of the breeding are also of research interest (Dimitrov, 1988; Marinova, 1976; Slavov, 2007).

In the recent years there has been an increased interest in the Ile-de-France sheep breed in Bulgaria. Deeper and up-to-date studies are therefore necessary for the purpose of maintaining the parameters of the main productive features in compliance with the Selection program for breeding the Ile-de-France in Bulgaria (Dimitrov et al., 2016).

The aim of the present study is to ascertain the growth of the lambs of the Ile-de-France breed until weaning and the influence of basic genetic and paratypical factors on it.

MATERIALS AND METHODS

The study was carried out in 3 farms rearing pure-breed sheep of the Ile-de-France breed. The farms are situated in Northern Bulgaria.

The animals in Farms № 1 and № 2 are both shed and pasture reared. During the shed period, silage feeds are also added to the main

ration in Farm № 1. Both farms practise natural mating of groups of 30-35 female animals with 1 main ram and 1 substitute ram.

The flock in the first farm is separated into two groups where the first group mates in the period April - May, and the second one - August - September. The mating in the second farm is performed in the period April - May. The animals in Farm № 3 are entirely shed-bred with coarse feed from feeding crib. The flock is divided into two groups where the first group are inseminated in the spring and the second one - in the autumn.

Hormonal oestrus synchronization and hand service are used. Again, the sheep are separated into groups but 1 ram is used for a group of 5 sheep. In 2018, laparoscopic artificial insemination with frozen semen from elite rams was performed in Farms 1 and 3 to, respectively 50 and 100 animals.

The pellets are purchased from "Artificial Insemination and Ram testing Station" in France. Five and respectively ten brood rams of different lineage were used in the first farm and the second farm.

The study involves 1,662 lambs - 851 male and 811 female ones born in 2017 (n - 783) and 2018 (n - 879).

In compliance with the technology used, the ewes generally yean in the autumn (n - 1042), in September (n - 406), October (n - 613), November (n - 23) and the winter (n - 480) - in January (n - 358) and February (n - 122). 140 are the lambs born in the spring - in April (n - 45) and May (n - 95).

According to the Instruction on the Control of the productive qualities and valuation, endorsed in the Selection program for breeding the Ile-de-France in Bulgaria (Dimitrov et al., 2016), the lambs are weighed at birth, at 30 days old, and at 70 days upon weaning.

The data are processed via one-way (M1) and multi- factor analysis of variance whose models are the following:

$Y=\mu+Yi+Mj+Fk+SXl+YMij+YFik+YSil+MFjk+MSXjl+FSXkl+YMFijk+YMSXijl+YFSXikl+MFSXjkl+YMFSXijkl+eijkl$ (M2),

$Y=\mu+Yi+SZm+Fk+SXl+YSZim+YFik+YSXil+SZFmk+SZSXml+FSXkl+YFSXikl+SZFSXmkl+eimkl$ (M3),

$Y=\mu+SRn+MDo+Fk+SXl+FSXkl+SXSRln+ejkln$ (M4),

Where: Y, M, F, SX, SZ, SR, MD are fixed effects of, respectively i - year of birth, j - month of birth, k - farm, l - gender, m - season of birth, n - father and o - mother; YM, Y**, M**, F**, SZ**, SXSR - are random effects of interaction between the respective factors.

The statistical processing is performed via SPSS 21.

RESULTS AND DISCUSSIONS

The lambs from the population examined by us were born with an average live weight of 4.497 ± 0.030 kg, and the male lambs were 1.5% heavier than the female ones (Table 1), however, the difference is unreliable (Table 2). The variation is in a relatively wide range of 27.0% for the male and 27.9% for the female lambs. Upon studying the population in a previous period, Achkakanova and Staykova (2019) ascertained similar live weight at birth of female lambs - 4.564 kg, which comes to prove that this parameter is stable around that level.

Table 1. Ile-de-France breed live weight at birth, at 30 days old and 70 days old

Age	Gender	Mean	Std. Er.	Std. Dev.
At birth	Male	4.531	0.042	1.225
	Female	4.462	0.043	1.247
30 days	Male	14.90	0.078	2.271
	Female	14.74	0.085	2.424
70 days	Male	27.31	0.155	4.598
	Female	27.56	0.158	4.514

Of all paratypical factors examined by us, the farm is the only one having reliable influence on the live weight of lambs at birth ($P < 0.001$) with an average live weight difference between the different farms from 1.52 to 19.5% (Table 3). There was also a reliable difference in the live weight of the lambs, reared in the different farms, which were born in one and the same month ($P < 0.01$).

Our study corroborates the influence of the farm ascertained by Achkakanova and Staykova (2019), but does not take into account the influence of the year of birth on the live weight at birth of lambs of the Ile-de-France breed and their growth during the next periods. Considerable influence of the year on the live weight at birth and the growth until weaning is

also ascertained by Dimitrov (1978), Dimitrov et al. (1982).

The factors year of birth, month of birth and farm, however, probably have varied impact on the live weight at birth as, within the year, the reliable effect of the "farm" factor turns pale (Table 2).

Similar interactions to those mentioned above are also observed when the months are grouped into seasons. Like the month of birth, the season does not affect the live weight of lambs upon birth on its own, but the farm is a reliable source of variation within the season. The interaction farm*season*year of birth is close to the interaction farm*month of birth*year of birth- within the year and the season, the farms are not a reliable source of variation in the live weight at birth.

Along with the farm, the father also considerably affects ($P < 0.05$) the live weight at birth (Table 4). The same analysis model indicates differences ($P < 0.01$) between the genders, however, the male and female lambs in the separate farms have not appreciably differed in their live weight at birth. The fathers have not exhibited reliable influence within the different genders which shows that the father influences the size of lambs in general.

Achkakanova and Staykova (2019) have ascertained the influence of the farms on the live weight at birth as well as at all other ages examined.

Along with the reliable difference at birth, the lambs from the different farms also varied in their growth until weaning. The lambs with biggest live weight at birth also had the biggest live weight at 30 days old (Farm № 3). The difference with the other two farms where the live weight at birth is similar is 27.2 and 33.0%. In the second farm, there is a relative slowdown in growth around 30 days old which is compensated in the next period until weaning. The difference with the farm with biggest live weight of lambs at birth, however, is not compensated and even increases to 38.8%.

Dimitrov et al. (1982) and Ivanova et al. (2017) report bigger average live weight at birth, but the values at the other ages are lower, i.e. just like we observed in the second farm, there is a certain slowdown in the growth around day 30 which is compensated until weaning.

The results of Raycheva et al. (200%) are similar - lower live weight at birth (4.370 kg), at 30 days old (11.826 kg) and at 70 days old (20.750 kg), whereas Laleva et al. (2006) publishes data indicating even lower average weight at birth - 3.570 kg but the results regarding the other ages are close to those of Raycheva et al. (2005).

Dimitrov (1978) specifies slightly lower values of the parameter examined at birth and at 1 month old with reference to the lambs of introduced mothers, and results close to ours with reference to the lambs of ewes born and reared in Bulgaria. Achkakanova and Staykova (2019) report observations performed and results gained regarding the live weight parameter as follows- the live weight of lambs of the Ile-de-France breed at birth is 4.457 kg, at 30 days old 15.164, at 70 days old - 23.736 kg, at 9 months - 54.761 kg and at 24 months - 70.939 kg. The results we obtained in the present study on the ascertainment of the growth of lambs of the Ile-de-France breed from birth to weaning are also close to those announced in the Ile-de-France Breeders Association in Bulgaria (AILFB) report for 2018 in which results of the entire population reared in Bulgaria are presented. During all periods examined, the "farm" factor keeps its reliable influence on the live weight of the lambs. Certain differences are also noticed in the growth of the lambs from the two genders born in different months but, as a whole, the gender is not a reliable source of variation of the growth.

A range of specific interactions have a considerable effect on the weight at weaning: the farm within the month and year of birth ($P < 0.05$), the gender within the farms and the year of birth ($P < 0.05$), the gender within the scope of the farm, month and year of birth ($P < 0.05$).

Upon grouping the months into seasons, the specific effects disappear which shows that, upon specifying the breeding value, the factors farm, year and month of birth should be included in the linear models.

The descendants of the different stock-breeding farms differ considerably both in their live weight at birth and in their growth and weight upon weaning.

Table 2. Influence of major paratypical factors on the live weight at birth and the growth of lambs of the Ile-de-France breed until weaning

Model	Factor	Age					
		At birth		30 days		70 days	
		F	Sig.	F	Sig.	F	Sig.
M1	gender	1.287	0.257	1.861	0.173	1.276	0.259
M2	year of birth	0.323	0.570	0.000	0.996	2.884	0.090
	month of birth	0.652	0.753	1.528	0.132	0.537	0.848
	farm	9.958	0.000	137.764	0.000	324.704	0.000
	gender	1.084	0.298	1.527	0.217	0.003	0.956
	year * month	0.718	0.541	1.336	0.261	1.823	0.141
	year * farm	1.280	0.278	15.296	0.000	0.249	0.780
	year * gender	0.529	0.467	4.245	0.040	0.735	0.391
	month * farm	6.291	0.000	5.929	0.000	5.570	0.000
	month * gender	1.511	0.159	2.147	0.036	2.030	0.048
	farm * gender	0.229	0.795	0.364	0.695	0.813	0.444
	year * month * farm	0.136	0.713	0.968	0.325	4.794	0.029
	year * month * gender	1.224	0.269	7.090	0.008	3.369	0.067
	year * farm* gender	1.119	0.327	0.497	0.608	3.188	0.042
	month * farms * gender	1.590	0.123	1.515	0.147	1.806	0.072
	year * month * farm * gender	3.330	0.068	0.255	0.613	6.271	0.012
M3	Year of birth	0.001	0.982	0.012	0.914	0.023	0.880
	Season of birth	0.290	0.748	1.497	0.224	0.798	0.450
	Farm	43.835	0.000	692.576	0.000	1541.587	0.000
	Gender	0.068	0.795	0.000	0.988	1.020	0.313
	year * season	0.003	0.957	0.615	0.433	0.005	0.944
	year * farm	0.444	0.642	16.718	0.000	4.001	0.018
	year * gender	0.337	0.562	4.884	0.027	1.314	0.252
	season * farm	18.936	0.000	11.347	0.000	2.588	0.052
	season * gender	0.447	0.639	2.816	0.060	0.918	0.400
	farm * gender	0.122	0.885	0.711	0.491	0.516	0.597
	year * farm * gender	0.216	0.806	3.241	0.039	0.594	0.552
	season * farm* gender	1.324	0.265	1.637	0.179	0.057	0.982

Table 3. Live weight at birth and growth of lambs of the Ile-de-France breed until weaning in different farms

Age	gender	Farm					
		1 (36)		2 (46)		3 (37)	
At birth	male	4.360	1.286	4.421	1.012	5.241	1.186
	female	4.299	1.352	4.361	0.858	5.110	1.221
	Total	4.329	1.320	4.395	0.947	5.175	1.203
30 days	male	14.41	1.500	13.91	1.639	18.26	2.138
	female	14.25	1.663	13.44	1.577	18.21	2.280
	Total	14.33	1.587	13.71	1.627	18.23	2.207
70 days	male	25.69	2.691	25.44	2.620	35.67	2.301
	female	25.68	2.327	26.01	2.863	35.64	2.168
	Total	25.69	2.509	25.69	2.741	35.65	2.231

Table 4. Influence of the mother and the father on the live weight at birth and the growth of lambs of the Ile-de-France breed until weaning (Model 4)

Factor	Age					
	At birth		30 days		70 days	
	F	Sig.	F	Sig.	F	Sig.
Farm	6.329	0.012	118.699	0.000	343.810	0.000
Gender	7.534	0.006	0.549	0.459	0.014	0.908
Father	1.442	0.021	2.486	0.000	1.438	0.021
Mother	1.223	0.269	4.546	0.033	1.937	0.164
farm * gender	0.128	0.720	3.033	0.082	0.007	0.932
gender * father	0.841	0.770	1.033	0.413	0.803	0.828

CONCLUSIONS

The male lambs of the Ile-de-France breed reared in flocks in Northern Bulgaria are born with an average live weight of 4.531 ± 0.042 kg, at 30 days old they reach 14.90 ± 0.078 kg, at 70 days - 27.31 ± 0.155 kg and the female ones - 4.462 ± 0.043, 14.74 ± 0.085 and 27.56 ± 0.158, respectively. The gender is not a reliable source of variation of the parameters examined.

The farm year season and month of birth father and mother factors are in a complex network of interaction but as a whole it is the farm and the father that influence considerably the live weight at birth and the growth of the lambs until weaning.

Upon calculating the breeding value in terms of growth of the lambs until weaning the year and month of birth need to be included in the linear model along with the farm.

REFERENCES

Achkakanova, E. Staykova, G. (2018). Evaluation of the main productive traits of Ile de France sheep in Bulgaria. *Book of abstracts*. ISC on AB. 24.04.2018. Sliven. ISBN 978-619-160-981-9.

Achkakanova, E., Staykova, G. (2019). Evaluation of the main productive traits of Ile-de-France sheep in Bulgaria. *Bulgarian Journal of Agricultural Science*, 25 Suppl.1, 69-72.

Achkakanova, E., Staykova, G. (2019). Effect of Different Sources of Specific Variance on Live Weight and Daily Gain of Ile-de-France Sheep in Bulgaria 1-1017. ISSN 1311-0489 Agricultural Academy, *Journal of Mountain Agriculture on the Balkans*, 22(2), 1-267.

Dimitrov, Il. (1978). Characteristics of selection signs of the Ile-de-France breed, bred in Bulgaria. I. Fertility weight development and meat properties of lambs. *Animal Sciences*, 4, 58-67.

Dimitrov, I. (1988). Development of a specialized synthetic line and use of the Ile-de-France breed for improving the meat properties of lambs. *Dissertation*.

Dimitrov, I. (1991). Introduction of indices sheep selection by fertility and by breeding for meat. *Animal sciences*, 1-4, 36-39.

Dimitrov, I., Slavov, R., Achkakanova-Dimitrova, E. (2016). Selection program for breeding the Ile-de-France. Stara Zagora.

Dimitrov, I., Kaleva, S. (1987). Analysis of live weight and fertility signs of the Ile-de-France breed. *Animal Sciences*, 10, 3-9.

Dimitrov, I., Stankov, I., Slavov, R., Achkakanova–Dimitrova, E. (2011). Selection program for breeding the Ile-de-France. Stara Zagora.

Dimitrov, I., Georgieva, E., Ivanov., I. (1982). Phenotypic parameters of basic productivity qualities of sheep from the Ile-de-France breed, bred in the Institute of Cattle and Sheep Breeding - Stara Zagora. *Animal Sciences*, 3, 20-26.

France Génétique Elevage. 2020. http://en.france-genetique-elevage.org/INSEM OVIN.2020. https://www.insemovin.com/? lang=fr.

Ivanova, T., Raicheva, E. (2017). Analysis of the live weight and the gain of lambs from the flock of Ile-de-France breed according to genealogical lines. *Journal of Animal Science*, 2, 3-9 (BG).

Metodiev, N., Todorov, N., Raicheva, E. (2010). Sexual activity and use of non-hormonal methods for synchronization of fertility and increasing litter size of ewes from the Ile-de-France breed. *Journal of Animal Science*, 3, 15-23 (BG).

Raicheva, E., Ivanova, T. (2005). Effect of lambing. Type of birth and sex on the live weight and the gain of Ile de France lambs. *Journal of Animal Science*, 5, 222-226 (BG).

Raicheva, E., Ivanova, T. (2010). Live weight and gain at sheep from Ile-de-France breed according to some factors. *Journal of Animal Science*, 5, 10-15 (BG).

Slavov, R. (2007). Opportunities for improvement of sheep from the North-Eastern Bulgarian fine-fleece breed - Dobrudjan type. *PhD Dissertation*. Stara Zagora.

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SILKWORMS (*BOMBYX MORI* L.) REARING USING ARTIFICIAL DIET DURING THE SUMMER

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Abstract

The purpose of the research is to investigate new methods of silkworms rearing for a longer period during the year. To accomplish our main goal, we used artificial diet for silkworms rearing during the summer. The tasks of our research were to apply an artificial diet from the first to the third instars, and in the fourth and instars we used mulberry leaves. There is a trend showing that the hatchability in September is higher than in July and August. The average values of the pupation rate and larval period duration also remained relatively high in September. Considering the fresh cocoon yield by one box of eggs, we found that the lowest values are in August and the highest - in September again. Based on the results obtained, it can be concluded that the rearing of the silkworms using artificial diet in the young instars is most appropriate in September. It is economically viable, leads to greater employment and to increasing of the income of sericulture farmers.

Key words*: artificial diet, Bombyx mori L., Morus alba L.*

INTRODUCTION

The change of the organizational structure of agriculture in 1990s in Bulgaria and its intensive development, as well as the climate changes and the decrease of the rainfall in a temperate climate zone in the last 10 years created serious difficulties related to the silkworms rearing (*Bombyx mori* L.).

Rainfall in the period May-September is one of the main factors influencing the growing of mulberries under non-irrigation conditions, as is the case in widespread practice in Bulgaria. Humidity plays an important role in the synthesis of organic compounds and the transfer of mineral elements and substances to different parts and organs of the tree, as well as in the course of growth processes and cooling of the leaves when heated by the sun. Synthesis of 1 kg of dry matter requires the consumption of 700-800 l of water. The shoots usually contain more than 60% water and the leaves contain 70-85%. This shows the extreme importance of sufficient water in the soil and in the air while maintaining the necessary turgor, succulence and tenderness of the leaves in order to use them to produce a quality leaf mass. On the other hand, the labour force during the period May-September is very often insufficient. This necessitates exploring of other opportunities for lucrative and cost effective methods of silkworms rearing during this period. One way to solve this problem is to use artificial diet, which on its turn could be able to minimize the need for labour.

Although the Mulberry (*Morus* sp.) leaf is still considered as the traditional food for silkworms, many attempts have been made to establish rearing on artificial diet. Nowadays, sericulture research developed number of supplement nutrients with mulberry leaves for silkworm rearing. There are number of foods used as an ingredient for artificial diet of silkworm. Artificial diet encourages the small landless farmers to take up sericulture and it also helps to reduce labour cost for mulberry cultivation (Bhattacharyya et al., 2016).

There are insects for which it is possible to grow large populations in laboratory conditions, such as *Drosophila*, which has been used for many years as a major target for genetic research. Large-scale farming of *Bombyx mori* L. and *Apis mellifera* have been described as being widely spread and practiced decades ago (Cohen, 2004).

According to Panizzi et al. (2012), 1,300 species of insects are farmed using artificial

diet, 85% out of which are represented by Lepidoptera, Coleoptera and Diptera species.
Sahay et al. (2011) also developed artificial diet for rearing of *Anthereaea mylitta* D. under controlled conditions.
The use of artificial diet for *Ostrinia furnacalis* and *Harmonia axyridis* rearing has been reported in the literature (Teguh et al., 2018; Yuan-Xing Sun et al., 2019) used artificial diet for rearing, and the results obtained are similar to those of field-grown insects, allowing the production of a large number of insects in laboratory conditions and their use for biological control.
According to Petkov et al. (1980), one of the reasons for the unattractiveness of the silkworm industry is its extensive nature, which is associated with the consumption of a lot of labour for a short-term. In this respect, the cost of feed constitutes about 60% of the total cost, with 27-32% falling on the pruning and delivery of the mulberry leaves and 31-36% on the feeding process - mainly during the fourth and fifth instars.
Silkworm rearing on artificial diet has many advantages over existing practices, since it provides balanced nutrition and disease-free conditions, regardless of the seasons (Jula et al., 2011). This is especially important in the first, second and third instars.
According to Nair et al. (2013), mulberry leaves, especially in tropical conditions, does not always contain the necessary balanced nutrients that meet the needs of silkworms throughout the year.
It is widely accepted that the high cost of the artificial diet is a major problem for the spread of silkworm rearing in cooperative houses, especially in the young instars. The cost of the artificial diet amounts to about 35% and 50% of the total cost of silkworms rearing in cooperative houses from the first to the second instars and from the first to the third instars, respectively. Therefore, it is important to reduce the costs of artificial diet in order to extend the period of rearing so that farmers to be able to develop more robust breeding management systems (Shinbo et al, 1994).
Tzenov et al. (2010) creates a balanced artificial diet for rearing over the whole larval period. Silkworms reared on artificial diet have a shorter larvae period during the fifth instar and during the rearing period as a whole. Considering the other characters, there are no significant differences to the silkworms reared on mulberry leaves. Artificial diet is appropriate to be applied during all seasons of the year, which, on its turn will lead to higher incomes for the silkworm rearers.
We consider all those facts as reasonable enough to set as our main objective to explore new methods of silkworms rearing for a longer periods during the year and thus, for higher employment of the farm workers.

MATERIALS AND METHODS

Strains. Strains named Svila 1 and Svila 2 were tested. Three replicates with 200 larvae each were employed. As a reference we used silkworms reared on mulberry leaves only. Svila is uni-bivoltine 4 molting pure line, created in Bulgaria in 2005. The egg serosa color is greenish gray, chorion color is yellow and the eggs are sticky. The last instar silkworm larvae are bluish-white in color and plain. The body shape is thicker and shorter. The cocoons are white in color, with oval shape.
Environmental conditions. The incubation of silkworm eggs was carried out in accordance to the standard method for the summer season.
The standard conditions for silkworm rearing with mulberry leaf are shown in Table 1.

Table 1. Environmental conditions (larvae fed on fresh mulberry leaves)

Instar	Temperature, °C	Relative humidity, %
I	26-27	85-90
II	26-27	85-90
III	25-26	80-85
IV	23-25	70-75
V	20-25	65-70
Cocooning	24-26	70-75

The environmental conditions required for the rearing on artificial diets are slightly different than those established for the rearing on fresh mulberry leaves. The conditions for silkworm rearing with artificial diet are as shown in Table 2 and it can be seen that the temperatures are slightly higher during the all instars and cocooning.
Immediately after removal from the refrigeration chamber, the silkworm eggs were

placed at a temperature of 24-25°C. From the third day the temperature was raised to 26-27°C. During the first and the second instars, the temperature was set to be such as 29 to 30°C, being higher by 1-2°C than the one that has been set for mulberry-reared larvae. The same difference can be observed in regards to the temperature during the third instar. It is assumed that at a high temperature larvae might gain more weight than at a low temperature. The rearing temperature is recommended to be higher in the fourth instar also (compared to mulberry-fed rearing). It should be anyway kept low, such as at 24°C, in the fifth instar, similar to the case of mulberry rearing where the optimum temperature interval is established to be 20- 25°C.

Table 2. Environmental conditions (larvae fed on artificial diet)

Instar	Temperature, °C	Relative humidity, %
I	29–30	90
II	29–30	90
III	27–28	80
IV	26	70–75
V	24	70
Cocooning	25–27	55–60

The relative humidity was kept as required (50-85%). Higher humidity in the rearing room is recommended at least in order to prevent the drying of diet.

The incubation is carried out in room which the natural light can enter; artificial lighting is rather not used. 12-hours light and 12-hours dark rhythm is recommended as photoperiodism can also affect the silkworm rearing at some extend.

On the 10th day after the incubation has started, some silkworm eggs turn white and the first larvae appear. On the 11th day the hatching begins and, in order to prevent the larvae from crawling out, a few mulberry leaves or artificial food are placed around them. On the 12th day a "mass" hatching is observed and then the larvae can be fed with artificial diet for first time. Only the larvae, hatched on the day of "mass" hatching are brushed for rearing.

Experimental plan. The experiments were conducted during the period 2017-2018 at the Agricultural University of Plovdiv. For the achievement of our main objective, we tested the use of artificial diet in silkworms rearing during the summer. The tasks of our research were to feed the silkworms on artificial diet during the first, second and third instars, and the silkworms in their fourth and fifth instars we fed on mulberry leaves. The main technological characters were studied: fresh cocoon weight; cocoon shell weight; filament length and shell ratio.

RESULTS AND DISCUSSIONS

Table 3 shows the mean values of the hatchability of the employed strains Svila 1 and Svila 2 during the months of July, August and September. The average values for Svila 1 range from 1,109 to 1,683, indicating that the values of hatchability in 2017 are within acceptable limits, with the average error ranging from 0.671 to 1.011.

Table 3. Hatchability (%)

Strains	Months	2017			2018		
		$\bar{x}$	*Sx*	*Vc*	$\bar{x}$	*Sx*	*Vc*
Svila 1	July	85.56	0.67	1.11	84.35	0.621	1.04
	August	82.24	0.76	1.31	82.95	1.00	1.70
	September	84.91	1.01	1.68	85.23	0.35	0.58
Svila 2	July	83.44	0.90	1.53	81.25	0.73	1.27
	August	82.88	2.50	4.26	82.32	0.27	0.46
	September	87.53	0.40	0.65	85.30	0.43	0.71

We have the lowest results of the character in August 2017 for Svila 1 with a value of 82.24% and with a value 81.25% in July 2018. For Svila 2, the highest hatching rate of 87.53% was observed in September 2017, being with 2.23% more than in the same month of the following year. In Svila 1, the difference in hatching in September in both years is minimal and has a value below 1%, and in both strains we noticed a slight decrease in values in

August, and then in the following month they increase. The lowest value of the hatchability character was recorded in Svila 2 in July 2018 with a value of 81.25%, and the highest one - 87.53%, was registered in September 2017 for by Svila 2 again (the difference between them being 6.28%).

There is a trend showing that the hatchability in July is lower than in September, which is most likely due to the negative impact of the high temperatures in July and August.

The average values of the pupation rate of the both studied strains remained relatively high in September (Table 4). There is a greater variation of the values of this character for the Svila 1 in July 2017 and for the Svila 2 in September 2018. The difference between the average values of the pupation rates in September (in terms of the surveys conducted in two consecutive years), is minimal with a value of 0.13% for the Svila 1 and 0.73% for Svila 2.

One of the factors that influence the pupation rate is the diseases during the larval stage. In regards to this character, there are slight differences between the larvae fed on artificial diet and those fed on fresh mulberry leaves. This difference is due to the fact that when feeding on artificial diet we can better control the humidity and the development of microorganisms that can cause some diseases. For larvae fed on mulberry leaves, the mortality is slightly higher and hence a slight decrease in the pupation rate might be observed.

Table 4. Pupation rate (%)

Strains	Months	2017			2018		
		$\bar{x}$	*Sx*	*Vc*	$\bar{x}$	*Sx*	*Vc*
Svila 1	July	87.33	0.820	1.33	86.67	0.41	0.68
	July (reference)	87.70	0.25	0.41	86.93	0.10	0.17
	August	86.23	0.55	0.90	85.29	0.24	0.40
	August (reference)	86.17	0.18	0.29	86.03	0.10	0.18
	September	89.40	0.37	0.59	89.53	0.39	0.62
	September (reference)	88.80	0.14	0.22	88.73	0.177	0.28
Svila 2	July	88.57	0.41	0.66	88.27	0.45	0.73
	July (reference)	87.90	0.19	0.30	86.60	0.39	0.64
	August	85.30	0.43	0.71	86.80	0.14	0.23
	August (reference)	85.37	0.25	0.41	86.96	0.15	0.23
	September	89.07	0.15	0.23	89.80	1.04	1.64
	September (reference)	88.90	0.07	0.11	89.16	0.47	0.74

The results on the larval period duration show a variation of the character from 0.897 to 1.97 for Svila 1 and from 0.71 to 1.22 for Svila 2 (Table 5).

The lowest values of the variation coefficient for both Svila 1 and Svila 2 strains are observed in September. The deviations are insignificant and no firm conclusions can be drawn. There is no trend or significant difference compared to the reference values.

For Svila 1 strain fed on artificial diet, we observed a difference in the duration of the larval period of 92 h.

The lowest duration was in September 2018 with values of 776.67 h. For the reference of the same strain, a difference of 50 h between the lowest and the highest value is observed.

For Svila 2 strain, the difference in larval period duration is relatively small, with a value of 36.67 h for the larvae fed on artificial diet, and 63.33 h for the reference.

In silkworms rearing, the only character that targets lower values is the larval period duration. With longer larval period duration, an extended workflow for feeding and rearing can be expected, and hence a higher labor costs.

Table 5. Larval period duration (h)

Strains	Months	2017			2018		
		$\bar{x}$	*Sx*	*Vc*	$\bar{x}$	*Sx*	*Vc*
Svila 1	July	851.67	5.40	0.89	863.33	10.80	1.77
	July (reference)	850	7.07	1.17	870	7.07	1.14
	August	868.67	8.52	1.38	830	7.07	1.20
	August (reference)	856	8.60	1.42	826.66	4.08	0.69
	September	803.33	10.80	1.90	776.67	10.80	1.97
	September (reference)	830	7.07	1.20	800	14.14	2.8
Svila 2	July	870	7.07	1.14	820	7.07	1.22
	July (reference)	870	7.07	1.14	826.66	14.71	2.48
	August	876.67	10.80	1.74	863.33	4.08	0.74
	August (reference)	876.66	4.08	0.65	836.66	14.71	2.48
	September	813.33	4.08	0.71	783.33	4.08	0.74
	September (reference)	813.33	8.16	1.41	826.66	26.77	4.58

With reference to the fresh cocoon yield by one box of eggs, it was found that the highest values for both tested strains are in September (Table 6).

Larvae being fed on artificial diet show no significant differences compared to the reference - 0.37 kg for Svila 1 in 2017 and 0.46 kg in 2018. For Svila 2 the difference is 0.33 kg in 2017 to 0.67 kg in 2018.

The lowest values are in August, with a variance from 0.680 to 2.350.

These differences are probably due to the optimal conditions created during the young instars.

This finding proves the fact that the conditions during the first instars lead to higher yields in the latter instars.

Table 6. Fresh cocoon yield by one box of eggs (kg)

Strains	Months	2017			2018		
		$\bar{x}$	*Sx*	*Vc*	$\bar{x}$	*Sx*	*Vc*
Svila 1	July	23.74	0.61	3.68	23.13	0.39	2.38
	July (reference)	24.26	0.86	5.03	25.16	0.21	1.21
	August	22.47	0.10	0.68	23.03	0.29	1.81
	August (reference)	23.23	0.34	2.12	23.76	0.177	1.06
	September	25.93	0.04	0.22	25.47	0.39	2.16
	September (reference)	25.56	0.34	1.93	25.93	0.10	0.58
Svila 2	July	24.43	0.33	1.93	25.3	0.31	1.72
	July (reference)	24.43	0.28	1.65	24.56	0.35	2.00
	August	23.43	0.38	2.35	23.13	0.23	1.39
	August (reference)	23.3	0.43	2.61	24.03	0.22	1.27
	September	26.57	0.41	2.20	26.13	0.47	2.55
	September (reference)	25.9	0.07	0.38	25.8	0.18	1.02

The fresh cocoon yield by one box of eggs character is the indicator that shows the actual results of the rearing. Higher values of the character are aimed at each test and selection. While with the larval period duration lowering the values is the aim, here we consider the higher values as the best results.

CONCLUSIONS

Silkworm rearing during the summer season (from July to August) by applying artificial diet during the first three instars and mulberry leaves during the later instars does not lead to significant differences compared to the rearing of silkworms on mulberry leaves only.

Therefore, the results obtained allow us to recommend silkworm rearing during the summer season in Bulgaria on artificial diet during their first three instars and then with mulberry leaves up to the cocooning, which on its own turn will provide optimal nutrition and sanitary conditions, as well as reducing labor costs and raising the income of the silkworms rearers.

REFERENCES

Bhattacharyya, P., Jha, S., Mandal, P., Ghosh, A. (2016). Artificial diet based silkworm rearing system-A review. *Int. J. Pure App. Biosci*., 4(6), 114-122.

Cohen, A. C. (2004). *Insect diet; Science and Technology*, Boca Raton, Fl:CRC Press.

Jula, S.N., Nirmal Kumar, S. (2011). Development of bivoltine pure strains of silkworm, *Bombyx mori* L. to rear exclusively on artificial diet during young instar. *Journal of Biological Sciences*, 11(6), 423-427.

Nair, J.S., Nirmal Kumar, S., Nair, K.S., Babu, A.M. (2013). Scaning Electron microscopic studies on the mouth parts of silkworm, *Bombyx mori* L. developed on artificial diet. *Sericologia*, 53(2), 95-102.

Panizzi, A.R., Parra, J.R.P. (2012). *Insect bioecology and nutrition for integrated pest management*, CRC Press, Taylor and Francis Group, Boca Ration, London, New York .

Shinbo, I., Yanagaw, H. (1994). Low-cost artificial diets for poliphagous silkworm. *JARQ*, 28, 262-267.

Teguh Rahayu, Y., Witjaksono, A.T. (2018). Fitness of Asian corner borer, *Ostrinia furnacalis* (Lepidoptera: Crambidae) reared in an artificial diet. *Journal of Asia-Pacific Entomology*, 21, 823-828.

Yuan-Xing, Sun, Ya-Nan, Hao, Chang-Zhong, Liu, Sen –Shan, Wang (2019). Artificial diet is fruitful pre-stirage nutrition for long-term cold storage of laboratory-reared *Harmonia axyridis* (Pallas) adults. *Biological control*, 139.

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ASSESSMENT OF NON-GENETIC EFFECTS ON THE FATTENING AND SLAUGHTER TRAITS FOR DANUBE WHITE PIGS BASED ON THE PERFORMANCE TEST EVALUATION

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Abstract

An evaluation based on performance tests was carried out on 639 male and female (castrated) animals from the Danube White breed in Agricultural Institute Shumen. The testing of animals was done at 90 kg live weight with the "Pig log 105" apparatus. Significant sources of specific variance in different levels of probability were established for line, year, sex and replacement animals. Significant effects of sublevels of the studied environmental factors were also established. The results, presented by us, emphasized the necessity of fixed trait evaluations and determination of their effects during the genetic evaluation of the Danube White population.

Key words*: Danube White pigs, environmental effects, performance test.*

INTRODUCTION

Swine from the Danube White breed were locally selected, with a narrow range and limited number of animals – two herds with around 500 swine. In recent years, there have been significant changes in fattening and slaughter qualities that make animals competitive on the internal market. A large number of studies for assessing the origin influence, year of testing, season and, mostly, the effect of gender on the evaluated performance traits have been available. In some of the studies, differences between the slaughter traits in males and females were not found (Alonso et al., 2009; Gispert et al., 2010), while other authors pointed out that meat qualities were influenced by gender (Bridi et al., 2006; Franco et al., 2008). Significant influence on traits characterizing growth capabilities was caused by origin, herd, year of testing and their interaction.

The selective breeding programs in pig farming used variation rates to improve fattening and slaughter traits. Variation in turn was represented by genetic and environmental components. Environmental variation, although not transferred from the parents to the offspring, has been essential for the productivity of the tested animals. The assessment of non-genetic factors gives assistance to the standardization and productivity organization (management) (Dube et al., 2011). Therefore, using environmental factors in pig breeding plans has been imperative (Habeanu et al., 2018). Numerous similar studies for assessing the influence of non-genetic effects have been available (Mungate et al., 1999; Serrano et al., 2008).

Occasional monitoring of the breed has been imposed by the market supply and demand, which influences selection.

The aim of this research was to study some non-genetic effects on fattening and slaughter traits for Danube White pigs based on the performance test evaluation

MATERIALS AND METHODS

The study was carried out in the period 2013-2019 in Agricultural Institute - Shumen. The animals were fed with fodder mixtures for the corresponding category according to Bulgarian State Standards. The animals were provided with adequate floor space for movement.

An evaluation based on performance tests of 639 male and female animals from the Danube White breed was made. Animal testing was carried out at 90 kg live weight with Pig log 105 apparatus; for each kilogram below or over 90 kg was added or deducted 0.7 days from the age according to the Regulation for Evaluation

of Breeding Value, Productivity and Classification of Breeding Pigs, Shumen.

The analyzed traits were: backfat thickness in points X_1 and X_2, thickness of *Musculus longissimus dorsi* (MLD) and growth rate intensity to 90 kg live weight.

Backfat thickness in point X_1 (located between the third and fourth lumbar vertebra, 7 cm from the medial line) and X_2 (located between the last third and fourth ribs, 10 cm from the medial line).

Data processing was made with LSMLMW MIXMDL software, version Pc-2.

The following statistical model was used:

$$\gamma = \mu + L_{i(1-8)} + Y_{j(1-7)} + R_{k(1-2)} + S_{i(1-2)} + e_{(i-)}$$

where: μ - average

L - Fixed line effect;

Y - Fixed year effect;

R - Fixed effect of replacement animals;

S - Fixed gender effect;

e - Residual effect.

The reliability of differences between the levels of studied factors was established according to distribution levels by Student (Hayter, 1984).

RESULTS AND DISCUSSIONS

Results from the performance test evaluation and the analysis of variance for productive traits are shown in (Table 1). Analysis of the results for the backfat thickness traits in points X_1 and X_2 present the same range from 11 to 15 mm. *Musculus longissimus dorsi* (MLD) was 44.45 mm and the age at 90 kg live weight was 205 days.

Statistically significant sources of specific variance with different rates of probability were established for all studied factors. The chosen replacement animals for own breeding have had significant and highly significant effect on traits: back fat thickness in point X_2 ($P \leq 0.05$) and age at 90 kg live weight ($P \leq 0.01$).

Gender had significantly influenced all of the studied traits including back fat thickness in point X_1 and age, as well as *Musculus longissimus dorsi* (MLD) thickness and X_2 ($P \leq 0{,}05$). Origin based on lines had a significant influence for the back fat thickness trait in point X_2 ($P \leq 0.05$), while no significant differences were established for the other traits. Regarding the year of testing trait, significant and highly significant effect was established for the following traits: back fat thickness in point X_2, *Musculus longissimus dorsi* (MLD) thickness and age at 90 kg live weight ($P \leq 0.01$, $P \leq 0.001$).

Determination coefficient were with comparatively high values for the backfat thickness trait in points X_1 and X_2 and age at 90 kg live weight ($R^2 = 0.77$, $R^2 = 0.81$, $R^2 = 0.66$) which showed that the studied factors accurately reflects the trait variations in the model. Regarding *Musculus longissimus dorsi* (MLD) thickness, this indicator was with low values.

Table 1. Results from the performance evaluations and variance analysis of the studied factors

Traits	Number, n/degree of freedom df	Backfat thicknes		*Musculus longissimus dorsi*, mm	Age, days
		X_1	X_2		
$\bar{X}$		15.20	11.76	44.45	205
SD		4.18	3.17	4.91	27.82
CV		13.22	12.00	9.41	8.06
R		0.77	0.81	0.30	0.66
Total	639				
Total reduction	88				
Replace-ment	1	n.s	*	n.s	***
SEX	1	**	*	*	**
Line	7	n.s	+	n.s	n.s
Year of test	6	n.s	***	**	***

Significance of differences: *** - P≤0.001, ** - P≤0.01, * - P≤0.05; n.s. - no significances

Influences of the line, year of testing, number of called and replacement animals and gender on studied traits from the evaluation for individual productivity are indicated in (Table 2).

It was established that the thinnest fat was in point X_2 in pigs from the first line (11.5 mm) as differences between them and those from fifth and seventh line were significant and highly significant ($P \leq 0.05$, $P \leq 0.01$).

Significant differences were established for the same trait between the fifth and seventh line with the second, third and sixth ($P \leq 0.05$). Backfat thickness in point X_1 was the thinnest in line seven, as differences with first, second and third line were significant ($P \leq 0.05$).

The same degree of significance was established in third line with fourth and fifth

(P≤0.05). *Musculus longissimus dorsi* (MLD) thickness was with the highest values in the seventh line (45.42 mm). The highly significant differences between the seventh line with first and second were P≤0.01, but those with third, fourth and fifth were with significance of P≤0.05. For the same trait were established significant differences between sixth line with first, second and fourth (P≤0.05).

Regarding the trait for growth rate intensity to 90 kg live weight, it was established that age had the lowest values for line eight (203 days) and between her and the other lines small and insignificant differences were established.

Differences between years of testing, presented in the same table, were significant and highly significant in regards to almost all studied traits (P≤0.01, P≤0.001), except backfat thickness in point X_1.

Backfat thickness in point X_2 was the thinnest in 2014 (11.38 mm) and between that year and 2013, 2018 and 2019, differences with high significance were established (P≤0.001). *Musculus longissimus dorsi* (MLD) thickness was with the highest values in 2019 (46.69 mm) and age at 90 kg live weight was also the lowest values in 2019 (194 days), as the differences between them and other years were significant (P≤0.001).

An intensive increase (11 days) was established in replacement animals compared to the rest of the studied population (P≤0.001).

In regards to gender, significant differences were established for the *Musculus longissimus dorsi* (MLD) thickness and age at 90 kg live weight traits (P≤0.01). The established regularly regression values of the studied traits backfat thickness in points X_1 and X_2, *Musculus longissimus dorsi* (MLD) thickness and age at 90 kg live weight were small and insignificant.

Growth intensity and lean meat content from farrowing up until the end of the test period was determined by growth and muscle fiber characteristics (Larzul et al., 1997; Gentry et al., 2004).

The influence from the environment on the performance productivity tests suggested presence of differences for the studied fattening and slaughter traits. These differences often occur due to changes in raising during different seasons and years, work organization, which requires optimization of procedures for swine raising and selection.

Dube et al. (2011) established similar results to ours in regards to fattening and slaughter traits, which were significantly influenced by year of testing (P≤0.001).

In regards to the daily gain, respectively, growth intensity was better in female animals, where as in our study male castrated pigs were characterized by higher growth intensity with approximately 6 days.

Similar results were established by Augspurger et al. (2002), where males had more intensive growth rates. The established significant differences for the studied traits between separate years were probably due to differences in the conditions of the production process and organization.

In our study no substantial differences were established, in regards to fat thickness between the two genders. In unison with our results, Lee et al. (2019) did not establish essential differences in backfat thickness, while Dube et al. (2011) found that the male animals were characterized by thinner backfat compared to females (P<0.001). Similar results in which male swine had a higher content of hypodermic fat were established by Cassady et al. (2004) and Bahelka et al. (2007).

Table 2. Factor influence on the studied traits

Factors		n	Backfat thickness, mm		MLD, mm	AP, day	X_1	X_2	MLD	AP
			X_1	X_2						
LSC		639	14.54±0.25	12.08±0.18	43.73±0.53	209.1±2.11				
Line	1	91	14.97±0.42	11.54±0.29	43.17±0.88	212.0±3.49	1-7*	1-5*	1-6*	
	2	94	15.04±0.48	11.71±0.34	43.02±1.01	210.4±4.01	2-7*	1-7**	1-7**	
	3	92	15.16±0.45	11.60±0.31	43.67±0.93	207.8±3.72	3-4*	2-5*	2-6**	
	4	114	14.34±0.45	12.11±0.31	43.29±0.93	209.4±3.71	3-5*	2-7*	2-7**	
	5	102	14.27±0.43	12.32±0.30	43.62±0.90	208.3±3.58	3-7*	3-5*	3-7*	
	6	116	14.74±0.39	11.94±0.27	44.25±0.81	208.8±3.24		3-7*	4-6*	
	7	19	13.58±0.65	13.10±0.46	45.42±1.36	213.1±5.41		6-7*	4-7*	
	8	11	14.23±0.71	12.28±0.50	43.39±1.49	203.1±5.92			5-7*	
YT	2013	110	14.27±0.36	12.68±0.25	43.28±0.76	217.6±3.03		1-2***	1-6,7***	1-2, 5, 7***
	2014	108	14.86±0.41	11.38±0.29	42.73±0.86	218.3±3.42		1-3*	2-4**	2-3, 5, 7***
	2015	44	14.35±0.50	11.89±0.35	42.13±1.04	198.0±4.12		1-5**	2-6, 7***	3-4, 6***
	2016	155	14.95±0.35	11.86±0.24	44.08±0.73	217.6±2.91		2-6, 7***	3-4, 6, 7***	4-5, 7***
	2017	65	14.40±0.42	11.65±0.30	42.30±0.89	201.8±3.53		4-6, 7*	4-5,7***	6-7***
	2018	115	14.40±0.38	12.40±0.27	44.89±0.80	215.9±3.17		5-6, 7*	4-6*	5-7*
	2019	42	14.54±0.52	12.68±0.36	46.69±1.08	194.2±4.29			5-6, 7***6-7*	6-7***
REM	1	451	14.70±0.26	11.89±0.18	43.60±0.55	203.4±2.20				***
	2	188	14.38±0.28	12.26±0.20	43.86±0.59	214.7±2.35				
SEX	1	85	14.08±0.33	12.26±0.23	43.16±0.68	206.0±2.73			**	**
	2	554	14.10±0.24	11.89±0.17	44.30±0.51	212.1±2.04				

Significance of differences: *** - P≤0.001, ** - P≤0.01,* - P≤0.05

CONCLUSIONS

Results from the study on non-genetic effects on the lifetime assessment of fattening and slaughter traits for Danube White pigs established that optimal conditions for raising and managing were a necessary requirement for effective selection.

Significant sources of specific variance in different levels of likelihood were established for the studied traits: replacement, sex, line and year of testing.

The results, presented in this research, emphasized the necessity of fixed trait evaluations and determination of their effects during the genetic evaluation of Danube White population.

REFERENCES

Alonso, V., del Mar Campo, M., Español, S., Roncalés, P., Beltrán, J. A. (2009). Effect of crossbreeding and gender on meat quality and fatty acid composition in pork. *Meat Sci*., 81, 209–217.

Augspurger, N. R., Ellis, M., Hamilton, D. N., Wolter, B. F., Beverly, J. L., Wilson, E. R. (2002). The effect of sire line on the feeding patterns of grow-finish pigs. *Appl. Anim. Behav. Sci*., 75, 103–114.

Bahelka, E., Hanusova, D., Peškovičova, P. (2007). The effect of sex and slaughter weight on intramuscular fat content and its relationship to carcass traits of pigs. *Czech J. Anim. Sci*., 52(5), 122–129.

Bridi, A. M., Oliveira, A. R., Fonseca, N. A. N., Shimokomaki, M., Coutinho, L. L., Silva, C. A. (2006). Effect of halothane genotype, ractopamine and sex on pork meat quality. *Rev. Bras. Zootec*., 35(5), 2027–2033 (in Portuguese, English abstract).

Cassady, J. P., Robison, O. W., Johnson, R. K., Mabry, J. W., Christian, L. L., Tokach, M. D, Miller, R. K., Goodwinn, R. N. (2004). National pork producers council maternal line genetic evaluation: A comparison of growth and carcass traits in terminal progeny. *J. Anim. Sci*., 82, 3482–3485.

Dube, B., Mulugeta, S. D., van der Westhuizen, R.R., Dzama, K. (2011). Non-genetic factors affecting growth performance and carcass characteristics of two South African pig breeds. *South African Journal of Animal Science*, 41(2*).*

Franco, M. M., Antunes, R. C., Borges, M., Melo, E. O., Goulart, L. R. (2008). Influence of breed, sex and growth hormone and halothane genotypes on carcass composition and meat quality traits in pigs. *J. Muscle. Foods*, 19, 34–49.

Gentry, J. G., McGlone, J. J., Miller, M. F., Blanton, J. R. Jr. (2004). Environmental effects on pig performance, meat quality, and muscle characteristics. *J. Anim. Sci*., 82, 209–217.

Gispert, M., Angels Oliver, M., Velarde, A., Suarez, P., Pérez, J., Font i Furnols, M. (2010). Carcass and meat quality characteristics of immunocastrated male, surgically castrated male, entire male and female pigs. *Meat Sci*., 85, 664–670.

Habeanu, M., Gheorghe, A., Ropota, M., Toma, S.M., Marin, M. (2018). Changes in fatty acids composition of *Longissimus dorsi* muscle, brain, liver and heart as effect to hempseed addition in pigs feeding. *AgroLife Scientific Journal*, 7(2), 61–67.

Larzul, C., Lefaucheur, L., Ecolan, P., Gogue, J., Talmant, A., Sellier, P., Le Roy, P., Monin, G. (1997). Phenotypic and genetic parameters for longissimus muscle fibre characteristics in relation to growth, carcass, and meat quality traits in Large White pigs. *J. Anim. Sci*., 75, 3126–3137.

Lee1, J. H., Song, M.H., Yun, W., Liu, S.D., Lee, C.H., Oh, H.J., An, J.S., Yu, D.J., Kim H.B., Cho, J.H. (2019). Influence of season of birth, sex and paternal line on growth performance and carcass traits in pigs. *South African Journal of Animal Science*, 49(2).

Mungate, F., Dzama, K., Mandisodza K., Shoniwa, A. (1999). Some non-genetic factors affecting commercial pig production in Zimbabwe. *S. Afr. J. Anim. Sci.*, 29, 164–173.

Serrano, M. P., Valencia, D. G., Fuentetaj, A., Lázaro R., Mateos, G. G. (2008). Effect of gender and castration of females and slaughter weight on performance and carcass and meat quality of Iberian pigs reared under intensive management systems. *Meat Sci*., 80, 1122–1128.

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CORRELATION LINK OF INDICES OF DAIRY PRODUCTIVITY OF COWS OF HOLSTEIN BREED OF DIFFERENT ORIGIN

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Abstract

The article presents the results of a study of the productive qualities of Holstein cows of the Dutch and German breeding, the presence of a correlative relationship between milk yield, the content and amount of milk fat, live weight, average daily milk yield and milk ratio. It is established the superiority of cows of Dutch breeding for milk yield for 305 days of lactation over cows of German breeding by 458 kg of milk (the third lactation), the difference is not significant. A comparative analysis of the results of studies of the productive qualities of cows of the first lactation of the Holstein breed of the Dutch and German breeding showed that the heifers - the descendants of the first generation exceeded their peer sin milk yield by 855 and 1444 kg of milk respectively, the difference is highly reliable (P<0.001). Low correlation coefficients between milk yield and live weight (positive - German breeding and descendants of the first generation) and (negative - Dutch breeding) indicate the non-linear nature of the relationships between them, and characterizes the uniformity of the herd of Joint-Stock Company „Aydyn" in live weight.

Key words*: descendants of the first generation, milk yield, fat, live weight, correlation coefficient.*

INTRODUCTION

At present, in countries with developed dairy cattle breeding, the genetic improvement of herds largely depends on the direction of the strength of the relationship between the signs of productivity. Genes that influence the development of body systems and cause signs of productivity, act interconnected, that is, correlate (Dragotoiu et al., 2015). The study of correlative relationships allows to predict undesirable consequences when conducting selection on one sign or to enhance the effect of selection on others.

When breeding Holstein cattle, it was found that in the selection process the variability of one of the phenotypic indicators depends on the variability of other economically useful signs. The variability of the mass fraction of fat in milk depends on the variability of cows' milk yield per lactation.

In turn, the variability of milk production depends on live weight, the age of the animals at the first calving, the length of the dry and service periods, calving season and other factors (Akhmetzyanova, 2015).

If there is a positive correlation between breeding signs, selection by one sign automatically leads to the improvement of another. With a negative correlation, selection leads to the deterioration of one of the traits. In the absence of communication, it should be considered that the selection of animals according to the main character does not affect the development of other characters. The rate of genetic improvement of herds depends on the direction and strength of the relationship between the signs. Thereby, it is important to establish to what extent these or those signs are interconnected with milk yield of cows (Efimova et al., 2017).

A study of the relationship between breeding signs at cows (Nazarchenko, 2011) between milk yield and percentage of fat in milk, milk yield and amount of protein, percentage of protein and fat showed that a deterioration of one of the signs does not entail a deterioration of the other.

The correlation between milk yield and fat content in milk is most often negative - with an increase of milk yield, the fat content in milk decreases (Efimova, 2010). However, in each herd there are animals at which high milk yield is combined with a high fat content in milk and this feature is inherited by offspring (Beauty et al., 1999). The selection of animals by the total amount of milk fat or milk protein is equivalent to one-sided selection by milk yield, the authors consider. The correlation coefficients between these signs range from 0.84 to 0.99.

Lepyokhina (2012) believes that correlation between productive signs is a biological regularity and is relatively stable inherited in generations. Even with high selection intensities, they did not change for a long time. The heritability of milk yield by the mother (r = + 0.11) in three lactations was very high, and the milk fat content (r = 0.20) was even higher (Rudziev, 2001; 2003; 2006).

There is not always a direct connection between the productive qualities and the breeding qualities of record cows, and therefore the good origin of each cow does not always guarantee its high breeding and productive qualities. However, many authors (Ernst and Chemm, 1970; Vorobyova, 2010) argue that the most productive animals are mainly descended from the same highly productive ancestors and that the best animals give the best offspring. According to Antal (2004), "the most important moment of work is the selection of the most worthy females, from which it is worth and should be received male offspring for its possible use in improving the population of dairy cows".

One of the factors that influence the milk productivity of cows is their live weight. In each breed, in each herd, the best part of animals in productivity, as a rule, has a higher live weight than the average for the breed, the average for the herd (Silver, 2017; Bakay et al., 2016). For the best of breed milk yield recorders is characteristic and higher live weight, the variability of live weight of cows can reach 15% (Adzhibekov, 1995; Kuznetsov, 2002; Vorobyova et al., 2010). But this does not mean that the biggest animals should be high dairy. It has been established that for each breed there is a certain optimum of live weight (Kuziv and Fedorovich, 2014; Pogadaev and Gadzhiev, 2001). The increase of live weight of cows to this indicator has a positive effect on milk productivity. But if the live weight is above the limit of the breed optimum, then its increase does not affect the increase in milk yield.

The research of the herd of the Joint Stock Company "Aydyn" revealed the relationship with dairy productivity of some indicators of the exterior of cows for the third lactation (Konstandoglo et al., 2019).

The aim of these studies was to identify the correlation between the main indicators of dairy productivity of Holstein cows of different origins.

MATERIALS AND METHODS

Studies were carried out in a herd of Joint-Stock Company (J.S.C.) „Aydyn" on Holstein cows imported from Holland and Germany, as well as the first generation descendants received from bulls Kiperush 79, Maker 891 and Leicester DE 05.804.011478. All the analyzed number of cows was kept in optimal conditions of feeding and keeping in accordance with the basic zootechnical and hygiene requirements.

The main data on the milk production of animals were taken from forms of zootechnical and pedigree accounting. Were used zoo-technical research methods with biometric processing of materials by the method of variation statistics according to Plokhinsky (1978) and Merkurieva (1983): arithmetic mean (X), arithmetic mean error (Sx), correlation coefficient (r) and correlation coefficient error (m_r). Phenotypic correlations were estimated using the procedure of the Statistical Analysis System (SAS Version 6.1, 2007). The relation-ship between milk productivity indicators and milk quality, live weight was determined by calculating the correlation coefficient using Microsoft Excel.

The milk coefficient (MC), proposed by Startsev (1966), was calculated by the formula: MC = MY/LW, where: MC is the milk coefficient, kg; MY - milk yield for 305 days of lactation, kg; LW - live weight, kg. The data obtained during the research were processed biometrically on a personal computer using Microsoft Excel programs; the reliability of the indicators was determined by Student.

RESULTS AND DISCUSSIONS

A comparative assessment of the milk productivity of cows, depending on the selection, showed that the highest milk yield for 305 days of lactation was at Dutch cows (Table 1), which is more by 458 kg of milk (the third lactation) than at German cows (Table 2), and on average in the samples - by 434 kg, the difference is unreliable.

Table 1. Dynamics of milk productivity J.S.C. "Aydyn" Dutch origin (X ± Sx)

Lactation	Number of cows, n	Milk yield		Fat		Live weight, kg	Milk ratio, kg
		The average daily, kg	For 305 days, kg	Mass fraction, %	Quantity, kg		
III	30	34.5±0.8	10560±255.5	3.85±0.03	402±9.9	698±4.4	1508±34.5**
IV	31	33.3±0.6	10194±206.5	3.92±0.03	394.2±8.7	697±3.9	1461±30.2
Average	64	34.0±0.5	10370±160.6	3.88±0.02	379.2±6.4	698±2.8	1490±22.1***

Note: ** - P <0.01; *** - P <0.001

Table 2. Dynamics of milk production of J.S.C. "Aydyn" German origin (X ± Sx)

Lactation	Number of cows, n	Milk yield		Fat		Live weight, kg	Milkratio, kg
		The average daily, kg	For 305 days, kg	Mass fraction, %	Quantity, kg		
II	15	28.8±1.1	8794±333.6	3.87±0.04	339±12.8	716±8.1	1219±41.4
III	106	32.7±0.6	10102±185.6	3.92±0.01	384.7±6.6	720±2.4	1387±22.2
Average	125	32.3±0.05	9936±166.1	3.92±0.01	379.6±6.1	721±2.3	1369±20.2

The live weight of cows of German breeding was by 22 kg more than cows of Dutch breeding, the difference was significant at P<0.001.
In terms of milk yield, cows of the Dutch breeding exceeded the cows of the German breeding by 121 kg (in III lactation and on average in the samples), the difference was significant at P<0.01 and P<0.001, respectively.

A preliminary assessment of the milk productivity of the first generation heifers, the descendants of the first generation, obtained from the result of using bulls Kiperush 79, Maker 891 and Leicester DE 05.804.011478 showed that the average milk yield of heifers was 8658 kg of milk with a fat content of 3.77%, milk fat - 324.3 kg (Table 3).

Table 3. Characteristics of cows - descendants of the first generation, as well as heifers of different breeding by milk productivity, the first lactation (X ± Sx)

Indicators		Descendants of the first generation (MD)	Dutch breeding*	German breeding*
Number of cows, n		38	120	129
Milk yield	average daily, kg	28.7±0.5	25.6±0.3	23.6±0.32
	for 305 days, kg	8658±143.6***	7803±90.1	7214±96.3
Fat	mass fraction, %	3.77±0.03	3.76±0.02	3.77±0.04
	quantity, kg	324.3±5.6	293.4±3.44	272.3±3.86
Live weight, kg		625±5.7	637±2.5	638±4.2
Milk ratio, kg		1364±15.1	1228±13.9	1125±25.7

Note: * - Foksha et al., 2017; *** - P<0.001

As it can be seen, the milk yield coefficient averaged 1364 kg. Milking yields of heifers ranged from 7490 kg to 9455 kg of milk, the average daily milk yield 24.5-31 kg of milk.
A comparative analysis of the results of studies of the productive qualities of cows of the first lactation of the Holstein breed of the Dutch and German breeding (Foksha et al., 2017) showed that the heifers - the descendants of the first generation exceeded in milk yield their peers by 855 and 1444 kg of milk, respectively, the difference is highly reliable (P<0.001). It should be noted that the female ancestors of bulls (fathers of heifers of the first generation)

combined high milk yield and butterfat, which is desirable in transferring valuable qualities to offspring. So, mother's milk yield Kiperush 79, averaged 10915 kg of milk with a fat percentage of 4.42; mother of his father, respectively 11485 kg and 3.60%. Mother's milk yield of Leicester DE 05.804.011478 for highest lactation was 15186 kg of milk with a fat content of 3.81%, mother's father - p11017 kg of milk, 4.59% fat, and milk yield of the mother of the bull Maker 891-12572 kg of milk with a fat content of 3.65%, mother of his father - 11842 kg, fat content of 4.66%.

For the relationship character analysis between the main productivity indicators at cows of different lactations and breeds of J.S.C. "Aydyn" herd was conducted a study of the correlation between milk yield, milk fat content and amount, live weight, average daily milk yield and milk ratio (Table 4).

Table 4. Correlation between the main indicators of the productivity of cows of German breeding, r ± m

No	The correlated sign	II lactation	III lactation	IV lactation
1.	milk yield - mass fraction of fat, %	-0.212±0.3	-0.060±0.1	+0.875±0.5
2.	milk yield - the amount of milk fat, kg	+0.964±0.07	+0.970±0.09	+0.882±0.5
3.	live weight - milk yield 305 days	+0.290±0.26	+0.253±0.09	+0.164±0.6
4.	live weight - mass fraction of fat, %	+0.289±0.26	-0.083±0.1	+0.155±0.6
5.	live weight - fat, kg	+0.393±0.25	+0.143±0.09	+0.401±0.5
6.	milk coefficient - milk yield 305 days	+0.972±0.06	+0.904±0.02	+0.912±0.2
7.	milk ratio - live weight	+0.079±0.28	+0.014±0.1	-0.253±0.6
8.	milk ratio - fat, %	-0.281±0.27	+0.013±0.1	+0.779±0.4
9.	milk ratio - fat, kg	+0.911±0.11	+0.849±0.03	+0.710±0.4
10.	average daily milk yield - milk yield 305 days	+0.999±0.01	+0.999±0.0	+0.999±0.03
11.	average daily milk yield - fat, %	-0.053±0.27	-0.053±0.19	+0.876±0.3
12.	average daily milk yield - fat, kg	+0.966±0.07	+0.966±0.01	+0.881±0.3
13.	average daily milk yield - milk ratio	+0.971±0.07	+0.919±0.01	+0.912±0.3

An analysis of the data between the studied indicators of cows of German breeding shows that between milk yield - the amount of milk fat, milk ratio - milk yield for 305 days, milk ratio - the amount of milk fat, the average daily milk yield - the amount of milk fat, the average daily milk yield - the milk ratio is established a very high positive relationship. With each subsequent lactation, the correlation coefficients between the above indicators slightly decreased. The correlation coefficient between the average daily milk yield, milk yield per 305 days of lactation and the fat content in milk is negative, weak and varies from -0.053 to -0.212. The relationship between live weight and milk yield over 305 days of lactation, mass fraction of fat and the amount of milk fat is positive from weak (+0.164) to moderate (+0.401). A weak positive relationship has been established between the milk ratio and live weight.

The results of studied relationship between the main indicators of the productivity of cows of the Dutch breeding are given in Table 5.

Table 5. Correlation between the main indicators of productivity of cows of the Dutch breeding, r±m

No	The correlated sign	The third lactation	The fourth lactation
1.	milk yield - mass fraction of fat, %	+0.122±0.19	-0.160±0.18
2.	milk yield - the amount of milk fat, kg	+0.962±0.05	+0.927±0.07
3.	live weight - milk yield 305 days	-0.294±0.19	-0.136±0.18
4.	live weight - mass fraction of fat, %	+0.343±0.18	-0.121± 0.18
5.	live weight - the amount of milk fat, kg	+0.284±0.18	-0.024±0.18
6.	milk ratio - milk yield	+0.957±0.05	+0.965±0.05
7.	milk ratio - live weight	-0.283±0.18	-0.372±0.17
8.	milk ratio - fat, %	-0.015±0.19	-0.125±0.18
9.	milk ratio - fat, kg	-0.552±0.16	+0.802±0.11
10.	average daily milk yield - milk yield 305 days	+0.999±0.01	+0.999±0.01
11.	average daily milk yield - fat, %	+0.041±0.18	-0.132±0.19
12.	average daily milk yield - fat, kg	-0.501±0.16	+0.824±0.1
13.	average daily milk yield - milk ratio	+ 0.961±0.05	+0.964±0.05

As it is seen from the table, between the milk yield and the mass fraction of fat, was revealed a weak positive relationship (the third lactation) and a weak negative (the fourth lactation), which confirms the difference of 366 kg of milk between lactations. It was established a very high positive relationship between the milk yield for 305 days - the amount of milk fat, the milk yield coefficient - the milk yield for 305 days, the average daily milk yield - the milk yield coefficient. A negative correlation was found between live weight and milk yield for 305 days of lactation, live weight and milk coefficient, the tightness of communication varies from weak (-0.136) to moderate (-0.372).

The correlation between live weight and fat mass fraction, as well as between live weight - the amount of milk fat is weak positive (the third lactation), weak negative (the fourth lactation).

The results of studying the correlation between the productivity indicators of heifers, first-generation, are shown in Table 6.

Table 6. Correlation between the main productivity indicators of heifers of the offspring of the first generation, r ± m

No	The correlated sign	The first lactation
1.	milk yield - mass fraction of fat, %	-0.176±0.16
2.	milk yield - the amount of milk fat, kg	+0.884±0.08
3.	live weight - milk yield	+0.193±0.16
4.	live weight - mass fraction of fat, %	+0.298±0.16
5.	live weight - the amount of milk fat, kg	+0.324±0.16
6.	milk ratio - milk yield	+0.788±0.1
7.	milk ratio - live weight	-0.554±0.13
8.	milk ratio - mass fraction of fat, %	-0.137±0.16
9.	milk ratio - the amount of milk fat, kg	+0.517±0.02
10.	average daily milk yield - milk yield in 305 days	+0.999±0.01
11.	average daily milk yield - fat, %	+0.015±0.17
12.	average daily milk yield - fat, kg	+0.736±0.11
13.	average daily milk yield - milk ratio	+0.828±0.09

A weak negative correlation was found between milk yield - mass fraction of fat (-0.176), milk ratio - mass fraction of fat (-0.137), a noticeable negative relationship between milk ratio - live weight (-0.554). The relationship between live weight and milk yield for 305 days of lactation, as well as the mass fraction of fat and the amount of milk fat, is positive, weakly expressed. It should be noted that among the descendants of the first generation, as well as cows of German and Dutch breeding, the correlation between milk yield and the amount of milk fat, milk yield and milk yield, as well as between the average daily milk yield and milk yield coefficient is positive, the connection tightness is high.

Thus, the established negative relationship between the mass fraction of fat and milk yield, the mass fraction of fat and the milk yield coefficient of cows of German and Dutch breeding and offspring of the I generation indicates that further selection for milk production in herd of J.S.C. “Aydyn” must be carried out taking into account the mass fraction of fat.

Low coefficients correlation between milk yield and live weight (positive - German selection and descendants of the first generation) and (negative - Dutch selection) indicate the non-linear nature of the relationships between them, and characterizes the uniformity of herd of J.S.C. “Aydyn” in live weight.

CONCLUSIONS

Milking for 305 days of lactation (third lactation) at Dutch cows amounted to 10,560 kg of milk, which is by 458 kg of milk more than at German cows, the difference is not significant.

According to the milk yield coefficient, cows of Dutch breeding exceeded German breeding cows by 121 kg (the third lactation), the difference was significant at $P<0.01$.

A comparative analysis of the results of studies of the productive qualities of cows of the first lactation of the Holstein breed of the Dutch and German breeding showed that the heifers-descendants of the first generation exceeded by 855 (Dutch breeding) and 1444 (German breeding) kg of milk in milk yield, the difference is highly reliable (P<0.001).

At cows of German breeding, a negative correlation was found between the average daily milk yield, milk yield per 305 days of lactation and the fat content in milk, the connection tightness is weak and varies from -0.053 to -0.212.

At Dutch cows, between a milk yield and a mass fraction of fat was revealed a weak positive relationship (the third lactation) and a weak negative relationship (the fourth lactation), which confirms the difference of 366 kg of milk between lactations.

At the descendants of the first generation between the live weight and the milk yield for 305 days of lactation, as well as the mass fraction of fat and the amount of milk fat, revealed a positive relationship, the tightness of the connection is weakly expressed.

REFERENCES

Adzhibekov, K.K. (1995). The effectiveness of the use of the Holstein breed by the improvement of the black-motley cattle of the Middle Volga. *Abstract Doctor of Agricultural Sciences*, Moscow, 44.

Antal, L. (2004). Reflection on the theme: "The bull is half the herd." *Dairy and beef cattle breeding*, 1, 16-20.

Akhmetzyanova, G.R. (2015). Interrelation of milk productivity and milk quality indicators at Holstein cows in industrial technology. *News of the Orenburg State Agrarian University*, 6(56), 143-144.

Bakay, F.R., Krovikova, A.N., Mkrchtyan G.V. (2016). Correlation between live weight and milk yield at cows of heifers of black-motley breed in PZ Povadino CJSC. *Achievements of university science*, (2)2, 119-121.

Drăgotoiu, T., Isfa, N., Stroilescu, L., Drăgotoiu, D., Marin, M., Oprea, I. (2015). Study on the heritability of milk production in a line of Holstein Freise. *Agrolife Scientific Journal*, 4(1), 56-63.

Efimova, L.V. (2010). The influence of milk productivity on the reproductive qualities of cows. *Siberian Bulletin of Agricultural Science*, 4(208), 45-50.

Efimova, L.V., Kulakova, T.V., Ivanova, O.V., Ivanov, E.A. (2017). The relationship between the signs of a linear assessment of the exterior and milk production of cows. *Bulletin of the Journal of Novosibirsk State Agrarian University*, 3(44), 115-124.

Ernst, K.L., Chemm, V.A. (1972). *Modern methods for improving dairy cattle*. Publishing House Kolos, 375.

Foksha, V., Konstandoglo, A., Morar, G., Peykov, G., Kurulyuk, V., Tataru, G. (2017). The analysis of production and reproduction parameters of Holstein cows from Holland and Germany in J.-S.C. „Aydyn". *Scientific Papers. Series D. Animal Science,* LX, 14-20.

Krasota V.F., Lobanov V.T., Dzhaparidze E.G. (1990). *Breeding farm animals*. Moscow, RU: Agropromizdat Publishing House, 463.

Konstandoglo, A., Foksha, V., Granaci, V. (2019). The relationship of exterior and Dairy productivity of cows of the Holstein breed of various breeding. *Scientific papers. Series D. Animal Science*, LXII(2), 29-33.

Kuziv, M.I., Fedorovich, S.I. (2014). Dependence of dairy productivity of cows of Ukrainian black-rippled dairy breeds of live weight in the period of their cultivation. *Bulletin of Sumy National Agrarian University.* Livestock production series. Sumy., 2(2), 68-72.

Kuznetsov, V.M. (2002). Genetic variability and interrelation of signs of milk productivity of animals of Kholmogorsk and black-motley breeds. *Reports of the Russian Academy of Agricultural Sciences,* (2), 42-45.

Lepyokhina, T.V. (2011). The reproductive ability of mother cows and their daughters and its relationship with milk production. *Problems of biology of productive animals*, (4), 72-75.

Merkuryeva, E.K., Shangin-Berezovsky, G.N. (1983). *Genetics with the basics of biometrics*. Moscow, RU: Kolos Publishing House, 400.

Nazarchenko, O.V. (2011). The relationship between live weight and milk productivity of Holstein cows at the daughters of bulls of the Holstein lines of the Trans-Urals. *Bulletin of the Krasnodar State Agrarian University*, (10), 164-167.

Pogodaev, S.F., Gadzhiev, A.M. (2001). The live weight of cows determines the potential for milk yield. *Zootechnics*, 1, 29-31.

Plokhinsky, N.A. (1978). *Mathematical methods in biology*. Moscow, RU: Moscow State University Publishing House, 265.

Ruziev, T.B. (2001). Inheritance of milk production by daughters of bulls of Holstein breed of different ecogenesis. *The problem of the development of agricultural science of the Republic of Tajikistan: materials of the conference dedicated to the 85th anniversary of Academician Aliyev G.A.* Dushanbe, 1, 85-89.

Ruziev, T.B. (2003). Inheritance of milk yield and fat content of cow milk for lactation. *Collection of scientific papers "60 years of the Faculty of Zoo Engineering"*, Dushanbe, 49-51.

Ruziev, T.B. (2006). Inheritance of milk productivity by daughters of Tajik type bulls in subsidiary farms of pedigree farms. *News of the Academy of Sciences of the Republic of Tajikistan*, Dushanbe, 4(157), 57-61.

SAS, (2007). Developer Stat Soft, Platform: PC. Version 6.1. SAS institute, inc., Cary, NC.

Startsev, D.I. (1965). Direction of pedigree work when breeding livestock of Simmental breed in foreign countries. *Agriculture abroad Livestock*, 6, 11-16.

Vorobyova, N.V., Loginova T.P., Koval L.L. (2010). Interrelation of live weight and milk yield of cows in the conditions of the ,,Pushkin'' breeding farm. *Zootechnics*, 7, 9-10.

Wilver, D.S. (2017). The relationship of milk productivity with live weight. *Materials of the All-Russian scientific-practical conference "Modern problems of animal husbandry in the conditions of innovative development of the industry",* Kurgan State Agricultural Academy, 33-37.

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PHENOTYPIC AND GENETIC VARIATION IN VITAMIN B12 CONTENT OF BOVINE MILK

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Abstract

Vitamin B-12 (cobalamin) is essential for human health and current intake level of this vitamin is too low. Bovine milk is an important dietary source of vitamin B-12 and natural enrichment of the milk vitamin B-12 content may help to increase the intake levels. The aim of this study was to quantify the genetic variation in levels of vitamin B-12 in the milk of dairy cows. In this study milk (n= 194) samples were collected from first lactation Holstein Friesian cows, and analyzed for vitamin B-12 content. Vitamin B-12 content varied from 1.08 to 9.66 mg/l with a mean of 3.93 ± 1.58 mg/l. The amount of genetic variation between cows in vitamin B-12 content in milk was reflected by an estimated heritability of 0.36. This heritability of 0.36, combined with a coefficient of variation of 40% for vitamin B-12 content in milk indicates that average milk vitamin B-12 content of the cow Holstein Friesian population can be increased by genetic selection.

Key words*: dairy cows, milk, vitamin B-12, genetic variation.*

INTRODUCTION

Only bacteria and archaebacteria are able to synthesize vitamin B12 if cobalt supply is sufficient (Martens et al., 2002).

The natural source of vitamin B12 in human diets comes from animal-product, especially those from ruminants because of the close link between the animal and bacteria dwelling in its rumen.

The cow milk is a significant contributor to vitamin B12 intake.

In countries with a high dairy consumption approximately 40% of dairy vitamin B12 intake is from dairy products, and contributes to the low prevalence of vitamin B12 deficiency.

Low vitamin B12 status in people may be associated with megaloblastic anemia and neurologic disorders (Vogiatzoglou et al., 2009; Kim et al., 2008).

Also, vitamin B12 plays a role in prevention of osteoporosis, neurocognitive decline, cardio-vascular diseases and neural tube defects in newborns.

Vitamin B12 acts as coenzyme in only two metabolic reactions.

One of these vitamin B12 is dependent enzyme, methylmalonyl - coenzyme.

A mutase, plays a major role for the entry of propionate in the Krebs cycle and gluconeogenesis (McDowell, 2000).

Beside this role, the vitamin is a coenzyme for the methionine synthase, the critical interface between folic acid and vitamin B12 metabolism.

Vitamin B12 is the largest of the B complex vitamins, with a molecular weight of over 1000.

It consist of corrin ring made up of four pyrroles with cobalt at the center of the ring (Weir and Scott, 1999).

In nature there are two other forms of vitamin B12: hydroxocobalamin and aquacobalamin, where hydroxyl and water groups, respectively, are attached to the cobalt.

The synthetic form of vitamin B12 found in supplements and fortified foods is cyanocobalamin, which has cyanide attached to the cobalt.

These three forms of B12 are enzymatically activated to the methyl- or deoxyadenosylcobalamins in all mammalian cells.

Most microorganism, including bacteria and algal, synthesizes vitamin B12 and they constitute the only source of the vitamin B12 (Chanarin, 1979).

The vitamin B12 synthesized in microorganism enters the human food chain through incorporation into food of animal origin.
Products from herbivores animals, such as milk, meat etc., constitutes important dietary source of the vitamin B12.
Some observational studies suggest that vitamin B12 from dairy products is better bioavailable than vitamin B12 from other sources (Tucker et al., 2000; Vogiatzoglou et al., 2009).
The absorption of vitamin B12 in humans is complex (Weir and Scott, 1999).
Vitamin B12 in food is bound to proteins and is released from the proteins by the action of a high concentration of hydrochloric acid present in the stomach.
This process results in the free form of the vitamin B12, which is immediately bound to a mixture of glycoproteins secreted by the stomach and salivary glands.
These glycoproteins, called R - binders, protect vitamin B12 from chemical denaturation in the stomach.
The stomach parietal cells, which secrete hydrochloric acid, also secrete a glycoprotein called intrinsic factor.
Intrinsic factor binds vitamin B12 and ultimately enables its active absorption.
Russell et al. (2001) reported mean absorption of 55%, when radioactive vitamin B12 dissolved in milk was administered to human subjects.
Bioavailability of the synthetic form of vitamin B12 was reported to be poor (<4%) in humans and animals (Zittoun, 1996).
Ruminants require dietary cobalt (Co) for synthesis of vitamin B12 is the rumen. The dietary requirement of dairy cows for Co is 0.11 mg/kg (NRC, 2001).
Tiffany et al. (2006) recorded increased synthesis of vitamin B12 as the Co concentration of the diet increased from 0.1 to 1.0 mg/kg.
Thus, natural enrichment of the vitamin B12 in milk, by increasing cobalt levels of dairy feed may be a good way to increase dietary vitamin B12 intake of human consumer. This way seems limited, because it has been shown that milk vitamin B12 content level off between 4.2 mg/l, despite significantly higher cobalt levels of the cow's feed (0.93 vs. 0.57 mg/kg dry matter) (Kincaid and Socha, 2007).
Enhancing micronutrient (vitamin and mineral) concentration within milk and serum from dairy cows is important for both the health pf the cow and the nutritive value of the milk for human consumption (Knaus, 2013).
Natural enrichment of the cow milk vitamin B12 content could be achieved through genetic selection.
The aim of this study was to quantify phenotypic and genetic variation in levels of vitamin B12 in the milk of Holstein Friesian dairy cows.

MATERIALS AND METHODS

Animals involved in this study were from Agriculture Research and Development Station (ARDS) Simnic - Craiova, Romania.
All Holstein Friesian cows are results of a long-term selection experiment for genotype X environment interactions.
The selected cows have average to high genetic merit for kilograms of fat plus protein yield. The diet consists of high energy ration based on some by-products and one homegrown component (forage maize, grazed grass, alfalfa, forage beet, maize silage, grains and beans).
Additionally the ration is balanced with purchased minerals.
Average (minimum-maximum) nutrient and major ingredient composition was as follows: crude protein, 17% (15-19.5%); fat: 3.9% (3-5%); acid detergent fiber: 20% (16-26%); neutral detergent fiber 34% (23-41%); cobalt 0.52 mg/kg (0.19-1.27 mg/kg); net energy (lactation) 1.70 Mcal/kg (1.64-1.80 Mcal/kg); concentration: 50.2% (40-52%); corn silage 19% (0.00-47%) and hay 7% (0.00-24%).
Cows are milked two times daily (morning and evening) and for the present study milk samples were taken from first-location cows at morning milking. Milk samples (n = 194) were collected on 18 separate occasions between February 2014 to January 2019 at 60.4 ± 4.0 dais in milk (DIM).
After collection, milk samples were poured into 15 ml conical tubes.
At the laboratory they were frozen at: -20°C and stored until analysis. Vitamin B12 in milk was analyzed in duplicate by enzyme immunoassay using a commercial kit

(RIDASCREEN FAST Vitamin B12, R-Bio pharm AG, and Germany).
Data analyses were performed using M.O. Excel. To estimate the genetic parameters and variance components the REML approach was used.

RESULTS AND DISCUSSIONS

Vitamin B12 content in the milk of Holstein Friesian cows varied from 1.08 to 9.66 mg/l, with a mean of 3.93 ± 1.58 mg/l.
Figure 1 illustrates the large variation in milk vitamin B12 content.

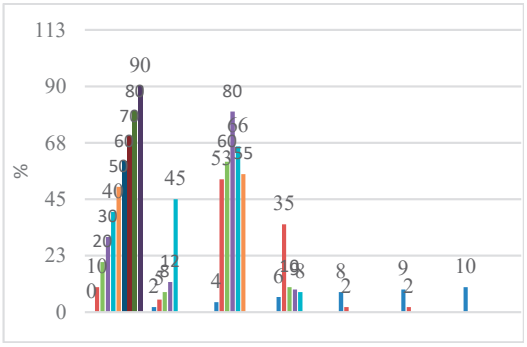

Figure 1. Vitamin B12 content in bovine milk samples (frequency distribution)

The lowest 25% of milk samples had an average vitamin B12 content of 2.07 ± 0.48 mg/l and the highest 25% of milk samples had an average content of 5.90 ± 1.41 mg/l, a difference of 3.83 mg/l.
Rutten et al. (2013) reported a variation of vitamin B12 from 1.0 to 12.9 mg/l, with a mean of 4.40 mg/l in raw sample of Dutch Holstein Friesian cows.
Duplessis et al. (2016) reported concentrations of vitamin B12 in milk from 2.309 to 3.878 mg/l in 15 commercial herds (386 Holstein and 13 Jersey cows in Canada). Vitamin B12 concentration in milk ranging from 1.575 to 4.781 mg/l has been reported by Preynat et al. (2009), and Akins et al. (2013).
Substantial amount of genetic variance in vitamin B12 content in milk was detected among the 194 milk sample.
Variance components and heritability of vitamin B12 content in milk of first lactation Holstein Friesian cows from ARDS Simnic - Craiova, Romania are presented in Table 1.

Table 1. Variance components and heritability of vitamin B12 content in milk of Holstein Friesian cows from ARD Simnic - Craiova, Romania

Source of variation	Vitamin B12
Genetic (6^2 animal)	0.92 ± 0.22
Residual (6^2 e)	1.59 ± 0.33
Phenotypic ($6p = 6^2(animal)+6^2e$	2.51 ± 0.18
Heritability ($h^2 = 6^2animal/6^2p$)	0.36 ± 0.21

In this study the phenotypic variance was 2.51 mg/l, and the genetic variance was 0.92 mg/l (Table 1).
Genetic variation between cows indicates that there is ample opportunity to influence the vitamin B12 content in milk. In the present study the heritability (the proportion of variation due to genetic variation) was 0.36, suggesting that genetic selection could modify milk vitamin B12 concentration (Table 1).
The genotype of the cow affects the amount of vitamin B12 that ends up in its milk.
Genotype of cows influences the microbial process in its rumen and after processes such as vitamin B12 absorbance from the digestive tract or secretion by the mammary gland.
Vitamin B12 concentration in milk varied among sampling months.
In this study, it was greater during spring and fall months than during winter and summer months (Figure 2).

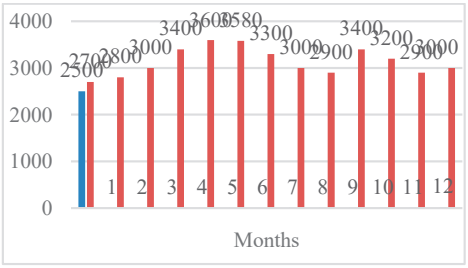

Figure 2. Vitamin B12 concentration in milk of cows among sampling months

Different species of microorganism in the rumen are involved in the degradation of forage and concentrate.
Petri et al. (2012) showed that some strains of ruminal bacteria could synthesize a greater amount of vitamin B12 than others.

As vitamin B12 is synthesized by ruminal microbes and a proportion of the synthesized vitamin is secreted into milk, we could reasonably hypothesize that what affects apparent vitamin B12 ruminal synthesis would also affect milk vitamin B12 concentration.

Apparent ruminal synthesis of vitamin B12 was positively correlated with dietary Neutral Detergent Fiber (NDF) and sugar contents and negatively correlated with Non Fiber Carbohydrate (NFC) contents in the trial of Schwab et al. (2006).

Santschi et al. (2005) observed that apparent ruminal synthesis of biologically active vitamin B12 was greater with a 60:40 forage - to - concentrate ratio diet.

Similarly, apparent ruminal synthesis was about 3 - fold greater for cows receiving a high - fiber diet compared with cows receiving a high - starch diet (Beaudet et al., 2016).

Duplessis et al. (2016) showed that negative relationship was observed between vitamin B12 concentration in milk and ration crude protein (CP).

Beaudet et al. (2016) concluded that apparent ruminal synthesis of vitamin B12 was not affected by 2 level of CP in the ration: 11.1% and 14.3% CP on a dry matter (DM) basis.

Present day genetic selection by dairy breeding organizations involves estimation of breeding values which requires phenotypes of many thousands of animals.

Phenotypes for vitamin B12 content in milk as determined in the current study, are too expensive for such large - sale collection.

Less expensive alternative phenotypes that correlate with milk vitamin B12 content are not currently available.

However, phenotype - based selection can also be substituted by genotype - based selection.

Genotype - based selection require knowledge on regions of the bovine genome associated with vitamin B12 content in milk.

Rutten et al. (2013) reported on a genome - wide association study to identify regions of the bovine genome associated with milk vitamin B12 content.

Identification of associated genomic regions also contributes to the understanding of the biological mechanism responsible for the observed genetic variation in vitamin B12 content in milk.

Significant association [-log 10 (P - value) >3] was found between 68 SNP and vitamin B12 content in raw milk of 487 first – lactation Dutch Holstein Friesian cows (Rutten et. al., 2013).

Significantly associated SNP were spread over 16 *Bos taurus* (BTA) chromosomes.

Among the 68 significantly associated SNP, cluster of at least 3 significantly associated SNP, could be discriminated on BTA 5, BTA 8, BTA 10, BTA 13, BTA 14 and BTA 26.

Table 2 shows that most of the candidate genes for vitamin B12 were actually genotyped for one or more SNP as port of the genome wide association study (Rutten et al., 2013), and none of these SNP were significantly associated with vitamin B12 in milk.

The lack of association between SNP and most known candidate genes for vitamin B12 indicates that variation in other genes causes most of the observed genetic variation in vitamin B12 content in milk (Rutten et al., 2013).

One glass (250 ml) of milk from cows in this study, would have provided between 25-45% of the recommended daily allowance of vitamin B12.

Among individual cows, however, this prevision varied between 20 and 62% of the recommendation.

Our results will need to be confirmed, but they open new research avenues on factors affecting the vitamin B12 concentration in milk of dairy cows.

CONCLUSIONS

Genetic variation between Holstein Friesian cows in vitamin B12 content in milk was established.

The average heritability of 0.36 combined with a coefficient of variation of 40% for vitamin B12 content in milk indicates that the average milk vitamin B12 content of Holstein Friesian cow population can be increased by genetic selection.

Our results will need to be confirmed on a large Holstein Friesian cow population.

Table 2. Candidate genes for vitamin B12 content in bovine milk with their genetic position and significance of genotype SNP

Gene symbol	Gene name	Chromosome	Genomic position (Kbp)[a]	#SNP in gene[b]	Highest -log 10 (p-value)[b]
LRP2	low density lipoprotein receptor - related protein 2 (Megalin)	2	27, 603, 435.27, 857, 248	3	0.747
MMADNC	methylmalonic aciduria (cobalamin deficiency)	2	47, 991, 127.48, 006, 635	1	1.481
MMACHC	methylmalonic aciduria	3	105, 516, 664, 106, 521, 668.	0	-
CD320	CD320	7	15, 415, 307.15, 421, 023	0	-
LMBRD1	LMBR1 domain containing 1	9	8, 373, 879.8, 506, 806	4	0.614
CUBN	Cubilin	13	31, 053, 631.31, 312, 338	10	2699
GIF	gastric intrinsic factor	15	83, 763, 128.83, 782, 200	0	-
TCN1	transcobalamin I haptocorrin	15	83, 791, 407.83.806, 715	1	13.19
MMAA	methylmalonic aciduria	17	73, 590, 935.13, 603, 835	1	0.169
MMAB	methylmalonic aciduria	17	66, 719, 631.66, 730, 086	0	-
TCN2	transcobalamin II	17	72, 841, 020.72, 857,003	0	-
MTRR	5-methyltetrahydrofolate-homocysteine methyltransferase reductase	20	68, 917, 318.68, 946, 683	1	1051
AMN	Amnionless	21	67, 751, 621.67, 762, 225	0	-
MUT	Methylmalonyl CoA mutase	23	22, 488, 908.22, 530, 197	1	0.206
ABCC1	ATP - binding cassette, sub-family C	25	15, 453, 547.15, 606, 736	2	0.656
MTR	5-methyltetrahydrofolate-homocysteine methyltransferase	28	7, 953, 588.8, 080, 514	2	0.599

a - based on genome assembly BTAU 461
b - number of genotyped SNP in the gene and - log 10 (P - value) of the SNP that was most significantly associated with milk vitamin B12 content.

REFERENCES

Akins, M.S., Bertics, S.J., Socha, M.T., Shaver, R.D. (2013). Effects of cobalt supplementation and vitamin B-12 injections on location performance and metabolism in Holstein Friesian dairy cow. *J. Dairy Sci.*, 96, 1755–1768.

Beaudet, V., Gervais, R., Graulet, B., Noziere, P., Doreau, M., Fanchone, A., Castagnion, D.S., Girard, C.L. (2016). Effects of dietary nitrogen levels and carbohydrate source on apparent ruminal synthesis of some B vitamins in dairy cows. *J. Dairy Sci*., 99, 2730-2739.

Chanarin, I. (1979). *The Megaloblastic Anemia 2nd* edition. London, UK: Blackwell Scientific Oxford Publishing House.

Duplessis, M., Pellerin, D., Cuc, R.I., Girard, C.L. (2016). Short communication: Factors affecting vitamin B-12 concentration in milk of commercial dairy herds. An exploratory study. *Journal of Dairy Science*, 99, 4886-4892.

Kim, J.M., Stewart, R., Kim, S.W., Yang, S.J., Shin, I.S., Yoon, J.S. (2008). Predictive value of falate, vitamin B-12 and homocysteine levels in late–life depression. *Br. J. Psychiatry*, 192, 268 – 274.

Kincaid, R.L., Socha, M.T. (2007). Effect of cobalt supplementation during late gestation and early lactation on milk and serum measures. *Journal of Dairy Science*, 90, 1880-1886.

Knaus, W. (2013). Re-thinking dairy cow feeding in light of food security. *AgroLife Scientific Journal*, II(1), 36-40.

Martens J.H., Barg, H., Warren, M., Jahn, D. (2002). Microbial production of vitamin B-12. *Appl. Microbial. Biotechnol.*, 58, 275-285.

McDowell, L.R. (2000). *Vitamin in animal and human nutrition.* 2 nd ed. Iowa State University Press Publishing House, Ames. U.S.A.

National Research Council (NRC) 2001. *Nutrient Requirements of Dairy Cattle: 7th Rev. Ed.*, National Academies Press, Washington DC, USA.

Petri, R.M., Forster, R.J., Yang, W., McKinnon J.J., McAlister, T.A. (2012). Characterization of rumen bacterial diversity and fermentation parameters in concentrate fed cattle with and without forage. *J. Appl. Microbiol.*, 112, 1152-1162.

Preynat, A., Lapierre, H., Thivierge, M.C., Palin, M.F., Matte, J.J., Desrochers, A., Girard, C.L. (2009). Influence of methionine supply on the response of lactational performance of dairy cows to supplementary folic acid and vitamin B-12. *Journal of Dairy Science*, 92, 1685-1695.

Russell, R.M., Baik, H., Kehayias, J.J. (2001). Older men and women efficiently absorb vitamin B-12 from and fortified bread. *J. Nutr*., 131, 291-293.

Rutten, M.J.M., Aniek, C., Bouwman, R., Sprong, C., Van Arendonk, J.A.M., Visker, M.H.P.W. (2013). *Genetic Variation in Vitamin B-12 Content of Bovine Milk and Its Association with SNP along the Bovine Genome.* PLOSONE; 8: e 62382.

Santschi, D.E., Chiquette, J., Berthiaume, R., Martireau, R., Matte, J.J., Mustafa, A.F., Girard, C.L. (2005). Effects of the forage to concentrate ratio on B-vitamin concentrations in different ruminal factors of dairy cows. Cam. *J. Anim. Sci*., 85, 389-399.

Schwab, E.C., Schwab, C.G., Shauer, R.D., Girard, C.L., Putnam, D.E., Whitehouse, N.L. (2006). Dietary forage and non-fibre carbohydrate contents influence B-vitamin intake, duodenal flow, and apparent ruminal synthesis in lactating dairy cows. *J. Dairy Sci*., 89, 174-187.

Tiffany, M.E., Fellner, V., Spears, J.W. (2006). Influence of cobalt concentrations on vitamin B-12 production and fermentation of mixed microorganisms grown in continuous culture flow- through fermenters. *J. Anim. Sci*., 84, 635 -340.

Tucker, K.L., Rich, S., Rosenberg, I., et al. (2000). Plasma vitamin B-12 concentrations relate to intake source in the Framingham Offspring study. *Am. J. Clin. Nutr*., 71, 514-522.

Vogiatzoglou, A, Smith, A.D., Nurk, E., Berstad, P., Drevon, C.A., et al. (2009). Dietary sources of vitamin B-12 and their association with plasma B-12 concentrations in the general populations: the Hordaland Homocysteine Study. *Am. J. Clin. Nutr*., 89, 1078- 1087.

Vogiatzoglou, A., Refsum, H., Jonston C., et al. (2008). Vitamin B-12 status and rate of brain volume 10SS in community - dwelling elderly. *Neurology*, 71, 826-832.

Weir, D.G., Scott, J.M. (1999). Cobalamins Physiology, Dietary Sources and Requirements. In: Sadler M.J., J.J. Strair, B. Caballero, eds. *Encyclopedia of human Nutrition*, 1, 394-401.

Zittoun, J. (1996). Cobalamins: an update of fundamental and clinical data. *Hematologie*, 2, 119-129.

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GROWTH PERFORMANCE AND MEAT QUALITY CHARACTERISTICS OF LAMBS FROM DIFFERENT GENOTYPE

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Abstract

Thirty male lambs from three genotype, Tsurcana (TA) und its crossbreed with Palas Meat Breed (PMB) and German Blackface (GBF) (ten from each genotype) were used to determine the effects of breed on growth performance and meat quality characteristics. Average daily gain in the period 0-153 days, final weight and warm carcass weight were higher in GBF x TA lambs than to the lambs from TA and PMB x TA groups, and hot slaughter yield were higher in TA compared to the lambs from GBF x TA and PMB x TA groups. Furthermore, except average daily gain and weight at 28 days (W28), 56 days (W56) and weaning weight (WW), the lambs from the three genotype did not show significant differences for the other traits (P>0.05). PMB x TA lambs had the lowest scores in terms of differences of juiciness and appearance than GBF x TA when compared with TA group, but higher scores in terms of differences of tenderness, flavour, the difference of specific lamb taste and overall difference. In conclusion, when the breed is to be decided, the PMB x TA lambs should be by consumers appreciated for meat quality and GBF x TA for growth performances.

Key words*: flavor, juiciness, German Blackface, Palas Meat Breed, Tsurcana.*

INTRODUCTION

With a population of 9.98 million sheep, Romania occupies in the year 2020 (after Brexit) the second place in sheep breeding sector in UE, the first place being occupied from Spain (FAOSTAT, 2020). Sheep breeding in Romania is usually performed by the use of native breeds (Tsurcana, Tsigai and Merino) and also using traditional methods - which are similar to many countries in the Middle East. Tsurcana sheep breeds are usually triple-purpose breeds, being reared for meat, milk and wool. Romanian indigenous Tsurcana, accounting over 6 million heads (Dărăban et al., 2009; Ilișiu et al., 2010). Its characteristics are the following: adult body weight of 45-50 kg in ewes and 70-75 kg in rams, growth rates of 110-160 g/day and 105-115% prolificacy (Georgescu et al., 2000).

The German Blackface breed is characterized by a birth rate of 94.11%, a prolificity of 150.5%, a percentage of weaned lambs of 132.4% (Dărăban, 2006). It was introduced first in Romania starting in autumn 1993 when a nucleus of males from Germany was brought to the Research Station for Sheep and Goats Reghin, in order to determine the combinability value for meat production with the Tsigai breed, existing in the unit. The crossbreed lambs obtained between German Blackface and the Tsigai breed achieved an average daily gain of 270 g.

The Palas Meat Breed is a new Romanian breed, approved in 2012, which was formed by crossing of the Ile-de-France and Merino of Palas breeds, followed by breeding isolation and selection in the direction of increasing meat production. The lambs in the pure breed realize at intensive fattening an average daily gain of 280-300 g and a slaughter yield of 45-48%.

The globalization of markets has resulted in greater economic integration, but at the same time has imposed the need to meet quality requirements to satisfy consumer demands. The meat industry and sheep producers must comply with certain quality standards to meet consumer demands and remain competitive in the global market. Eating quality of lamb and sheep meat has been examined by many

researchers throughout the years (Weller et al., 1962; Dransfield et al., 1979; Crouse et al., 1981; Jeremiah et al. 1993; Braggins, 1996; Young et al., 2003; Gkarane et al. 2017) and has been shown to be affected by many pre- and post-slaughter factors such as gender, castration, diet, maturity, breed, processing methods, aging, freezing, and packaging. However, the method of action and influence of these factors and their possible interactions on lamb eating quality have frequently remained unclear.

Studies that have examined breed effects on lamb flavor characteristics have produced inconsistent results. Cramer et al. (1970) compared the flavor of meat produced by Rambouillet, Targhee, Columbia, and Hampshire lambs and reported that among-breed differences in meat flavor intensity paralleled differences in wool fineness - increased wool fineness was associated with more intense meat flavor. Based on these findings, they concluded that mutton flavor was most detectable in meat from fine wool breeds of sheep (Cramer et al., 1970). Similarly, Safari et al. (2001) reported a stronger meat flavor for straight-bred Merino lambs compared with Border Leicester × Merino crossbred lambs. In contrast, other reports suggest that breed has no effect on lamb flavor (Dransfield et al., 1979; Crouse et al., 1981; Duckett et al., 1999). In a more recent study, Shackelford et al. (2012) compared sensory properties of lamb produced by progeny of several different sire breeds, including Dorper, Dorset, Finnsheep, Katahdin, Rambouillet, Romanov, Suffolk, Texel, White Dorper, and Composite (1/2 Columbia, 1/4 Suffolk, 1/4 Hampshire). When compared at the same age, lamb flavor intensity scores were greater for progeny of Katahdin, Romanov, and Texel sires than for progeny of Suffolk, Composite, and Rambouillet sires; however, it was noted by the researchers that the observed breed differences in lamb flavor intensity were relatively small (Shackelford et al., 2012).

In this context, it is necessary to know how the breed affects the main characteristics of meat and carcass quality. Investigations have determined that breed (Crouse et al., 1981; Hopkins and Fogarty, 1998), can affect the characteristics of carcass and meat.

The aim of this study was to determine the growth performance and meat quality of Tsurcana lambs and its crossbreed lambs with Palas Meat Breed and German Blackface.

MATERIALS AND METHODS

Animals and experimental design

The study was conducted at a private sheep farm in Mureș County, 46°46' N/ 22°42'E; 395 m altitude; annual rain fall varies between 650-700 mm; average temperatures 19/–3°C during summer/winter, in the period September 2018-September 2019. A total of 45 ewes were separated from the herd and put into three groups at the beginning of the breeding season (15 ewes/groups), and mated with rams from three genotype, as follows: Tsurcana breed (TA), Palas Meat Breed (PMB) and German Blackface (GBF) breed. 30 male lambs from above mentioned genotype (10 from each group) were used in the research. Lambs were born in January-March interval. At birth or shortly thereafter, lambs were identified with ear tags and weighed (± 0.1 kg). Sex, date of birth, type of birth, dam and ram group were recorded. The lambs were also weighed monthly (± 0.1 kg) up to 5 month age. Ewes and their lambs were kept together under the same management condition. Up to weaning, the lambs were creep fed (*ad libitum*, 16% crude protein pellets) and weaned at 75 days of age. After weaning up to 5 month (153 days), the lambs are raised on pastures, and the diet consisted of pastures *ad libitum* and 300 g/head pellets with 16% crude protein content.

The research activities were performed in accordance with the European Union' Directive for animal experimentation (Directive 2010/63/EU).

Slaughter procedure, carcass characteristics and dissections

At the end of rearing, 3 male lambs from each genotype were brought to the abattoir for small and large animals from Reghin City. The lambs were weighed and then slaughtered. After the removal of non-carcass components, the carcass weights were recorded. Hot carcass yield was calculated based on pre-slaughter live body weight (LBW) and warm carcass weight (WCW), after formula:

HSY% = WCW x 100/LBW.

Meat sensory evaluation

In order to be used in meat quality analyses, LD muscle was removed from right side of the carcasses at 2 h post-slaughter and were packed. For sensory evaluation, meat samples were frozen and kept at –18°C until the day before of panel evaluation. Meat samples, which were served to untrained panelists, were prepared according to the methodology described by AMSA (2015). Sensory characteristics of cooked samples were assessed by 24 panelists using the degree of difference test. The panelists assessed the lambs breed difference in juiciness, tenderness, flavour, appearance, the difference of specific lamb taste and overall difference. The scale used has a seven point category (scale 1 = no difference, 2 = very small difference, 3 = small difference, 4 = moderate difference, 5 = big difference, 6 = very big difference, 7 = extremely big difference). Were evaluated meat from GBF x TA and PMB x TA crossbreed lambs, compared to TA pure breed.

Traits Definition

The traits investigated were classified as lamb, carcass, and meat traits. Early growth traits consisted of birth weight (BW); weight at 28 day (W28); weight at 56 day (W56); weaning weight (WW); post-weaning weights at 5 months (W5M).

Carcass traits included warm carcass weight (WCW) and hot slaughter yield (HSY).

Meat sensory characteristic refers to juiciness, tenderness, flavor, appearance the difference of specific lamb taste and overall difference.

Statistical analyses

In order to determine the effect of breed on growth performance, carcass and meat quality characteristics, the mean comparisons between the three groups of the variables were carried out using independent samples Student t-test of the JASP procedure.

RESULTS AND DISCUSSIONS

The effects of breed on lamb growth performance are presented in Table 1. Significant differences (P< 0.05) were recorded between the crossbreed lambs PMB x TA and the others two groups with regard at W28, W56 and WW. Also, significant differences (P<0.05) were found between PMB x TA and GBF x TA with regard at W5M.

Table 1. Means ± SE (standard errors) for body weight evolution from birth up to 5 months for the lambs from different genotype (kg)

Characteristics	GBF x TA	PMB x TA	TA x TA
BW	4.04 ± 0.22^{a}	4.09 ± 0.10^{a}	4.04 ± 0.11^{a}
W28	12.45 ± 0.71^{a}	10.21 ± 0.66^{ab}	12.95 ± 0.91^{a}
W56	19.00 ± 0.87^{a}	15.98 ± 1.06^{ab}	20.40 ± 1.51^{a}
WW	23.19 ± 1.33^{a}	18.90 ± 1.38^{ab}	24.27 ± 1.69^{a}
W5M	32.79 ± 2.49^{a}	25.86 ± 1.80^{b}	28.68 ± 1.65^{a}

^{a,b}Means in the same line with different superscripts are significantly different (P<0.05).

The average daily gain (Table 2) were significantly higher in Tsurcana lambs than in GBF x TA crossbreed lambs (P<0.001) in birth-weaning period and in GBF x TA compared with PMB x TA in birth - 28 days (P<0.01) and birth - 5 months (P<0.05). Also, significant differences (P<0.05) were found between PMB x TA and TA x TA with regard at ADG birth - 28 days and ADG birth - weaning.

The effects of the breed on carcass quality characteristics are shown in Table 3. TA x TA lambs presented higher hot slaughter yield. However, there were no significant differences between the three genotypes in terms of these traits.

Table 2. Means ± SE (standard error) for average daily gain (ADG - g) evolution from birth up to 5 months for lambs from different genotype

Characteristics	GBF x TA	PMB x TA	TA x TA
ADG birth - 28 days	300.36 ± 20.00^{a}	218.57 ± 24.3^{ab}	318.21± 32.36^{a}
ADG 28 - 56 days	233.94 ± 26.69^{a}	206.07 ± 20.18^{a}	266.07 ± 36.88^{a}
ADG birth - weaning	253.59 ± 13.60^{a}	226.00± 17.00^{ab}	271.00 ± 17.00^{b}
ADG birth - 5 months	182.20 ± 14.10^{a}	147.12 ± 10.76^{b}	157.48 ± 9.58^{a}

^{a,b}Means in the same line with different superscripts are significantly different (P<0.05, P<0.01, P<0.001).

Table 3. Means ± SE for hot slaughter yield of lambs from different genotype

Characteristics	GBF x TA	PMB x TA	TA x TA
LBW, kg	38.33 ± 1.59	35.50 ± 1.26	36.50 ± 0.29
WCW, kg	18.05 ± 1.59	17.08 ± 0.68	17.80 ± 0.17
HSY, %	46.90 ± 2.20	48.37 ± 3.52	48.78 ± 0.86

GBF x TA crossbreed lambs were slaughtered at higher slaughter weight than PMB x TA and TA x TA lambs, although all lambs were at similar ages in the research. These differences in final live weight might be explained by the effect of breed. The average daily gain for the two period (pre-weaning and post-weaning) were higher in the suckling period, and

between groups, were higher for TA x TA lambs compared to GBF x TA and PMB x TA. Post-weaning, the ADG were higher for GBF x TA lambs.

Although GBF x TA crossbreed lambs had higher live body weight and warm carcass weight, the hot carcass yield were higher to TA x TA breed, followed of PMB x TA lambs.

Sensory characteristics of meat are presented in Table 4.

Table 4. Means ± SE for meat sensory characteristics of lambs from different genotype

Characteristics	N	Genotype	
		GBF x TA	PMB x TA
Juiciness	24	3.67 ± 0.34	3.29 ± 0.25
Tenderness	24	4.13 ± 0.34	4.18 ± 0.27
Flavour	24	3.58 ± 0.31	3.92 ± 0.27
Appearance	24	3.29 ± 0.32	2.88 ± 0.23
The difference of specific lamb taste	24	3.46 ± 0.37	4.08 ± 0.32
Overall difference	24	3.83 ± 0.32	4.21 ± 0.28

Discussions

GBF x TA crossbreed lambs were slaughtered at higher slaughter weight than PMB x TA and TA x TA lambs, although all lambs were at similar ages in the research. These differences in final live weight might be explained by the effect of breed. The average daily gain for the two period (pre-weaning and post-weaning) were higher in the suckling period, and between groups, were higher for TA x TA lambs compared to GBF x TA and PMB x TA. Post-weaning, the ADG were higher for GBF x TA lambs. Although GBF x TA crossbreed lambs had higher live body weight and warm carcass weight, the hot carcass yield were higher to TA x TA breed, followed of PMB x TA lambs.

Lamb flavour, tenderness, the differences of specific lamb taste and overall difference perception of panelists were influenced by breed in the present study. Meat preferences of consumers are associated with socio-economic factors, ethics or religious believe and tradition (Font-i-Furnols and Guerrero, 2014). For instance, a highly preferred meat flavour in one culture, region or country may be perceived as less preferable or unacceptable in another (Schreurs et al., 2008). Overall difference scores given to lamb could be reflection of the meat tenderness, flavour intensity and quality perception of panelists (Ekiz et al., 2012). In particular, flavour which can be the determining feature in acceptance or rejection of the meat, is an important aspect for consumer preferences (Schreurs et al., 2008). The highest scores in terms of flavour and tenderness difference, as well as for the differences of specific lamb taste and overall difference were given to meat from PMB x TA crossbreed lambs.

The effect of breed on lamb flavor has been a topic of interest for many years (Jacobson and Koehler, 1963; Duckett et al., 1999; Elmore et al., 2000; Sanudo et al., 2000) many of which have reported no differences in lamb flavor due to breed or sire breed in crossbred studies (Fox et al., 1962; Dransfield et al., 1979; Mendenhall and Ercanbrack, 1979; Crouse et al., 1981).

Researchers who have found significant differences in flavor based on breed or sire breed have hypothesized why breed may or may not have an influence on flavor. Cramer (1983) suggested that wooled sheep might possess a mechanism for sulphur (S) storage, because wool is abundant in the amino acid cysteine. It is known that cysteine contains disulfide bonds between their thiol groups which in theory would cause sheep to have a higher S requirement than other meat producing livestock.

CONCLUSIONS

We found that growth rate and carcass weight were higher for GBF x TA crossbreed lambs. On the other hand, TA pure breed and PMB x TA lambs had higher values in terms of hot slaughter yields than GBF x TA lambs. Furthermore, results of sensory analyses indicate that meat from PMB x TA lambs had higher value of tenderness, flavour, the difference of specific lamb taste and overall difference as meat from GBF x TA lambs, when compared with meat from TA pure breed lambs. The results of the current study indicate that GBF x TA lambs had higher growth performances than TA pure breed and PMB x TA lambs, and meat of PMB x TA is better appreciated by consumers.

REFERENCES

American Meat Science Association (2015). *Research guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of meat*. Retrieved March 3, 2020, from

www.https://pdfs.semanticscholar.org/e8a4/22c39a85c4af95e65d1c95be565673cc0585.pdf.

Braggins, T. J. (1996). Effect of stress related changes in sheepmeat ultimate pH on cooked odor and flavour. *Journal of Agricultural and Food Chemistry*, 44, 2352-2360.

Cramer, D. A., Pruett, J. B., Kattnig, R. M, Schwartz, W. C. (1970). Comparing breeds of sheep: flavor differences. *Proceedings of the Western Section American Society of Animal Science*, 21, 267-271.

Cramer, D. A. (1983). Chemical compounds implicated in lamb flavor. *Food Technology*, 37, 249- 257.

Crouse, J. D., Busboom, J. R., Field R. A, Ferrell, C. L. (1981). The effects of breed, diet, sex, location and slaughter weight on lamb growth, carcass composition and meat flavor. *Journal of Animal Science*, 53, 376-386.

Dărăban, S., Coroian, C., Georgescu, B. (2009). Cluj Merino breeds' potential for meat production. *ABAH Bioflux*, 1, 57-62.

Dărăban, S. (2006). *Technology of sheep breeding*. Cluj-Napoca, RO: Risoprint Publishing House.

Duckett, S. K., Cuvala,S. L., Snowder, G. D. (1999). Effects of Dorper genetics on tenderness, fatty acid and cholesterol content of lamb. *Journal of Animal Science*, 77, 168.

Dransfield, E., Nute, G. R., MacDougall, D. B., Rhodes, D. N. (1979). Effect of sire breed on eating quality of crossbred lambs. *Journal of the Science of Food and Agriculture*, 3, 805-808.

Elmore, J. S., Mottram,D. S., Enser,M., Wood, J. D. (2000). The effects of diet and breed on the volatile compounds of cooked lamb. *Meat Science*, 55, 149-159.

Ekiz, B., Yilmaz, A., Ozcan, M., Kocak, O. (2012). Effect of production system on carcass measurement and meat quality of Kivircik lambs. *Meat Science*, 90, 465-471.

FAOSTAT, 2020. *Data Food and Agriculture Organization of the United Nations*, Live Animals. http://www.fao.org/faostat/en/#data/QA.

Font-I-Furnols, M., Guerrero, L. (2014). Consumer preference, behaviour and perception about meat products: An overview. *Meat Science*, 98, 361–371.

Fox, C. W., McArthur, J. A. B.,Sather, L. (1962). Effect of sire and breed on flavor scores from weaning lambs. *Proceedings of Western Section American Society of Animal Science*, 13, 37-38.

Georgescu, G., Banu, C., Croitoru, C., Savu, C., Tafta, V., Van, I., Lungu, S., Movileanu, G. (2000). *Treaty for the production, processing and capitalisation of meat*. Bucharest, RO: Ceres Publishing House.

Gkarane, V., Allen, P., Gravador, R. S., Diskin, M. G., Claffey, N. A., Fahey, A. G., Brunton, N. P., Farmer, L. J., Moloney, A. P., Monahan, F. J. (2017). Effect of castration and age at slaughter on sensory perception of lamb meat. *Small Ruminant Research*, 157, 65-74.

Hopkins, D. L., Hegarty, R. S., Walker, P. J., Pethick, D. W. (2006). Relationship between animal age, intramuscular fat, cooking loss, pH, shear force and eating quality of aged meat from sheep. *Australian Journal of Experimental Agriculture*, 46(6-7), 879-88.

Ilisiu, E., Daraban, S., Neascu, G., Ilisiu,V., Rahman, G. (2010). Improvement of lamb production in Romania by crossbreeding of local Tsigai breed with high performance breeds. *Landbauforschung - vTI Agriculture and Forestry Research*, 60, 259-266.

Jeremiah, L. E., Gibson,L. L., Tong, A. K. W. (1993). Retail acceptability of lamb as influenced by gender and slaughter weight. *Food Research International*, 26, 115-118.

Jacobson, M., Koehler, H. H. (1963). Components of the flavor of lamb. *Agricultural Food Chemistry*, 11, 336-339.

Mendenhall, V. T., Ercanbrack, S. K.(1979). Effect of carcass weight, sex, and breed on consumer acceptance of lamb. *Journal of Food Science*, 44, 1063-1066.

Safari, E., Channon,H. A.,Hopkins,D. L., Hall,D. G. Ven, R. (2001). A national audit of retail lamb loin quality in Australia. *Meat Science*, 61, 267-273.

Sanudo, C., Enser,M. E., Campo,M. M., Nute,G. R., Maria,G., Sierra,I., Wood, J. D. (2000). Fatty acid composition and sensory characteristics of lamb carcasses from Britain and Spain. *Meat Science*, 54, 339-346.

Schreurs, N. M., Lane,G. A., Tavendale,M. H., Barry,T. N., McNabb, W. C. (2008). Pastoral flavor in meat products from ruminants fed fresh forages and its amelioration by forage condensed tannins. *Animal Feed Science and Technology*, 146, 193-221.

Shackelford, S. D., Leymaster, K. A., Wheeler T. L., Koohmaraie, M. (2012). Effects of breed of sire on carcass composition and sensory traits of lamb. *Journal of Animal Science*, 90(11), 4131-4139.

Young, O. A., Lane,G. A., Priolo,A., Fraser, K. (2003). Pastoral and species flavour in lambs raised on pasture, lucerne or maize. *Journal of the Science of Food and Agriculture*, 83, 93-104.

Weller, M., Galgan, M. W., Jacobson, M. (1962). Flavor and tenderness of lamb as influenced by age. *Journal of Animal Science*, 21, 927-929.

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STUDY REGARDING FATHER'S INFLUENCE ON PERFORMANCE OF DAIRY COW'S POPULATION FROM RESEARCH AND DEVELOPMENT STATION FOR CATTLE REARING DANCU, IAȘI

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Abstract

It is known that the performances of the cows are influenced by the value of the mother and the value of the father for which it is important to know and group the performances so that the influence of the father can be highlighted. Therefore, the present study was conducted in order to identify the families of bulls, to study the performances of their daughters and also to determine their influence on these performances, analyzing the performances for milk production, percentage and quantity of fat and protein for cows that have completed maximum IV lactation. The biological material studied was grouped into 19 families with a total of 262 milk cows, the family with the highest milk production being given by the family with the Janther bull with an average production of 7975.80 ± 225.16 kg milk while the family with the smallest amount of milk was that of the bull Jecko Embriotransfer with an average production of 6474.20 ± 546.12 kg. The results obtained from this study indicate that the bull with the most influence on the descendants is the Janther bull, having the highest average yields on the quantity of milk, the quantity of fat in milk as well as on the quantity of proteins.

Key words*: fat, milk, quality, protein.*

INTRODUCTION

Cattle are the largest farm animals in the world and play an extremely important role globally both as a source of food and because they help increase and maintain the fertility and health of the earth (Acatincăi, 2004).

The importance of raising cattle is given by the variety of products they provide, they are divided into main products: milk and meat and by-products: skins, slaughterhouse by-products, horns, blood, hair, nails and manure (Șumovschi, 2016).

The importance of milk lies both in the special nutritional value and the fact that it can be transformed into a very large and diverse number of dairy products (over 1000 products) which contributes to the diversification of human nutrition.

The large milk productions that can be obtained nowadays from this species are the results of the work of man who, by applying methods of genetic improvement, has perfected the breeds of cattle (Ivancia, 2007; Creangă et al., 2008; Saraz et al., 2017).

Although the amount of milk is the main factor that gives profitability to a dairy farm, the quality of milk also has an important economic impact, especially the percentage of milk fat, which dictates its price (Atasever et al., 2018; Schlee et al., 1994; Bishop et al., 1994; Croiseau et al., 2009).

The percentage of milk protein and especially the casein fraction kappa - casein has a particularly important role in the cheese industry, this fraction having a special impact in the coagulation stage of milk (Ivancia, 2004; Aston et al., 1994).

Currently, in Romania, milk production does not meet the requirements at the national level, so it is necessary to increase milk production both by improving maintenance conditions and by improving the quantity and quality of milk (Drăgotoiu and Pop, 2015; Pîntea et al., 2008; De Brabander et al., 1999).

To achieve this goal, it is necessary to know the breeding value of cattle and their influence on offspring. In this paper we aimed to identify the families of bulls, to study the performance of their daughters and to determine what is their influence on these performances. Therefore, we analyzed the performance for milk production, analyzing the amount of milk, the percentage and the amount of fat and the percentage and the amount of protein, in cows that completed a maximum of IV lactation in 2017.

MATERIALS AND METHODS

The biological material was represented by 262 dairy cows of the Bălțată cu negru Românească (Romanian Black Spotted) breed located within the Dancu Iași Cattle Breeding Research and Development Station with lactations concluded between 01.10.2016 - 10.10.2017.

Some of the information was taken from the registers of "Classification of females according to milk production in MS, on standard lactation, with determined production and statistically relevant" between 01.10.2016 - 30.09.2017. According to the data taken, a sorting was performed that would allow us to form families by bulls.

Data on the amount of milk, percentage and amount of fat, percentage and amount of protein were statistically processed.

Compiling families according to father.

Bull River George Morty Keith, USA 00006159377, has 8 daughters inside the resort with lactations concluded between 01.10.2016 - 10.10.2017.

Bull Lantana, DE 000937712020, has 16 daughters inside the resort with lactations concluded between 01.10.2016 - 30.09.2017.

Mason Taurus, FR 002258172065, has 9 daughters inside the resort with lactations concluded between 01.10.2016 - 30.09.2017

Bull Aquolino, IT 004902500933, has 6 daughters inside the resort with lactations concluded between 01.10.2016 - 30.09.2017.

Bull Janther, DE 000343983068, has 5 daughters inside the resort with lactations concluded between 01.10.2016 - 30.09.2017

Bull Esprel, DE 000341614334, has 4 daughters inside the resort with lactations concluded between 01.10.2016 - 30.09.2017.

Bull Amedo E.T. DE 001601144673, has 8 daughters inside the resort with lactations concluded between 01.10.2016 - 30.09.2017**.**

Bull Harry E.T. USA 0060083723, has 6 daughters inside the resort with lactations concluded between 01.10.2016 - 30.09.2017**.**

Bull Alidio, IT 004902500933, has 5 daughters inside the resort with lactations concluded between 01.10.2016 - 30.09.2017.

Bull Master, DE 000940486348, has 5 daughters inside the resort with lactations concluded between 01.10.2016 - 30.09.2017**.**

Bull Matron, NL 00034904965, has 5 daughters inside the resort with lactations concluded between 01.10.2016 - 30.09.2017**.**

Bull Tunis, DE 001401422341, has 5 daughters inside the resort with lactations concluded between 01.10.2016 - 30.09.2017**.**

Bull Jobs E.T., DE 000347023454, has 6 daughters inside the resort with lactations concluded between 01.10.2016 - 30.09.2017.

Bull Enos E.T., DE 000113140063, has 3 daughters inside the resort with lactations concluded between 01.10.2016 - 30.09.2017.

Bull Jecko E.T., DE 000578914022, has 3 daughters inside the resort with lactations concluded between 01.10.2016 - 30.09.2017**.**

Bull Omajo Wideview Ballack, NL 000427218361, has 42 daughters inside the resort with lactations concluded between 01.10.2016 - 30.09.2017.

Bull Bonos, NL 000350854757, has 38 daughters inside the resort with lactations concluded between 01.10.2016 - 30.09.2017.

Bull Elevit, DE 000943121111, has 38 daughters inside the resort with lactations concluded between 01.10.2016 - 30.09.2017.

Jockmon E.T. bull, DE 000374303216, has 39 daughters inside the resort with lactations concluded between 01.10.2016 - 30.09.2017.

RESULTS AND DISCUSSIONS

The main characteristics pursued were represented by the performances of the families; in order to be able to observe the father's influence on the daughters' productions, it was necessary to calculate the family averages, the standard deviation from the average and the coefficient of variability within the families for each character studied.

A first index followed was represented by the quantity of milk, where it is found that the highest average was obtained in the Janther bull family, 7975.80 ± 225.16 kg of milk, the variations being between 7408 and 8495 kg of milk (Table 1).

Table 1. Estimators for the quantity of milk character (kg)

No	The name of the bull	No. daughters/bull	$\overline{X} \pm s_{\overline{X}}$ (kg)	V (%)	Limitation	
					Min.	Max
1	RIVER	8	7626.53±328.22	12.17	6650	8848
2	LANTANA	15	7586.35±145.15	7.65	6609	8470
3	MASON	9	7748.44±166.6	6.45	7085	8781
4	AQUOLINO	16	7269.27±184.86	10.17	5911	8594
5	JANTHER	5	7975.80±225.16	6.31	7408	8495
6	ESPREL	4	7735.00±306.90	7.94	6943	8338
7	AMEDO E.T.	8	7187.25±178.71	7.03	6676	8273
8	HARRY E.T.	6	7268.50±288.36	9.72	6099	8214
9	ALIDIO	5	7290.40±127.99	3.93	6865	7637
10	MASTER	5	6785.00±123.15	4.36	6302	7076
11	MATRON	5	6943.80±164.24	5.28	6488	7450
12	TUNIS	5	7805.20±341.08	9.77	6870	8585
13	JOBS E.T.	6	7251.67±263.87	8.91	5984	7747
14	ENOS E.T.	3	7564.67±178.00	4.07	7270	7885
15	JECKO E.T.	5	6474.20±546.12	18.86	5076	7781
16	BOBAS	38	7065.68±110.03	9.60	5396	9265
17	ELEVIT	38	7042.88±115.93	10.15	5273	8722
18	JOCKMON E.T.	39	7243.76±105.23	9.08	5761	9094
19	O.W. BALLACK	42	7535.62±117.44	10.10	4608	8848

Regarding the studied character, it presented a very good homogeneity, the value of the coefficient of variation being 6.31%.

As for the smallest amount of milk, it was obtained from the family of the bull Jecko E.T. namely, 6474.20 ± 546.12 kg of milk with variation limits between 5076 and 7781 kg of milk.

For the percentage of milk fat, it is found that the highest average was obtained in the Mason bull family, 4.25 ± 0.017% milk fat, varying between 4.12 and 4.25% (Table 2).

The lowest average fat percentage was obtained in the Matron bull family, namely, 4.10 ± 0.014% milk fat with variation limits between 4.08 and 4.16% milk fat (Table 2). Studying the coefficient of variation for the percentage of milk fat, it can be seen that the most homogeneous family is the family of the Harry Embriotransfer bull (V% = 0.32) and the most heterogeneous family is that of the bull Enos Embriotransfer (V% = 2.89) (Table 2).

For the amount of milk fat, it is found that the highest average was obtained in the Janther bull family, 330.00 ± 11.91 kg fat, ranging between 300 and 356 kg total fat in milk.

The lowest average amount of fat in milk was obtained in the family of the bull Jecko Embriotransfer, 277.20 ± 23.54 kg with variation limits between 213 and 230 kg total fat in milk (Table 3).

Table 2. Milk fat percentage (%) estimators

No	The name of the bull	No. daughters / bull	$\overline{X} \pm s_{\overline{X}}$ (%)	V (%)	Limitation	
					Min.	Max.
0	1	2	3	4	5	6
1	RIVER	8	4.15±0.022	1.53	4.05	4.26
2	LANTANA	15	4.14±0.011	1.05	4.03	4.24
3	MASON	9	4.25±0.017	1.20	4.12	4.29
4	AQUOLINO	16	4.15±0.018	1.73	3.94	4.25
5	JANTHER	5	4.13±0.04	1.45	4.04	4.19
6	ESPREL	4	4.17±0.04	2.04	4.08	4.29
7	AMEDO E.T.	8	4.14±0.014	0.93	4.09	4.20
8	HARRY E.T.	6	4.15±0.005	0.32	4.13	4.16
9	ALIDIO	5	4.18±0.009	0.47	4.16	4.21
10	MASTER	5	4.17±0.015	0.80	4.13	4.22
11	MATRON	5	4.15±0.014	0.78	4.08	4.16
12	TUNIS	5	4.12±0.021	1.16	4.07	4.17
13	JOBS E.T.	6	4.11±0.020	1.21	4.06	4.20
14	ENOS E.T.	3	4.20±0.07	2.89	4.13	4.34
15	JECKO E.T.	5	4.15±0.016	0.84	4.11	4.20
16	BOBAS	38	4.15±0.009	1.32	4.05	4.29
17	ELEVIT	38	4.15±0.008	1.14	4.05	4.27
18	JOCKMON E.T.	39	4.13±0.007	1.02	4.05	4.26
19	O.W. BALLACK	42	4.17±0.008	1.30	4.02	4.29

Table 3. Milk fat estimators (kg)

No	The name of the bull	No. daughters / bull	$\overline{X} \pm s_{\overline{X}}$ (kg)	V (%)	Limitation	
					Min.	Max.
1	RIVER	8	316.88±14.1	12.58	259	369
2	LANTANA	15	311.21±6.39	8.21	231	359
3	MASON	9	329.00±6.06	5.23	302	362
4	AQUOLINO	16	302.50±8.67	11.50	241	364
5	JANTHER	5	330.00±11.19	7.58	300	356
6	ESPREL	4	323.00±15.18	9.40	285	348
7	AMEDO E.T.	8	297.63±7.66	7.28	275	343
8	HARRY E.T.	6	308.50±7.42	5.89	294	340
9	ALIDIO	5	305.00±5.50	4.03	286	318
10	MASTER	5	291.00±4.15	3.19	280	305
11	MATRON	5	284.60±7.06	5.55	263	305
12	TUNIS	5	321.60±12.66	8.80	287	349
13	JOBS E.T.	6	304.83±3.81	3.14	294	318
14	ENOS E.T.	3	317.67±4.81	2.62	311	327
15	JECKO E.T.	5	277.20±23.54	18.99	213	320
16	BOBAS	38	290.06±4.43	9.41	224	378
17	ELEVIT	38	294.24±4.92	10.30	221	363
18	JOCKMON E.T.	39	300.02±4.36	9.07	237	376
19	O.W. BALLACK	42	315.22±4.91	10.10	193	366

Regarding the percentage of milk protein, it is found that although the highest average was equal to the families of River, Janther and Alidio bulls with an average production of 3.47%, the Alidio bull family has the lowest standard deviation of the average, of ± 0.14 compared to the standard deviation of the average River and Janther bulls that have a standard deviation of ± 0.18 of the percentage of milk protein (Table 4).

The lowest average protein percentage was obtained in the Tunis bull family with an average milk protein production of 3.39 ± 0.015 with limits between 3.37 and 3.45%.

The study of the coefficient of variation for the percentage of milk protein can be found that the most homogeneous family is the Lantana bull family (V% = 0.4), and the most heterogeneous family is that of the Enos Embryotransfer bull (V% = 1.91) (Table 4).

Table 4. Milk protein percentage (%) estimators

No	The name of the bull	No. daughters/bull	$\bar{X} \pm s_{\bar{X}}$ (%)	V (%)	Limitation	
					Min.	Max.
0	1	2	3	4	5	6
1	RIVER	8	3.47±0.018	1.46	3.39	3.55
2	LANTANA	15	3.45±0.003	0.40	3.36	3.52
3	MASON	9	3.34±0.021	1.82	3.37	3.56
4	AQUOLINO	16	3.45±0.005	0.61	3.42	3.50
5	JANTHER	5	3.47±0.018	1.18	3.40	3.51
6	ESPREL	4	3.43±0.015	0.87	3.40	3.47
7	AMEDO E.T.	8	3.47±0.012	1.01	3.42	3.51
8	HARRY E.T.	6	3.46±0.023	1.65	3.38	3.51
9	ALIDIO	5	3.47±0.014	0.92	3.43	3.52
10	MASTER	5	3.44±0.017	1.10	3.40	3.49
11	MATRON	5	3.45±0.009	0.60	3.43	3.48
12	TUNIS	5	3.39±0.015	0.97	3.37	3.45
13	JOBS E.T.	6	3.43±0.017	1.23	3.39	3.51
14	ENOS E.T.	3	3.44±0.034	1.91	3.38	3.51
15	JECKO E.T.	5	3.43±0.026	1.72	3.36	3.49
16	BOBAS	38	3.45±0.008	1.39	3.38	3.60
17	ELEVIT	38	3.44±0.006	1.15	3.38	3.55
18	JOCKMON E.T.	39	3.45±0.007	1.21	3.38	3.61
19	O.W. BALLACK	42	3.43±0.006	1.12	3.35	3.49

For the amount of protein, it is found that the highest average was obtained in the Esprel bull family, 265.75 ± 10.09 kg of protein (Table 5) ranging between 238 kg and 283 kg of protein in milk.

The lowest average amount of protein in milk was obtained in the family of the bull Jecko Embriotransfer, 228.40 ± 19.05 (Table 5) with limits between 177 and 264 kg of protein in milk.

Tablel 5. Protein quantity estimators (kg)

No	The name of the bull	No. daughters / bull	$\bar{X} \pm s_{\bar{X}}$ (%)	V (%)	Limitation	
					Min.	Max.
1	RIVER	8	264.38±10.81	11.57	218	303
2	LANTANA	15	260.75±4.89	7.5	226	295
3	MASON	9	268.00±5.23	5.86	248	297
4	AQUOLINO	16	259.16±5.07	7.83	204	297
5	JANTHER	5	276.80±8.42	6.80	252	296
6	ESPREL	4	265.75±10.09	7.59	238	283
7	AMEDO E.T.	8	249.00±5.94	6.74	229	284
8	HARRY E.T.	6	257.17±6.32	6.02	240	282
9	ALIDIO	5	253.20±5.12	4.52	235	265
10	MASTER	5	240.80±2.54	2.36	233	249
11	MATRON	5	240.00±5.93	5.53	223	259
12	TUNIS	5	264.60±10.78	9.12	237	289
13	JOBS E.T.	6	254.67±3.45	3.32	245	266
14	ENOS E.T.	3	260.33±5.33	3.55	255	271
15	JECKO E.T.	5	228.40±19.05	18.65	177	264
16	BOBAS	38	242.14±3.71	9.45	189	316
17	ELEVIT	38	243.61±3.91	9.88	187	301
18	JOCKMON E.T.	39	250.25±3.36	8.39	201	313
19	O.W. BALLACK	42	258.92±3.88	9.71	155	300

Regarding the coefficient of variation for the amount of milk protein, it can be seen that the most homogeneous family is the Master bull family (V% = 2.36) and the most heterogeneous family is that of the Jecko Embriotransfer bull (Table 5).

CONCLUSIONS

For the study, 19 families were grouped with a total of 262 dairy cows.

For the quantity of milk the family with the highest milk production was that of the Janther bull where the average production was 7975.80 ± 225.16 kg of milk ranging between 7408 and 8495 kg of milk while the family with the lowest average production was the bull family Jecko Embryotransfer with an average milk production of 6474.20 ± 546.12 kg, with limits between 5076 and 7781 kg milk.

Regarding the character of the percentage of fat in milk, it was found that the Manson bull family had the highest average production, of 4.25 ± 0.017% with limits between 4.12% and 4.25%.

Regarding the amount of fat in milk, the highest values were in the Janther gold family, with a production of 330.00 ± 11.19 kg of fat, while the lowest average amount of fat was identified in the Jecko Embriotransfer bull family, with a production of 277.20 ± 23.54 kg.

The highest average milk protein content was identified as belonging to the River, Janther and Alidio bull families, with an average production of 3.47% and an average standard deviation of ± 0.14 to ± 0.18, with variation limits between 3, 39% and 3.55%.

For the amount of milk protein, the Janther bull family was identified as having the highest average production, of 276.80 ± 8.42kg, while the Jecko Embriotransfer bull family had an average protein quantity of 228.40 ± 19.05kg.

The bull with the most bitter influence on the offspring is the Janther bull, having the highest average yields for the amount of milk, the amount of milk fat, the percentage of milk protein and the amount of milk protein.

REFERENCES

Acatincăi, S. (2004). *Cattle production/Producțiile bovinelor*, second edition. Timișoara, RO: Eurobit Publishing House.

Aston, K., Thomas, C., Daley, S. R., Sutton, J. D. (1994). Milk production from grass-silage diets. Effects of the composition of supplementary concentrates. *Anim. Prod.*, 59, 335–344.

Atasever, S., Erdem, H., Jonas, E.M. (2018). Somatic cell count of milk in Holstein cows raised in Turkey conditions: a comparative evaluation. *Scientific Papers. Series D. Animal Science*, LXI (1), 11-13.

Bishop, M.D., Kappes, S.D., Keele, J.W., Stone, R.T., Sunden, S.L.F., Hawkins, G.A., Solinas Toldo, S., Fries, R., Grosz, M.D., Yoo, J., Beattie, C.W. (1994). A genetic linkage map for cattle. *Genetics*, 136, 619-639.

Creangă, Ș., Maciuc, V., Pântea, M. (2008). *Genetic polymorphism in cattle breeding in Moldova/Polimorfismul genetic în ameliorarea bovinelor din zona Moldovei*. Iași, RO: Ion Ionescu de la Brad Publishing House.

Croiseau, P., Guillaume, F., Fritz, S., Ducroq, V. (2009). *Use of the Elastic-Net algorithm for genomic selection in dairy cattle.* Book of Abstracts of the 60th Annual Meeting of the European Association for Animal Production, Barcelona, Spain, August 24th-27th.

De Brabander, D. L., De Boever, J. L., Vanacker, J. M., Boucque, C. V., Botterman, S. M. (1999). Evaluation of physical structure in dairy cattle nutrition. Pages 111–145, in *Recent Advances in Animal Nutrition*, P. C. Garnsworthy and P. C. Wiseman, Nottingham, UK: Nottingham University Press Publishing House.

Drăgotoiu, D., Pop, I. M. (2015). *Principles of nutrition/Principii de alimentație*, Bucharest, RO: Granada Publishing House.

Ivancia, M. (2004). *Somatic cells - Milk quality indicator/Celule somatice - Indicator de calitate a laptelui*. Iași, RO: Alfa Publishing House.

Ivancia, M. (2007). *Animal breeding/Ameliorarea animalelor*. Iași, RO: Alfa Publishing House.

Pîntea, M., Maciuc, V., Creangă, Ș. (2008). *Genetic breeding of cattle in the cross - border region/Ameliorarea genetică a bovinelor în regiunea transfrontalieră*. Iași, RO: Ion Ionescu de la Brad Publishing House.

Saraz, I., Cenariu, M., Ciupe, S., Pop, R., Groza, I. (2017). Correlations between somatic cell count and milk quality in two dairy farms from North-Western Romania. *AgroLife Scientific Journal*, 6(1), 234-236.

Schlee, P., Graml, R., Rottmann, O., Pirchner, F. (1994a). Influence of growth-hormone genotypes on breeding values of Simmental bulls. *J. Anim. Breed. Genet*., 111, 253-256.

Șumovschi, D.C., (2016) *Management of dairy cows in medium-sized farms in the northeastern development region/Managementul exploatării vacilor de lapte în ferme de medie capacitate din regiunea de dezvoltare nord-est.* Iași, RO: Ion Ionescu de la Brad Publishing House.

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DETERMINING THE CURRENT STAGE OF IMPROVING CURL UNIFORMITY IN THE COLOR VARIETIES BELONGING TO THE KARAKUL OF BOTOŞANI BREED

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Abstract

Within the performance control activities of the skin production, for the analysis of the uniformity of the curls, we focus on the main characteristics that influence the expression and the externalization of a genotype with a certain type or form of curl with approximately equal values of the basic dimensions. To create conditions for genetic expression of the uniformity of the type of curls, it is necessary to follow the improvement of the following parameters: the length, the height, the width, the degree of closure, spiral direction, the contour and the distance of the curls on the skin surface. The method applied in the assessment of the specific characteristics of the research carried out was based on the technical norms specified in Section 1.4 and 1.5 of the MADR Order no. 22/20.01.2006, and the statistical processing of the data was based on the use of the computer program S.A.V.C. The obtained results show that between the average score determined for this character in the grayish variety and the black one, the difference is insignificant but the one between grayish and pink and grayish and grey is significant for P<0.01%, and the one between grayish and brown is significant for P<0.05. In the brown variety it is found that if at the first generation since the establishment of the breed register the proportion of those with maximum score was 46.33% at the generation evaluated in 2015, it is found that at almost 70% a good uniformity is obtained, resulting in a progress 2.35% genetically for each successive generation within that 10-year period.

Key words*: curl uniformity, Karakul of Botoşani sheep, pelts.*

INTRODUCTION

The uniformity of all the curls from the skin surface has always been an important objective of improving the Karakul of Botoşani sheep because it also influences the way of expressing other characters such as length, degree of closure, width and height and is expressed in an original way on the general appearance of the skin, influencing their aesthetic and commercial value.

This character is a special criteria and an important objective of improving the quality of skins. The trend and the desire of the breeder is to create the technical conditions for producing some types of skins, to meet the existence on their surface of curl's uniform as type, size, degree of closure, spiral direction, alignment mode etc. (Buzu, 2018; Hrincă et al., 2014; Iñiguez et al., 2008; Mochnacs et al., 1978, Nechifor et al., 2016; Pascal, 2001; Schoeman, 1998).

Enumerating these requirements that ensure uniformity emphasizes the complex aspect and the high degree of correlation of this character with other reasons for which the effect of the improvement is quantified with a lower intensity. Regarding this last remark it can be said that "no matter how rigorous the selection, it is not possible that within the same skins, even being elite there are only one or two forms of curls" (Taftă et al., 1996; Nechifor et al., 2019).

Another drawback that can reduce the effect of selection is because the uniformity of the curls is difficult to obtain due, they depend on many other characters.

Studies conducted in this regard show that on the surface of the skin the most uniform curls are arranged in the upper-posterior part (croup, shafts, spine); it follows in order of uniformity -

the lateral parts (the upper part of the thighs, flanks and ribs to the back); on the third place in the top of the expression of the desired type are the curls located on the chest and neck; the most uneven curls are arranged on the head, sternum and middle part of the abdomen (Evtodienco et al., 2019; Nechifor et al., 2014; Pascal et al., 1995; Pascal, 2015).

MATERIALS AND METHODS

The number of sheep from which the lambs came from, based on which the current status of the improvement was evaluated for the main characters on which the skins quality depends, is found in over 95% in the particular sector in the counties breeding area, the difference was represented by lamb obtained from SCDCOC Popăuți - Botoșani sheep. The biological material analyzed was represented by the purebred Karakul of Botoșani lambs belonging to all the varieties of color, obtained over the course of three successive generations, originating from breeding seasons that took place in 2013, 2014 and 2015.

Uniformity of curls is appreciated by observing the lamb from distance on both sides of the body, establishing if exist one or more types of curls, as well as the occupied surface and their distribution way on different corporal regions. Function of those characteristics curl's uniformity could be: good, medium or weak.

In order to increase the degree of evaluation and, implicitly, the accuracy of the data regarding the characters on which the quality of the skins depends, the following conditions were met:

- from each flock, all the lambs were evaluated in the same place and under the same conditions;
- all the assessments were made during the morning, until noon;
- from each flock, all the lambs were evaluated by a unique technical personnel so that there would be the same level of exigency;
- every day, the evaluation started with the lambs of a lighter color and continued with those belonging to the darker varieties.

The data obtained were compared with those obtained in the performance control of the first generation of lambs obtained from the establishment of the Genealogical Register, respectively the generation of lambs from 2005. The statistical processing of the data was based on the use of the computer software S.A.V.C. (Statistical Analysis of Variance and Covariance 2003). To test the statistical significance of the differences between the averages parameters values studied and the correlations between them, the variables analysis (ANOVA Single Factor) and the Pearson Correlation algorithms were used, both included in the computer program used.

RESULTS AND DISCUSSIONS

Curl's uniformity represents one of the features which had a direct influence on pelts value. As much as the totality of curls are more uniform as type and shape on its surface, with that much the pelt is more valuable. Existence of only one type of curl is impossible from biological point of view, but also the situations in which are founded over 4 curl types are not admissible.

On the pelt of Karakul of Botoșani lambs the quality of curls differs. So, the most valuable curls are disposed on the upper-posterior side (rump, loin, dorsum); followed in order of quality by lateral sides (upper part of thighs, flanks and ribs till back); on the third place in curl's quality top are situated the ones from chest and neck; the weakest curls are disposed on head, stern and middle part of abdomen (Nechifor et al., 2019; Pascal, 2015; Pascal, 2011).

Evaluation of this character is made in the first day of life and in relation to how the uniformity is perceived, 50 points are awarded when over 65% of the curls have relatively similar characters, only 25 points when the presence of similarities is identified at 40% and 65% of the total of the curls, another 20 points when maximum 30% of the curls have a uniformity easy to record.

Processing data shows that the average score of this character has values higher than 43 points for the black and grayish variety, lower for the grey (26.69 points) and intermediate for pink and brown (39.59 and 39.87 points) (Table 1).

In the black variety the statistical calculation of the data shows in the first place the existence of genetic progress and in the second a degree with a different rate of improvement from the

first generation entered in the Genealogical Register of the Karakul of Botoşani breed (the one from 2005) until the last generation of lambs analyzed in these researches (2015).
If in 2005 the population of lambs subjected to the productive evaluation found that at a proportion of only 9.40% the uniformity of the curling was good, due to the intensification of the selection it reached that in 2015 more than 65% of the analyzed individuals have a good uniformity.

Table 1. The average score and statistical parameters for the uniformity of the curl according to the color variety

Color variety	no.	$\overline{X} \pm s_{\bar{x}}$	s	V%
Black	1158	44.93±0.460	10.065	22.430
Grayish	1184	43.07±0.326	11.199	26.001
Brown	431	39.87±0.595	12.287	30.818
Grey	533	26.69±0.208	4.75	17.795
Pink	106	39.59±0.537	12.576	32.628

Also, compared to the same year, the cases in which the lambs presented an accepted modeling increased from 67.82% to almost 81% in 2013. Regarding this maximum level, reached in 2013, because the increased exigencies in appreciating this character there is a decrease of this lambs category by 7.98%.
By granting the maximum score to the lambs that present 75% of a high degree of uniformity it has the role of amplifying the externalization in genotype of this character, and the curls to have a higher degree of uniformity expression for length, width, height etc.
For the grayish variety, the average score calculated for this character was 43.07 ± 0.326, which indicates that the improvement is in real progress. The efficiency of selection and genetic progress is also supported by the fact that with each generation there is an increase in the proportion of individuals with good uniformity of curls. Compared to 2005, the proportion of those with a good uniformity increases from 68.79% to 86.89%, which indicates an improvement of this character by about 1.81% per generation (Table 2).
These data suggest that the process of breeding amelioration is performed in the desired sense and is easy to quantify, and is also supported by the fact that individuals who showed low uniformity were totally eliminated from the population.

To present the evolution of breeding degree for curl's uniformity function of season and color variety the obtained data for appreciation of the desired character are synthesized in Figure 1. Their analysis shows different situations for those five color varieties and implicit the fact that curl's breeding under the aspect of uniformity is situated on different levels.
At evaluation of the character represented by curl's uniformity was observed that in each of those three seasons from which lambs were gathered the highest score was obtained in the case of appreciation of black and grayish lambs.
In the case of both varieties the mean score for a good uniformity was higher than 72 points and the highest level of the score was recorded in the case of evaluation of grayish lambs obtained in the calving season from 2015. In this case, at effectuated evaluations was observed that a high rate of studied lambs, curl had an increased uniformity and in the case of majority of curls distributed on pelts surface those ones had almost the same shape and dimensions as length, height and width.
At pink and grey varieties due to the fact that those ones are composed colors, the white fibers located in curl's structure had a greater length face to the dark one, so curl is more irregular.
A diminishing of curl's uniformity could be observed at these varieties also regarding curl's shape, the ones with short, large and high tubes are dominant.
Generally, at brown, grey and pink variety the effectuated appreciations indicate a reduces degree of character breeding, aspect highlighted also by the fact that mean score was between 63.34 points at grey lambs appreciated in 2014 and 69.82 points at the brown ones evaluated in 2015.
Therefore, for increasing the uniformity degree of curls is imposed an intensification of breeding based on the increasing of selection degree and a better matching of mating.
Efficiency of color breeding in the case of homogenous mating is measurable when it is working with small batches or sub-populations, at which are assigned valuable breeders with features at least at the level of the animals from those batch (Pipernea, 1974, cited by Creangă, 2007).

Regarding the statistical significance of the differences, it is found that between the average score determined for the grayish variety and for other colored varieties (grey and pink) they are significant for the P<1 threshold and the one between the grayish and brown is significant for the 5% threshold, and between grayish and black no significance of the differences for the statistical thresholds taken into account is recorded (Table 3).

At the brown variety the process of improvement is in full development - an aspect that is easy to highlight from the analysis of the proportion of lambs from each generation that have met the minimum requirements for granting the maximum score. Thus, if at the first generation since the establishment of the breed register the proportion of those with the highest score was 46.33% at the evaluated generation in 2015, there is an increase of 23.49%, representing a genetic progress of 2.35% per generation newly obtained. Also, the lack of lambs with low uniformity shows the efficiency of selection and improvement of this character.

Continuing the selection, at least at this level of intensity, will cause the share of lambs with a phenotypic expression characteristic of an accepted uniformity to generate a constant increase in each generation, but also to increase the average score above the level of 39.87 ± 0.59 as it was obtained in 2015. Another argument of the good direction in which the improvement of this character takes place is represented by the lack of lambs whose uniformity is low.

In the pink and grey color varieties the process of improvement is slower because the current objective is to increase the size of the population. Therefore, only individuals who express in the genotype major deviations of the desired type are eliminated in the selection. This explains also that the average score has values less than 40 points in pink and less than 30 points in grey.

However, by the fact that for the pink variety the average score was 39.59 ± 0.53 and at the grey of 26.69 ± 0.20 it indicates a different level of improvement of the respective character in the two varieties of color. Keeping a minimum threshold for admission into the livestock and eliminating from the active population individuals who had a major deviation from the desired type has made the current generations subject to research, individuals whose uniformity type is undesirable to be greatly reduced or missing.

The improvement of this character in the grey variety is evidenced by the constant increase of the share of lambs with the desired type from 51.67% in the 2005 generation to 68.07% in 2015. In this case, the genetic progress achieved in the ten years was 1.64 which means that against the background of applied selection and breeding management, the number of their products will increase with a better expression for this character in phenotype.

Table 2. The frequency of individuals in relation to improving the curl's uniformity

Color variety	Curl's uniformity	Frequency of the desired type per evaluation season (%)							
		2005		2013		2014		2015	
		n	%	n	%	n	%	n	%
Black	Good	107	67.82	380	79.50	450	80.79	343	72.98
	Medium	36	22.78	98	20.20	107	19.21	127	27.02
	Low	15	9.40	-	-	-	-	-	-
Grayish	Good	141	68.79	237	72.70	330	79.52	385	86.86
	Medium	36	17.56	89	27.30	85	20.48	59	13.14
	Low	28	13.65	-	-	-	-	-	-
Brown	Good	67	46.33	88	67.70	125	64.77	74	69.82
	Medium	54	37.70	42	32.30	68	35.23	32	30.18
	Low	23	15.97	-	-	-	-	-	-
Grey	Good	31	51.67	110	66.27	133	63.34	65	68.07
	Medium	18	30.00	56	33.73	77	36.66	90	31.93
	Low	11	18.33	-	-	-	-	-	-
Pink	Good	18	48.00	28	68.70	25	65.79	10	65.52
	Medium	9	26.47	13	31.30	13	34.21	19	34.48
	Low	8	25.53	-	-	-	-	-	-

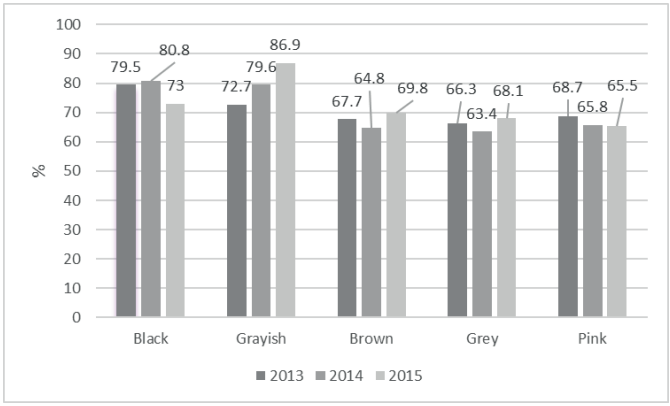

Figure 1. Evolution of the uniformity of the loop depending on the season and variety of color

Table 3. The difference and significance of difference for the uniformity of curl

Character 1	Character 2	Average difference	Difference meaning	Significance threshold
Grey	Grayish	3.48	significant	0.05
Grey	Brown	0.28	insignificant	-
Grey	Black	5.33	significant	0.01
Grey	Pink	1.05	insignificant	-
Pink	Grayish	4.53	significant	0.01
Pink	Brown	1.33	insignificant	-
Pink	Black	6.38	significant	0.01
Black	Grayish	1.85	insignificant	-
Black	Brown	5.06	significant	0.01
Brown	Grayish	3.20	significant	0.05

Statistical data processing shows that the difference between the mean scores was significant for the P<1 threshold between grey x grayish, pink x grayish, grey x black, pink x black and black x brown.

In lambs from the pink variety, the situation of the current level of improvement is in the same coordinates with that described at the grey, but the expression of this character is lower also on the background of the intensification of the selection and in the desire to increase the degree of similarity in the phenotypic expression of this character the proportion of lambs that had a uniformity that allowed the maximum score to be kept relatively at the same level, but with a tendency to reduce.

The data obtained highlight that the breeding program of the Karakul of Botoşani breed is efficient and allows a positive quantification of the way of expressing the specific characters of the skin production as a direct effect of the breeding. As a whole, all the data obtained converge in meaning with others found in the specialized literature determined for other populations of the Karakul of Botoşani breed (Cloete et al., 2014; Pascal, 2011; Pascal, 2001; Pascal et al., 1995) or for other breeds grown for fur production (Albertyn et al., 1993; Buzu, 2012; Buzu, 2014; Gligvashvili, 1998).

When the population will consolidate and the number of individuals within it will allow that by raising the selection criteria, the process of improvement will be accelerated and the proportion of lambs with uniform curls will have a progressive directional evolution.

CONCLUSIONS

For the uniformity of the curl, there is a different degree of improvement, being more advanced in the black variety and greyish (where more than 43 points were obtained), lower in the grey (26.69 points) and in an intermediate phase at pink and brown (39.59 and 39.87 points).

In the black variety the improvement of the uniformity of the curl registers higher quotas compared to other varieties of color and is at a

net level higher than in 2005 when the desired form was identified at only 9.40% and in 2015 at over 65% of the individuals analyzed.

For the greyish variety, compared to 2005 the proportion of lambs with the desired uniformity increases from 68.79 to 86.89% in 2015, indicating an improvement of this character by about 1.81% per generation and an improvement in progress.

Between the average score determined for this character in the greyish variety and the black one, the difference is insignificant but the one between greyish and pink and greyish and grey is significant for the threshold of 1%, and the one between the greyish and brown is significant for the threshold of 5%.

When assessing the uniformity of the curls in the brown lambs, it is found that if at the first generation since the establishment of the breed register the proportion of those with maximum score was 46.33% at the generation evaluated in 2015, it is found that at almost 70% a good uniformity is obtained, resulting in a 2.35% genetic progress for each successive generation over that 10-year period.

The evaluation of the uniformity of the curl for the grey variety allowed obtaining an average score of 39.59 ± 0.537 and at the grey of 26.69 ± 0.208, a level which indicates the existence of a different current threshold for improving the respective character in the two colour varieties.

The differences between the average score given to the evaluation of the uniformity of the curl were significant for 5% only between greyish - brown and greyish - grey**.**

REFERENCES

Albertyn, L.R., Schoeman, S.L., Groeneveld, H.T. (1993). Factors influencing the quality of Karakul pelts, with emphasis on discrete characteristics. *S. Afr. J. Anim. Sci*., 23, 183.

Buzu, I. (2018). Amelioration of the Karakul sheep race in different areas and countries of the world. *Scientific Papers, Animal Science Series: Lucrări Ştiinţifice, Seria Zootehnie*, 69, 19-32.

Buzu, I. (2014). Selection of Moldovan Karakul sheep by the body weight. *Scientific Papers. Series D. Animal Science*, LVII, 25-31.

Cloete, S.W.P., Olivier, J.J., Sandenbergh, L., Snyman, M.A. (2014). The adaption of the South Africa sheep industry to new trends in animal breeding and genetics: A review. *South African Journal of Animal Science,* 44(4).

Creangă, Şt. (2007). *Ereditate şi variabilitate/Heredity and variability*. Iaşi, RO: Alfa Publishing House.

Evtodienco, S., Maşner, O. (2019). Study of the quality of pelt at lambs of Moldovan Karakul type. *Lucrări Ştiinţifice, Seria Zootehnie, USAMV Iaşi*, 72(24), 104-109.

Gligvashvili, A.G. (1998). Crossbred sheep breeding and ways of improving this further. *Agricultural Sciences Doctorate Dissertation*. Georgian Zoo-Veterinarian Institute, Tbilisi, Georgia.

Hrincă, G., Georgescu, S.E., Vicovan, G., Nechifor, I. (2014). Genetic and pathological aspects of prion protein (PrP) in sheep belonging to Botosani Karakul breed. *AgroLife Scientific Journal*, 3(1), 69-74.

Iñiguez, L., Mueller, J. (2008). Characterization of Small Ruminant Breeds in Central Asia and the Caucasus. *International Center for Agricultural Research in the Dry Areas (ICARDA)*, Aleppo, Syria

Mochnacs, M., Taftă, V., Vintilă, I. (1978). *Genetica şi ameliorarea ovinelor/Genetics and breeding of sheep*. Bucharest, RO: Ceres Publishing House.

Nechifor, I., Florea, M.A., Pascal, C. (2019). Assessing the Current State of Curl Type Improvement for Karakul of Botoşani Breed in Relation to the Color Variety, *Animal Science and Biotechnologies*, Cluj-Napoca, 76(2), 87-93.

Nechifor, I., Hrincă, Gh., Nechifor, C.I., Florea, Al.M., Groza, M. (2014). Effect of selection pressure on fixing the qualitative features of lamb pelts of Karakul type. *Scientific papers. Animal Science. Series D*, LVII, 54-58.

Nechifor, I., Pascal, C. (2016). Research regarding the quality of pelts from Karakul lambs of Botoşani as effect of crossings between breeders belonging to the variety of brown color. *Scientific papers. Animal Science. Series D,* LIX, 218-222.

Pascal, C. (2015). *Tratat de creştere a ovinelor şi caprinelor/Treaty for the rearing of sheep and goats*. Iaşi. RO: Ion Ionescu de la Brad Publishing House.

Pascal, C. (2011). Researches regarding quality of sheep skins obtained from Karakul from Botoşani sheep, *Biotechnology in Animal Husbandry,* 27(3).

Pascal, C. (2001). Calitatea pielicelelor obţinute de la ovinele de rasa Karakul crescute şi exploatate în ferma Andrieşeni, jud. Iaşi/The quality of skins obtained from Karakul sheep raised and exploited in the Andrieşeni farm, Iaşi county. *Lucrări Ştiinţifice, Seria Zootehnie, USAMV Iaşi*, 43/44, 327-330.

Pascal, C., Gîlcă, I., Creangă, Şt., Vintilă, V. (1995). Cercetări comparative privind unele însuşiri ce influenţează calitatea pielicelelor la mieii de rasa Karakul şi metişi / Comparative research on some properties that influence the quality of skins in Karakul lambs and crossbreeds. *Lucrări Ştiinţifice, Seria Zootehnie, USAMV Iaşi*, 37/38, 216-221.

Schoeman, S.J., (2009). Genetic and environmental factors influencing the quality of pelt traits in Karakul sheep. *South African Journal Anim. Sci*., 3, 125-139.

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GROWTH OF FEMALE CALVES OF THE ABERDEEN ANGUS CATTLE BREED REARED IN AN ORGANIC FARM

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Abstract

The growth of female calves of the Aberdeen Angus cattle breed was studied in a certified organic farm. The animals were weighed on a monthly basis. It was ascertained that the calves are born with a live weight of approximately 31.6 ± 0.81 kg, upon weaning at 210 days they weigh 204.0 ± 5.93 kg, at 15 months - 363.7 ± 13.04 kg, and at 18 months - 433.0 ± 11.57 kg. The average daily individual gain widely varies reaching up to 2.090 kg. The growth of the animals as a whole and within the different age groups is influenced by the year, season and month of birth. Upon joint examination of the age-year-season and age-year-month factors, however, the effect of the separate factors "dillutes" and the complex influence may be considered unreliable. The female calves which are bred naturally under the conditions in Bulgaria, regardless of the gain fluctuations during the different periods, reach an optimal servicing live weight (330-360 kg) at an optimal age (15-16 months).

Key words: *Angus, growth calves, organic farm.*

INTRODUCTION

It can be observed that in search of solutions aimed at reducing the negative consequences of the industrialisation and intensification (Steinfeld, 2006; Chávez-Fuentes et al., 2017; Kamilaris et al., 2018; Zhang et al., 2018), more and more attention is paid to the organic farming which is considered a foundation for a sustainable animal production (Nikolov, 2003; Nikolova, 2006; 2013; 2020; Von Keyserlingk et al., 2013). In the recent years it has developed from a practical farmers movement to a serious business (Nikolova, 2020) practiced in 179 countries; in 88 of them there has already been effective national legislation (Zhou, 2018).

Breed selection is of extreme importance in the organic livestock breeding. Pointing out that the purpose of organic farming is the animals' welfare, Nikolov (2012) indicates that this deeply humane approach has a large scope and must be applied to all types and breeds of animals, otherwise the whole idea is pointless. The author believes that each breed has its advantages and disadvantages which make it more or less suitable for organic farming, and that none should be deprived of the chance to be bred in such way.

According to Hörning (2008) upon selection of a breed for beef cattle organic farming, one should not look for intensive growth, but choose a breed which is feasible with reference to small farms and is able to satisfy a special niche market with its unique produce. The Aberdeen Angus Cattle breed has a good adaptability and is suitable for organic farming. This breed's quality produce may have a specific marketability related to the region of breeding (Savić et al., 2013).

The Aberdeen Angus Cattle breed in Bulgaria has not been sufficiently studied yet. It was not until 2015 when a breeding program for the breed was approved. At the same time the import of animals which have different origin and are bred under different production systems continues. The aim of the present study is to examine the growth of female calves of the Aberdeen Angus Cattle breed under organic farming system.

MATERIALS AND METHODS

The study was carried out during 2016-2018 in a certified organic farm "Thraca Angus Farms" LTD. The farm is located in low mountainous, slightly hilly area between the West Predbalkan and the West part of the Danube Plain which is

suitable for breeding special beef animals. There are enriched pastures in the immediate vicinity of the farm which are used about 8 months of the year. Calving is all year round.

The study includes 54 female calves which were monthly weighed from their birth until attaining the age of 22 months.

The weighing is performed in a specialised crush with a weighing platform, and the reporting and registering of the live weight is automatic. The initial live weight is standardized in compliance with ICAR (2016) recommendations.

The data were processed via analysis of variance. Two models with the following statistical type were used: $Yijkl = \mu+AGi+YBij+SBijk+eijk(l)$; $Yijkl = \mu+AGi+YBij+MBijk+eijk(l)$, where: Yijkl - observation vector; μ - population average; Agi - age group fixed effect (i = 22); Ybij - year of birth random effect (j = 3); SBijκ and MBijk - a random effect of respectively the season (3) and month (11) of birth within the year and the season, eijkl - residuals. The data is processed statistically via SPSS 21.

RESULTS AND DISCUSSIONS

The live weight at birth is obligatory registered in the farm examined by us and serves for management of the factors affecting the embryonic and post embryonic growth of the calves. One of the leading factors is the physical condition of the mother which is related to the sustainable feeding during pregnancy (Funston et al., 2010).

Berger et al. (1992), consider that the birth weight is of significance with reference to the calving ease and the calves' survival during the first 24 hours after birth. They have ascertained that the new born calf live weight of 29 kg is the most suitable for the Aberdeen Angus cattle breed at a first calving age between 22-29 months. The average live weight at birth of the female calves studied by us is 31.6 ± 0.81 kg (Table 1).

Table 1. Live weight at birth, absolute and average daily gain of organically bred female calves of the Aberdeen Angus Cattle breed

Age, months		Live weight			Absolute gain			Average daily gain		
	N	LS	Sx	Sdev	LS	Sx	SDev	LS	Sx	SDev
At birth	54	31.6	0.81	5.96						
Standardized at birth	54	33.1	1.26	9.23						
1	54	54.3	1.61	11.85	21.20	1.122	8.245	0.713	0.039	0.286
2	53	74.6	1.95	14.21	20.54	1.004	7.306	0.711	0.028	0.205
3	52	98.7	2.69	19.36	24.09	1.338	9.647	0.756	0.036	0.263
4	51	123.2	3.52	25.13	24.58	1.336	9.540	0.834	0.039	0.281
5	50	149.3	3.99	28.24	26.18	1.617	11.437	0.912	0.041	0.287
6	46	172.8	5.22	35.41	25.18	2.069	14.035	0.836	0.043	0.289
7	46	204.0	5.93	40.21	31.12	1.985	13.466	0.951	0.046	0.309
8	44	225.7	6.31	41.85	22.21	1.587	10.530	0.714	0.051	0.338
9	40	244.2	6.65	42.06	20.66	1.853	11.719	0.681	0.064	0.405
10	35	261.1	7.46	44.14	19.62	2.193	12.972	0.629	0.072	0.426
11	31	278.1	9.11	50.70	16.75	2.645	14.729	0.541	0.091	0.509
12	27	301.6	9.94	51.63	19.07	3.432	17.832	0.631	0.110	0.572
13	27	322.9	9.88	51.34	21.27	2.802	14.560	0.667	0.086	0.447
14	25	346.1	12.13	60.67	23.27	3.102	15.508	0.764	0.102	0.510
15	23	363.7	13.04	62.53	17.49	2.418	11.596	0.570	0.081	0.390
16	21	387.8	9.69	44.41	13.47	3.145	14.413	0.423	0.106	0.487
17	17	407.6	11.60	47.82	27.28	2.503	10.319	0.902	0.083	0.341
18	16	433.1	11.57	46.27	22.40	3.809	15.238	0.704	0.116	0.463
19	16	458.8	13.03	52.12	25.75	3.968	15.872	0.835	0.140	0.561
20	13	481.9	14.14	50.97	26.38	5.258	18.958	0.866	0.163	0.589
21	11	475.5	12.53	41.57	-2.91	6.174	20.477	-0.051	0.204	0.675
22	5	458.2	16.93	37.86	1.40	5.870	13.126	0.094	0.209	0.468
Total	703	224.1	4.82	127.90	22.13	0.505	13.369	0.726	0.016	0.410

Similar live weight for the breed has also been ascertained by Jakubec et al. (2003) in Czech Republic - 29.22 kg, and lower was reported by Kolisnyk et al. (2018) in Ukraine - 26.5 kg for the females and 29.4 kg for the males. Apart from the mother, other factors influencing the weight at birth are the herd, year and season of calving, the breed, the gender of the calf, the pregnancy duration, the age of the mother, the genetic group of the cows (Holland et al., 1992; Waheed et al., 2003; Krupa et al., 2005; Pilarczyk et al., 2011) and so on.

We ascertained that under the conditions of organic farming and natural breeding, the year ($P<0.01$) and month of birth ($P<0.05$) affect the live weight at birth but the influence of the latter is specific during the different years ($P<0.05$). The difference in the average live weight at birth between the separate years is from 6.1 to 22.7% (Table 2). The season, both as a whole and within the year, is not a reliable source of variation in the live weight at birth. Thus, in 2017, the calves born in the spring are 3.8% heavier than those born in the winter while in 2018, they are 3.9% lighter - such differences are close to the statistical mistake.

Table 2. Influence of the year and the season of calving on the live weight upon birth of female calves of the Aberdeen Angus cattle breed

Year	Season	N	LS	Sx	Sdev
2016	Summer	11	37.28	0.85	2.68
	Autumn	6	32.33	2.20	4.93
	Total	17	35.47	1.05	4.21
2017	Spring	6	30.00	3.35	7.48
	Summer	7	28.86	0.98	2.41
	Autumn	11	27.91	2.02	6.38
	Winter	6	29.67	3.17	7.09
	Total	30	28.90	1.09	5.86
2018	Spring	3	32.67	1.47	2.08
	Winter	4	34.00	3.23	5.60
	Total	7	33.43	1.71	4.20
Total	Spring	9	30.89	2.18	6.15
	Summer	18	33.94	1.18	4.87
	Autumn	17	29.47	1.54	6.14
	Winter	10	31.40	2.19	6.59
	Total	54	31.56	0.82	5.96

In 2016 and 2017 the calves born during the summer were heavier than those born in the autumn with the difference during the first year reaching 15.3%.

Forster (2010) indicates that during the suckling period the growth of the calves from the Aberdeen Angus Cattle breed is strongly connected to the mothers' milk yield, which in turn depends on the season of calving and the live weight of the cow. Law et al. (2013) have found out that cows of Aberdeen Angus Cattle breed with higher live weight at calving and higher and sustainable milk yield had calves with higher live weight at weaning. Toušová et al. (2015) point out that during their third or fourth lactation, cows have: the highest milk yield, no problematic calvings, and good maternal instinct. Furthermore, the calves' death rate after birth is low. On the other hand, Gabidulin et al. (2018) have ascertained that the Aberdeen Angus cows had the highest milk yield at the age of 5 years.

After their birth and by reaching 3 months, the female calves studied by us increase their live weight almost twice (Table 1). Taking into account the fact that with the increase of the age, the growing needs of the calves may not be met by the mother's milk only, the farm provides access to individual feeder with a starter feed as early as the first month after birth. This provides the opportunity for maintenance of intensive growth during the entire suckling period (up to the age of 7 months) with a gradually expanding daily growth. During the first three months it is within 0.700-0.750 kg, during 4-7 months is 0.830-0.950 kg per month. It increases by 33.3% from the beginning until the end of the

suckling period. Similar gain during the suckling period was reported by Kolisnyk et al. (2018) upon examination of the Aberdeen Angus cattle breed in Ukraine.

As a whole the gain increase during the suckling period is gradual, with the exception of some fluctuations, due to which the absolute monthly gain is within 20-26 kg. At 7 months old the female calves reach approximately 32% of the live weight of the adult cows from the breed, at 12-50%, at 16-67%. This may be used to forecast the age of first service and first calving of the Aberdeen Angus Cattle breed heifers bred in the conditions of our country.

The growth of the beef cattle breeds' calves during the suckling period depends on the breed, gender, the father (Hoppe et al., 2010; Jakubec et al., 2003; Toušová et al., 2015) and so on. Thus, when comparing the growth of calves from the Aberdeen and Limousin cattle breed up to 3 months old in Belarus, Loban et al. (2018) found higher average daily growth in the former (97.7 g). On the other hand, Sukhanova et al. (2018) in Russia claim that during the respective period, the Aberdeen Angus had higher growth than that of the Hereford Cattle breed with a difference of 263 g, 309 g and 225 g for the first, second and third month respectively. Szabó et al. (2006) report that the year and season of calving, the breed, the age of the mother and the gender have influence on the weight upon weaning at the age of 205 days for the beef cattle calves.

The average live weight upon weaning of the cows studied by us is 204 kg. Higher live weight, 224-255 kg, depending on the year, is reported by Efimova (2018).

In the month following the weaning, the average daily gain of the calves examined by us diminishes by approximately 25%. While studying the growth of Aberdeen Angus cattle breed calves weaned at 205 days, Hassen et al. (2004) have found out that the average daily gain has continually increased from birth to the age of 250 days, and after that it starts to decrease. The same authors have ascertained that the female calves at the age of 261 days reach an average live weight of 271 kg.

In the case of the calves examined by us, the growth after weaning changes inconsistently. In the first three months it diminishes, then in the next three months it increases, and at 14 months it reaches the level which it had after weaning.

Taking the free access to concentrated feeds into account, we can explain the growth decrease after weaning only by the weaning stress which is probably strongly affected by the ecological factors of the season. As we already mentioned, in the farm examined by us the calving, and respectively the weaning of the calves is made all year round due to which the separate individuals are subjected to the influence of different factors during the different age periods.

Table 3 clearly shows that the year and the season of birth affect the growth of the calves

Table 3. Influence of major paratype factors on the growth of female calves of the Aberdeen Angus cattle breed bred organically (F - criteria and degree of reliability)

Factor	Absolute gain	Average daily gain
Year	2.228	6.511**
Season	3.550*	3.991**
Year*Season	0.047	0.016
Year	2.049	5.469**
Month	2.12*7	2.189*
Year*Month	1.281	1.656
Age*Year	2.132**	2.288**
Age*Season	4.479***	5.511***
Age*Year*Season	0.975	0.936
Age*Year	3.089***	2.577**
Age*Month	3.785***	3.520***
Age*Year*Month	2.525***	0.759

***P<0.001; ** P<0.01; * P<0.05

and during the different age periods the season has specific (age*season) and more reliable influence than the season as a whole.
Specifically strong influence, within the age, is also exercised by the month of birth but its effect on the growth is not taken into account.

Figure 1 shows that during the first 3-4 months after birth no significant differentiation is observed regarding the live weight of the claves born in different seasons. The calves born during the autumn have negligibly higher mass.

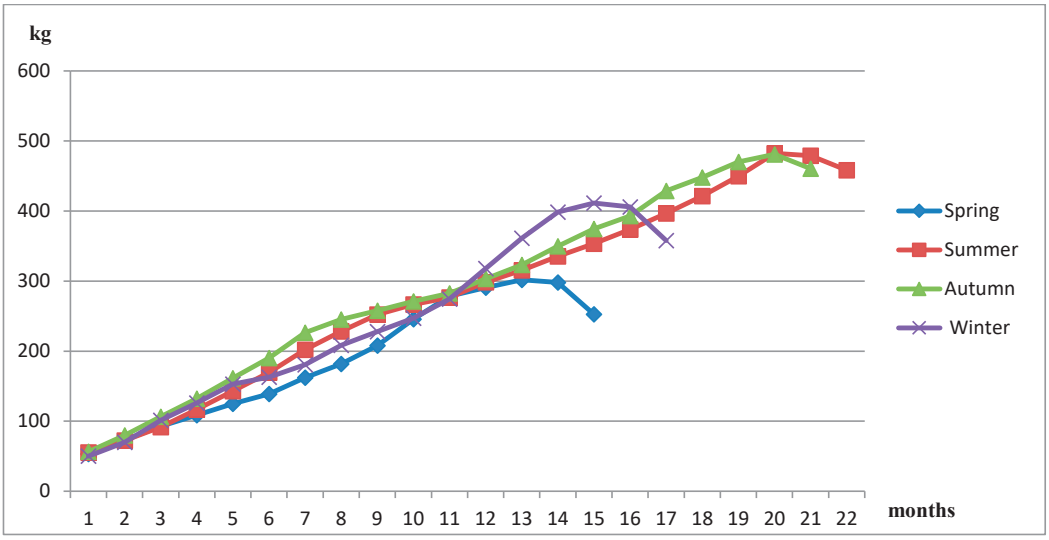

Figure 1. Influence of the season on the growth of the female calves of the Aberdeen Angus cattle breed

More considerable differences are observed after the fifth month. The calves born in the spring have the lowest weight until the tenth. This seems illogical at first sight but is not at all surprising for the conditions in Bulgaria. The calves born in the spring months (March-May) are weaned in September- November. These are the most unfavourable months in terms of feeding of the pasture animals. In this period, the natural pastures are entirely dry, and the feeding with winter feeds has begun. The figure shows that after the respective period there is a certain compensation of the growth slowdown and at one year old, the calves born in different seasons basically have the same live weight.
Until reaching the age of one year, the calves born in the autumn (September-November) show the highest growth rate. The first months of the suckling period coincide with the most unfavourable feeding period for the mothers but it is then that the needs of the calves are the fewest. The period of growth of the needs coincides with the transition to winter feed which is significantly more favourable than the autumn one. The weaning is in the spring when the pastures are at their best. The calves born in the autumn have become strong during the first year and develop better in the following months.
The cows born in the winter (December-February) indicate higher growth rate after reaching one year. The feeding of the one-year-old calves born in this period coincides with the beginning of the spring season which is favourable for the growth and development in the conditions of grassland feeding.
The calves born in the summer mark the most stable growth during the entire observation period. The weaning of these calves coincides with the beginning of the winter season during which the animals consume high-quality mixed ration which is balanced for the respective category.
After a relative stabilisation around 14 months, at 15 and 16 months the growth once again sharply decreases to approximately 0.420-0.570 kg. This period coincides with the reaching of commercial maturity and service of the heifers.

So that heifers of the Aberdeen Angus cattle breed be fit for breeding, they need to reach a minimum of 65% of the adult cows live weight so as to reach 85% of that mass upon first calving (Hall, 2006). The calves studied by us reach 65% of the live weight of the cows at exactly 15-16 months old. Obviously, regardless of the growth fluctuations, the female calves bred organically under the conditions in Bulgaria reach commercial maturity at the optimal breeding age.

CONCLUSIONS

In conclusion, it can be noted that under the conditions of a certified organic farm in Bulgaria, the female calves of the Aberdeen Angus cattle breed are born with live weight of 31.6 ± 0.81 kg, upon weaning at 210 days old they weigh 204.0 ± 5.93 kg, at 15 months - 363.7 ± 13.04 kg, and at 18 months - 433.0 ± 11.57 kg.

The average daily gain of the individuals varies greatly reaching up to 2.090 kg. The growth of the animals both as a whole and within the separate age groups is influenced by the year, season and month of birth, and the calves which were born in the autumn develop the best.

Irrespective of the growth fluctuations during the different periods, the female calves reach an optimal breeding live weight (330-360 kg) at an optimal (15-16 months) age.

REFERENCES

Berger, P. J., Cubas, A. C., Koehler, K. J., Healey, M. H. (1992). Factors affecting dystocia and early calf mortality in Angus cows and heifers. *Journal of Animal Science*, 70(6), 1775-1786.

Chávez-Fuentes, J. J., Capobianco, A., Barbušová, J., Hutňan, M. (2017). Manure from Our Agricultural Animals: A quantitative and qualitative analysis focused on biogas production. *Waste and Biomass Valorization*, 1-9, 10.1007/s12649-017-9970-5.

Efimova, L.V. (2018). Dynamics of the state of beef cattle in Krasnoyarsk region. *Scientific support of animal breeding of Siberia, Krasnoyarsk*, 112-116.

Forster, K. M., Pimentel, M. A., Moraes, J. C. F. (2010). Availability of net energy in the milk and weight performance in Hereford and Aberdeen Angus calves from birth to weaning. *Revista Brasileira de Zootecnia*, 39(11), 2545-2552.

Funston, R. N., Larson, D. M., Vonnahme, K. A. (2010). Effects of maternal nutrition on conceptus growth and offspring performance: implications for beef cattle production. *Journal of animal science*, 88(13), 205-215.

Gabidulin, V., Svetlana, A., Fanus, G. (2018). Relationship in some fertility traits of Aberdeen Angus cows and their offspring. *Bulletin of Bashkir University*, Ufa, RU, 1, 45-48.

Hall, J. (2006). Supplementation and Management Strategies to Optimize Reproductive Performance. Applied reproductive strategies in beef cattle. *Conference Proceedings* (August 30-31), 237-250.

Hassen, A. T., Wilson, D. E., Rouse, G. H., Tait Jr, R. G. (2004). Use of linear and non-linear growth curves to describe body weight changes of young Angus bulls and heifers. *Animal Industry Report*, 650(1), 28.

Holland, M. D., Odde, K. G. (1992). Factors affecting calf birth weight: a review. *Theriogenology*, 38(5), 769-798.

Hoppe, S., Brandt, H. R., König, S., Erhardt, G., Gauly, M. (2010). Temperament traits of beef calves measured under field conditions and their relationships to performance. *Journal of animal science*, 88(6), 1982-1989.

Hörning, B., Feige, M., Dollinger, J. (2008). Comparison of organic and conventional beef-suckler farms in Germany. *16 th IFOAM Organic World Congress, Modena*, Italy, June 16-20. /https://nanopdf.com/download/comparison-of-organic-and-conventional-beef_pdf

ICAR - International Committee for Animal Recording, (2016). International Agreement of Recording Practices, Copyright: ICAR p. 95-182.

Jakubec, V., Schlote, W., Říha, J., Majzlík, I. (2003). Comparison of growth traits of eight beef cattle breeds in the Czech Republic. *Arch. Tierzucht. Dummerstorf*, 46. Doi 10.5194/aab-46-143-2003.

Kamilaris, A., Assumpcio, A. A., Blasi, A. B., Prenafeta-Boldú, F. X. (2018). Assessing and mitigating the impact of livestock agriculture on the environment through geospatial and big data analysis. *Int. J. Sustainable Agricultural Management and Informatics*, 4(2), 98-120.

Kolisnyk, O. I., Prudnikov, V. G., Kryvoruch-ko Yu, I., (2018). Monitoring and evaluation of the meat diseases of the Aberdeen-Angus breed in Ukraine. *Bulletin of Poltava State Agrarian Academy*, UA, 3, 127-131.

Krupa, E., Oravcová, M., Polák, P., Huba, J., Krupová, Z. (2005). Factors affecting growth traits of beef cattle breeds raised in Slovakia. *Czech J. Anim. Sci*., 50(1), 14-21.

Law, N. L., Hickson, R. E., Lopez-Villalobos, N., Kenyon, P. R., Morris, S. T. (2013). Efficiency of beef breeding cows that vary in live weight and milking potential. *Proceedings of the New Zealand Society of Animal Production*, 73, 11-16.

Loban, R., Sidunov, S., Letkevich, V., Sidunova, M., Kozyr, A. (2018). Comparative characteristics of growth of young cattle from Limousine and Aberdeen Angus breed. *Scientific support of animal breeding of Siberia. Krasnoyarsk*, 162-167.

Nikolov, V. (2003). Livestock Production, In *Fundamentals of Organic Farming*. Plovdiv, BG: "Vasil Petrov" Publishing House, 237-386.

Nikolov, V. (2012). *Organic Animal Breeding*. Plovdiv, BG: Academic Publishers - Agrarian University, 83-101.

Nikolova, L. (2006). Organic production and its application in Aquaculture. *Animal Breeding Sciences*, 6, 65-72.

Nikolova, L. (2013). *Biological Aquaculture*. Plovdiv, BG: Academic Publishers - Agrarian University.

Nikolova, L. (2020). *Increasing the resilience of Aquaculture*. Plovdiv, BG: Academic Publishers - Agrarian University.

Pilarczyk, R., Wójcik, J., Binerowska, B., Kryskow, P., Czerniak, P. (2011). Influence of selected factors on maternal nursing performance of Aberdeen Angus calves. *Acta Scientiarum Polonorum. Zootechnica*, 10(1), 65-72.

Savić, M., Aleksić, S., Živković, D. (2013). Breeds of Choice in Organic Production System. *10th International Symposium*, 1205.

Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M., de Han, C. (2006). *Livestock's long shadow: environmental issues and option*. Rome, IT: FAO.

Sukhanova, S., Alekseeva, E., Lushnikov, N., Nazarchenko, O. (2018). Productivity of Young Aberdeen Angus and Hereford Breeds. *Advances in Engineering Research*, 151, 24-28.

Szabó, F., Nagy, L., Dákay, I., Márton, D., Török, M., Bene, S. Z. (2006). Effects of breed, age of dam, birth year, birth season and sex on weaning weight of beef calves. *Livestock science*, 103(1-2), 181-185.

Toušová, R., Ducháček, J., Stádník, L., Ptáček, M., Beran, J. (2015). The Selected Factors Influenced Growth Ability to Weaning of Aberdeen Angus Cattle. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 63(2), 457-461.

Von Keyserlingk, M.A.G., Martin, N.P., Kebreab, E., Knowlton, K.F., Grant, R.J., Stephenson, M., Sniffen, C.J., Harner, J.P., Wright, A.D., Smith, S.I. (2013). Sustainability of the US dairy industry. *Journal of Dairy Science*, 96, 5405–5425.

Waheed, A., Hyder, A. U., Khan, M. S. (2003). Genetic and phenotypic evaluation of the growth performance of Bhagnari and Droughtmaster X Bhagnari female calves in Pakistan. *Pakistan Veterinary Journal*, 23(3), 134-142.

Zhang, L., Yan, C., Guo, Q., Zhang, J., Ruiz-Menjivar, J., (2018). The impact of agricultural chemical inputs on environment: global evidence from informetrics analysis and visualization. *International Journal of Low-Carbon Technologies*, 13(4), 338–352.

Zhou, Z. (2018). Current status and development trend of organic agriculture in the world and Asia. Development of organic agriculture in Central Asia. *Proc. International Conference,* Uzbekistan, FAO, p. 334.

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INFLUENCE OF THE BREED AND AGE ON HEMATOLOGICAL AND BIOCHEMICAL INDICATORS OF MARES FROM PUREBRED ARABIAN AND EASTBULGARIAN BREEDS

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Abstract

In the mares of purebred Arabian horse (n-8) and East Bulgarian horse (n-12) breeds, raised in the State stud farm `Kabiuk` near Shumen, Bulgaria, were studied basic hematological and biochemical blood parameters in the beginning of spring (March). It was established that the average levels for the mares from both breeds are as follows: alanine aminotransferase ALT U/L - 73.00±14.47, lactate dehydrogenase LDH U/L - 1523.88±93.11, creatinine Creat. U/L - 284.56±53.08, calcium Ca U/L - 2.06±0.4, total bilirubin TBL U/L - 78.40±12.45, phosphorus P U/L - 6.63±0.51, white blood cells WBC - 8.87±0.71, lymphocyte count Lym - 32.94±2.48, monocytes Mon-3.98±0.30, granulocytes Gra - 61.45±2.74, red blood cells RBC - 8.01±0.14, erythrocyte volume MCV - 67.07±28.42, hematocrit Hct - 35.12±1.54, hemoglobin MCH - 12.34±0.16, erythrocyte distribution width RDW-16.90±1.42, hemoglobin Hb-11.16±0.67, platelets THR -255.17±40.66, platelet volume MPV -7.81±0.19, platelet distribution width PDW-9.22±0.30, hemoglobin in erythrocytes MCHC - 27.29±0.29. The breed is a reliable ($p<0.01$) source of variation of LDH, with higher values found in the East Bulgarian breed (EBB). The mares of different ages can differ significantly ($p<0.05$) in the content of Mon in the blood. The age group in the breed had a significant effect ($p<0.05$) on Ca and PDW, with the oldest mares from EBB (10-12 years old) had decreasing amount of Ca and increasing amount of POW.

Key words*: Arabian mares, biochemistry, blood indicators, hematology, sport horses.*

INTRODUCTION

The East Bulgarian Horse is a half-blooded breed created by complex reproductive crossing of native, Arab, Anglo-Arab and half-blooded English mares with half-blooded and Thoroughbred stallions (Sabeva et al., 2010).
It is suitable for the classic disciplines of equestrian sports - dressage, show jumping, eventing and amateur riding (Sabeva et al., 2018; Sabeva and Popova, 2019). The breed was recognized in 1951.
The diverse use of this breed implies different intensities of exercise and it is important to have an effective health-program. This requires a detailed study of its biological parameters and their dynamics over time.
Hematological and biochemical parameters in horses are mainly used in clinical diagnostics and therapeutic monitoring, but also as an indicator of nutritional value, assessment of animal metabolic states, etc. (Al-Bulushi et al., 2017; Shawaf, 2017; Shawaf et al., 2018). The composition of the blood is sufficiently labile and reflects the mechanisms of adaptation of the body to changing environmental conditions (Meyer and Harvey, 2004; Hristev and Zapryanova, 2017).
Of particular interest are the correlation between haematological indices and horse performance. Thus, lightweight breeds have higher erythrocytes (RBC), hemoglobin (Hb), and hematocrit (Hct) than heavy horses (Kramer, 2000; Grondin and Dewitt, 2010).
Blood factors are influenced by many factors related to the physiological status of horses as season, diet, reproductive status, geographical location, and more. (Burlikowska et al., 2015; Aiello and Moses, 2016; Aros et al., 2017; Padiha et al., 2017).
Many authors have examined the effect of age on biochemical and hematological parameters (Cebulj-Kadunc et al. 2002; Gurgoze and Icen,

2010; Shawaf et al., 2018; Nidl et al., 2017; Ono et al., 2019, etc.).

Hematologic and biochemical parameters are breed specific (Burlikowska et al., 2015; Cruz et al., 2017; Shawaf et al., 2018) and the use of inappropriate reference values increases the risk of incorrect conclusions and inappropriate actions (Tsanq et al., 1998).

The purpose of this study is to determine the parameters of major haematological and blood biochemical parameters in mares of the East Bulgarian breed and Purebred Arabian horse, under the same breeding conditions, as well as the influence of the breed and age on them.

MATERIALS AND METHODS

The study was conducted in the Kabiyuk National Park, Shumen, with mares from East Bulgarian (EBB) and Purebred Arabian (PAB) breeds. The animals were fed a ration including haymaking and rolled concentrated fodder (a mixture of triticale, corn and black oats). The haymaking is with dry matter - 33.23%, which includes - crude protein - 32.17%, crude cellulose - 27.57%, crude fat - 4.58%, BEV - 28.3% and crude ash - 7.38%. One kilogram of hay contains Ca - 5.45 g, Mg - 1.37 g, Cu - 6.84 mg, Fe - 0.36 g, Zn - 23.19 mg, Mn - 52.91 mg. The dry matter in the concentrated feed is 91.66%, in which the crude protein is 12.27%, the crude cellulose - 11.16%, the crude fat - 4.66%, BEV - 69.35%, crude ash - 2.26%. The content of Ca in one kilogram of concentrated feed is 61.85 mg, of Mg - <1.00 mg, Cu - 2.89 mg, Fe - 2.24 mg, Zn - 1.40 mg, Mn - 0.20 mg.

In March, at the beginning of the spring season, blood samples were taken from 12 mares from EBB and 8 mares from PAB. The blood was taken from the jugular vein between 6-8 in the morning before a meal, with the animal having constant access to water. Hematological parameters were examined with a MS4 + apparatus (Switzerland), biochemical analyses spectrophotometrically on a Mindray BA88 A using the following parameters and reagents: ALT-Tris buffer, 100 mM; pH 7.5, L Alanine 500 mM, 2 Oxoglutarate 15 mM, NADH 0.18 mM; LDH ≥ 1700 U/L and a wavelength of 340 nm; Ca-CPC 0.14 mM, 8-quinolinol 25 mM, HCl pH 1.20 and a wavelength of 575 nm; Creatinine-picnic acid 0.14 mM, NaOH 0.18M, Na tetraborate 10 mM and a wavelength of 510 nm; LDH-phosphate buffer 50 mM, pH 7.5, sodium pyrivate 0.60 mM, NADH 0.18 mM, wavelength 340 nm; P-ammonium molybdate 0.4 mmol/L, sulfuric acid 0.21 mol/L, wavelength 340 nm; total bilirubin-hydrochloric acid 0.1M, 3,5-dichlorphenyldiazonium salt 2 mM, wavelength 510 nm.

Data processing was done with the software product SPSS 21. A mixed linear model of the following statistical type was used:

$Yijk = \mu + BRi + AGj + BAij + eijk,$

where: Yijk - observation vector; μ - total mean constant; BR and AG have fixed effects of the breed (i = 2) and age group, respectively (k = 3: A - from 4-6 years; B - from 7-9 years; C - from 10-12 g .in.); BAij is a random effect of the age group in the breed; eijk … - residual variant.

RESULTS AND DISCUSSIONS

The studies show that, despite identical feeding and rearing conditions, the blood indices of the breeds studied differ to a lesser or greater extent (Table 1). In PAB, alanine aminotransferase (ALT) values, lymphocyte count (Lym), hematocrit (Hct), erythrocyte distribution width (RDW), hemoglobin (Hb), platelets (THR), relative thrombocyte ratio are higher. volume (PDW) and the concentration of hemoglobin in a number of red blood cells (MCHC). All other studied indicators are higher in mares from EBB. In both breeds, ALT, lactate dehydrogenase (LDH), creatinine (Creat), phosphorus (P), total bilirubin (TBL) indicators were elevated, while mean hemoglobin (MCH), mean hemoglobin in erythrocytes (MCHC) and calcium (Ca) are below normal.

The most significant and reliable difference is the activity of lactate dehydrogenase (LDH) (Table 2). The enzyme is responsible for the anaerobic conversion of pyruvate to lactate. Studying the haematological and biochemical parameters in 20 adult sport horses Burlikowska et al. (2015) report significantly lower than the values obtained by us 335.60 ± 24.80 U * L^{-1}. Similar to the authors' cited levels, LDH levels have been reported by

Gurgoze and Icen (2010). They examined 90 purebred Arab mares, ages 6-12 and 14-20, in Turkey, and obtained enzyme values of 236.66 ± 14.18 U/L and 416.77 ± 122.58 U/L for the respective age group.

Table 1. Hematological and biochemical parameters in both breed mares (Mean ± SE)

Parameters	Mean ± SE	Breed	
		PAB	EBB
LDH, U/L	1,524±93.11	1,090±130.8	1,813±128.4
ALT, U/L	73.00±14.47	75.63±20.33	71.25±19.95
Creat., µmol/L	284.6±53.08	262.1±74.56	299.5±73.17
Ca, mmol/L	2.06±0.41	1.27±0.58	2.59±0.57
TBL, µmol/L	78.40±12.45	61.05±17.49	89.97±17.17
P, mmol/L	6.63±0.51	6.15±0.72	6.95±0.71
WBC, 1000/ µL	8.87±0.71	8.21±1.00	9.31±0.98
Lym, %	32.94±2.48	34.69±3.48	31.78±3.41
Mon, %	3.98±0.30	3.81±0.42	4.09±0.42
Gra, %	61.45±2.74	60.83±3.85	61.87±3.78
RBC, m/mm^3	8.01±0.14	8.34±0.20	7.79±0.20
MCV, fl	67.07±28.42	44.43±39.92	82.17±39.18
Hct, %	35.12±1.54	36.85±2.16	33.96±2.12
MCH, pg	12.34±0.16	12.23±0.22	12.42±0.22
RDW, %	16.90±1.42	19.30±1.99	15.30±1.96
Hb, g/L	11.16±0.67	12.24±0.94	10.45±0.93
THR, m/mm^3	255.2±40.66	318.4±57.12	213.0±56.05
MPV, fl	7.81±0.19	7.89±0.27	7.76±0.26
PDW, %	9.22±0.30	9.84±0.42	8.81±0.41
MCHC, g/dL	27.29±0.29	27.68±0.41	27.03±0.40

In 39 Noma horses, Ono et al. (2019) have established values for LDH 488 ± 270 U/L (from 217-758 U/L), indicating that values are higher than those of the Kisso breed (431 ± 161 U/L) and lower than Japanese race horses - 550 ± 134 U/L. According to the authors, the differences are due to the different diet. Our LDH values are similar to those reported by Aros et al. (2017) - 807 ± 515 IU/L (353 to 1,746 IU/L) and from Winnicka (2011) - 520-1,480 U * L^{-1}.

Alanine aminotransferase (ALT) is an enzyme released by the cytoplasm from hepatocytes when they are destroyed and it is considered to be very specific for the liver. According to Mircheva (2006), this indicator is rarely an indicator of clinical certainty, unless it exceeds at least twice the upper limit of the reference values, which in horses are 3-25 U/L. The values obtained from the mares of both breeds are twice above the reference value specified by the author. Probably the reason is the significant breeding differences or temporary discomfort of the animals at the end of the winter. The latter is probably more likely, given the values of the indicator reported by other authors: Ju et al. (1993) - for 46 mares in Taiwan - 6.49 ± 3.14 µ/L; Burlikowska et al (2015) - 8.80 ± 0.61 IU * L^{-1} for competition horses. Gurgoze et al. (2010) examined the effect of age on blood counts of Purebred Arabian horses and reported slightly elevated ALT values in horses between 6-13 months of age - 31.66 ± 10.44 U/L. With age, the values of the indicator decreased, with mares at 6-12 years of age and 14-20 years of age ALT being 7.66 ± 2.96 U/L and 4.22 ± 0.82 U/L, respectively.

Table 2. Influence of the breed, age group, group in the breed on the hematological and biochemical parameters of EBB and PAB mares, F-criterion and degree of reliability

Parameters/factors	Breed	Age group	Age group in the breed
df	1	2	1
LDH, U/L	13.446**	0.192	1.911
ALT, U/L	0.211	0.975	0.569
Creat., µmol/L	0.050	0.040	2.427
Ca, mmol/L	1.462	2.606	4.042*
TBL, µmol/L	2.651	1.020	0.012
P, mmol/L	1.799	2.814	2.714
WBC, 1000/µL	2.369	1.949	0.040
Lym, %	1.185	0.895	0.226
Mon, %	0.218	3.120*	0.059
Gra, %	0.345	0.645	0.027
RBC, m/mm^3	1.683	1.170	1.724
MCV, fl	0.803	0.677	0.710
Hct, %	0.563	1.682	2.110
MCH, pg	1.669	1.513	0.005
RDW, %	3.087	0.726	0.349
Hb, g/L	2.075	0.549	0.001
THR, m/mm^3	1.130	0.287	0.518
MPV, fl	0.237	0.320	1.645
PDW, %	0.565	1.825	7.947*
MCHC, g/dL	0.924	1.228	0.213

Creatinine is a product of the breakdown of creatine phosphate in muscle tissue. It is exclusively excreted by glomerular filtration through the kidney. The values of this indicator reported in the literature vary widely. In the studies of Meyer and Harvey (2004), Boediker (1991) and Gurgoze and Cetin (2004), Creat is in the range of 88.4-167.96 µmol/L. Gurgoze and Icen (2010), in Purebred Arabian mares, found an increase in values of the indicator with increasing of the age - 66.30 ± 9.72 µmol/L in mares 14-20 years, 86.63 ± 5.30 µmol/L at 6-12 years and 97.24 ± 17.68 µmol/L for mares over 20 g. In the mares we studied, creatinine was elevated in both breeds - 262.1 ± 74.56 µmol/L at PAB and 299.5 ± 73.17 µmol/L at EBB, and according to Kaneko et al, (1997), creatinine was strongly influenced by diet and the muscle mass of an animal

The levels of Hb and Hct found by us are similar to those of other authors: - Ono et al. (2019) for the Noma breed - 12.8 ± 2.6 g/L and 35.5 ± 7.1%, respectively for Hb and Hct, for the Kisso breed - 11.6 ± 1.5 g/L and 32.9 ± 4.0%, and for Japanese race horses - 16.1 ± 1.4 g/L and 42.2 ± 4%; Aros et al. (2017) for local working horses in Chile - 120 ± 16.2 g/L and 33.6 ± 5.1%; Qadri et al. (2018) have found Hb values of 12.8 ± 0.68 g/L in mountain horses in Kashmir.

Mircheva (2006) indicates that total serum bilirubin in horses and ponies varies between 1 and 2 mg/dL (17-34 µM/L) and is dependent on the latest food intake. In the mares we studied, bilirubin was 61.05 ± 17.49 µM/L for PAB and 89.97 ± 17.17 µM/L for EBB.

The calcium in both breeds has significantly lower than those reported by other authors - 1.27 ± 0.58 mmol/L for PAB and 2.59 ± 0.57 mmol/L for EBB. Thus, Padilha et al. (2017) reported 13.22 ± 0.59 mg/dL in the Brazilian Sport Horse breed; Aros et al. (2017) report 4.5 ± 3.4 mmol/L in local horses in Chile. Among the main causes of hypocalcemia are insufficient Ca content in feed, low levels of magnesium in the blood, which reduces the activity of parathyroid hormones, etc. (Arnbjerg, 1980; Ramiro, 2011; Raimundo et al., 2017).

In contrast to the Ca, amount of P, exceed the limit of reference values, reported by (Mircheva, 2006). This disturbs the balance between Ca and P. Blood calcium levels can be adjusted for nutritional supplements. It is advisable to intake supplements with vitamin D and magnesium.

In experiment, made by Popov et al. (1974), who tested the effect of compound feed on the feeding of 18 Thoroughbred mares during pregnancy and lactation, found that the

granulated compound feed enriched with trace elements and vitamins that fed the mares from the experimental group had a beneficial effect on them both during pregnancy and on the growth and development of foals.

Table 3. Hematological and biochemical parameters of the mares by age group in the breed (Mean ± SD)

Breed	PAB		EBB		
Age group	A (4-6 y)	C (10-12 y)	A(4-6 y)	B(7-9 y)	C(10-12 y)
LDH, U/L	81.25±28.75	70.00±28.75	71.75±28.75	33.00±33.19	109.0±40.65
ALT, U/L	1,275±185.0	904.8±185.0	1,748±185.0	1,743±213.6	1,949±261.6
Creat., µmol/L	182.9±105.5	341.4±105.5	393.0±105.5	321.4±121.8	184.1±149.1
Ca, mmol/L	1.39±0.82	1.14±0.82	4.35±0.82	3.00±0.95	0.40±1.16
TBL, µmol/L	53.48±24.74	68.63±24.74	95.48±24.74	57.73±28.57	116.7±34.99
P, mmol/L	5.92±1.02	6.38±1.02	5.57±1.02	5.48±1.18	9.80±1.45
WBC, 1000/µL	7.96±1.42	8.47±1.42	10.71±1.42	6.63±1.63	10.59±2.00
Lym, %	37.40±4.92	31.98±4.92	28.80±4.92	37.93±5.68	28.60±6.96
Mon, %	3.53±0.60	4.10±0.60	3.05±0.60	5.27±0.69	3.95±0.85
Gra, %	59.08±5.45	62.58±5.45	61.65±5.45	56.80±6.29	67.15±7.70
RBC, m/mm^3	8.34±0.28	8.33±0.28	7.52±0.28	7.51±0.33	8.33±0.40
MCV, fl	43.58±56.46	45.28±56.46	153.4±56.46	44.50±65.19	48.65±79.84
Hct, %	36.20±3.06	37.50±3.06	28.68±3.06	33.30±3.53	39.90±4.32
MCH, pg	12.13±0.31	12.33±0.31	12.60±0.31	11.90±0.36	12.75±0.44
RDW, %	18.00±2.82	20.60±2.82	14.33±2.82	18.37±3.26	13.20±3.99
Hb, g/L	11.63±1.34	12.85±1.34	9.53±1.34	11.17±1.54	10.65±1.89
THR, m/mm^3	381.3±80.78	255.5±80.78	220.3±80.78	194.3±93.27	224.5±114.2
MPV, fl	8.33±0.38	7.46±0.38	7.58±0.38	7.90±0.44	7.80±0.54
PDW, %	10.50±0.59	9.18±0.59	8.13±0.59	7.77±0.69	10.55±0.84
MCHC, g/dL	28.03±0.58	27.33±0.58	27.70±0.58	27.00±0.67	26.40±0.82

Erythrocyte counts, such as mean erythrocyte volume (MCV), MCH (mean hemoglobin concentration in erythrocytes), MCHC (mean hemoglobin concentration in erythrocytes), show the efficiency of hemoglobin synthesis and its oxygen transport capacity. The results we have obtained for this parameters correspond to those reported by other authors. Burlikowska et al. (2015) found that for mares used for jumping, the MCV was 44.17 ± 1.05 fl, MCH - 16.75 ± 0.43 pg, MCHC - 37.88 ± 0.20 g * dl^{-1}; Shawaf et al. (2018) cite MCV 45 ± 1.8fl, MCH 9.1 ± 1.12 pg and MCHC 22.28 ± 0.20 g/dL for Scottish ponies in Saudi Arabia etc.

A number of studies have reported reductions in some blood parameters in older animals in different animal species (Nakai et al., 1992; Atanasova et al., 2008; Valcehv et al., 2009; Markova et al., 2018). Table 2 and Table 3 clearly shows that the age group in the breed has a significant effect on Ca levels. With advancement of age in both breeds the Ca content in the blood decreases as in the PAB reaches up to 1.14 ± 0.82 mmol/L and at EBB - 0.40 ± 1.16 mmol/L. Gurgoze and Icen (2010) reported that levels of Ca (2.80 ± 0.07 mmol/L) and P (0.84 ± 0.05 mmol/L) in PAB mares with advancing age (14-20 years) dramatically decrease as the cause is probably due to a decrease in bone metabolism. Young animals have been shown to absorb calcium from the food more efficiently and have much higher values than adult animals (Braithwaite, 1975).

With advancing of the age, after 10 years, TBL levels increased (by 22.07% for PAB and 50.53% for EBB), P (by 7.21% for PAB and 43.16% for EBB) and HcT (by 3.47% for PAB) and by 28.12% for EBB). The differences, although significant, are unreliable, with probable cause being the small number of animals and the wide variation in signs. The levels of LDH (69.72%) and ALT (10.31%) were also increasing in EBB mares after 10

years of age, and Creat (50.41%) and MCV (3.69%) levels increased in PAB mares. In the animals of the highest age group, MCV values were 68.29% lower than those of the youngest. Trending decreases in LDH and ALT values have been observed in PAB mares with advancing of the age.

CONCLUSIONS

It was found that in mares from the East Bulgarian and Purebred Arabian horse breeds, at the end of the winter period, when feeding with winter ration, the mean ALT level was 73.00 ± 14.47 U/L, of LDH - 1,523.88 ± 93.11 U/L, Creat. - 284.56 ± 53.08 U/L, Ca - 2.06 ± 0.4 U/L, TBL - 78.40 ± 12.45 U/L, P - 6.63 ± 0.51 U/L. WBC is 8.87 ± 0.71 μL, Lym is 32.94 ± 2.48%, Mon - 3.98 ± 0.30%, Gra - 61.45 ± 2.74%, RBC - 8.01 ± 0.14 m/mm^3, MCV - 67.07 ± 28.42 fl, Hct - 35.12 ± 1.54%, MCH - 12.34 ± 0.16 pg, RDW - 16.90 ± 1.42%, Hb - 11.16 ± 0.67 g/L, THR - 255.17 ± 40.66 m/mm^3, MPV - 7.81 ± 0.19 fl, PDW - 9.22 ± 0.30%, MCHC - 27.29 ± 0.29 g/dL.
The breed is a reliable ($p<0.01$) source of LDH variation, with higher values reported in the Eastbulgarian breed. The mares at different ages differed significantly ($p<0.05$) in blood content of Mon. The age group in the breed had a significant effect ($p<0.05$) on Ca and PDW, with the oldest EBB mares (10-12 years old) reducing the amount of Ca and increasing that of PDW.

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REFERENCES

Aiello, S., Moses, M. (2016). *The Merck Veterinary Manual*, 11th Edition, Wiley Publishing House, p. 3325.

Al-Bulushi, S., Shawaf, T., Al-Hasani, A. (2017). Some hematological and biochemical parameters of different goat breeds in Sultanate of Oman "A preliminary study". *Vet World*, 10, 461–466.

Arnbjerg, J. (1980). Hypocalcemia in the horse. A case report. *Nord Vet Med*., 32(5), 207-11.

Aros, K., Carrasco, J.,Briones, R., Tadich, A. (2017). Haematological and serum biochemical reference values for urban-working equines in Chile. *Austral journal of veterinary sciences*, 49(1), 27-33.

Atanasova, R., Hadjinikolova, L., Nikolova, L. (2008). Investigations on the biochemical composition of carp fish (Cyprinidae) blood serum at conditions of organic aquaculture. *Bulg. J. Agric. Sci*., 14, 117-120.

Braithwaite, G.D., (1975). Studies on the absorption and retention of calcium and phosphorus by young and mature Ca-deficient sheep. *Br J Nutr*, 34, 311-324.

Boediker, R. (1991). Die bestimmung der gamma-glutamyl-transferase (GGT) im serum alsindikator fur die kolostralmilch-versorgung des kalbes. *Tieraerztl.Umsch*., 46, 190–194.

Burlikowska, K., Bogusławska-Tryk, M., Szymeczko, R., Piotrowska, A. (2015). Haematological and biochemical blood parameters in horses used for sport and recreation. *JCEA*, 16(4), 370-382.

Cebulj-Kadunc, N., Bozic, M., Kosec, M., Cestnik, V. (2002). The influence of age and gender on haematological parameters in Lipizzan horses. *J Vet Med.*, 49, 217–221.

Cruz, A.M., Maninchedda, U.E., Burger, D., Wanda, S., Vidondo, B., (2017). Repeatability of gait pattern variables measured by use of extremity-mounted inertial measurement units in nonlame horses during trotting. *Am J Vet Res*, 78, 1011–1018.

Grondin, T.M., Dewitt, S.F. (2010). *Normal hematology of the horse and donkey*. Schalm´s Veterinary Hematology. Weiss, D.J. & Wardrop Publishing House, pp. 821-828.

Gurgoze, S., Icen, H. (2010). The Influence of Age on Clinical Biochemical Parameters in Purebred Arabian Mares. *Journal of Equine Veterinary Science*, 30(10), 569-574

Hristev, H., Zapryanova, I. (2017). Study of some morphological biochemical and immunological indicators of hybrid boars. *Journal of Mountain Agriculture on the Balkans*, 20(6), 96-103.

Ju, J.C., Cheng, S. P.,Fan, Y. K.,Hsu, J. C., Chiang, S. K., Chen, E. V., Chang, S. H., Chiou, S. C. (1993). Investigation of Equine hematological constituents in Central Taiwan. I. Distribution of the blood cell parameters and the biochemical compositions of serum. *AJAS*, 6(1), 147-153.

Kramer, J.W. (2000). *Normal hematology of the horse*. In: Shalm's Veterinary Hematology. Feldman, B.F., Zinkl, J.G. & Jain Publishing House, N.C. (eds.), pp. 1069-1074.

Markova, V., Zapryanova, I., Ivanova, R. (2018). Study on some biochemical indicators of the blood serum In terminal boars (preliminary data). *Agricultural University – Plovdiv, Scientific Works*, LXI(1), 65-72.

Meyer, D.J., Harvey, J.W. (2004). *Veterinary laboratory medicine: interpretation and diagnosis*, 3rd ed. St. Louis, MO: Saunders Publishing House.

Mircheva, T. (2006). *Basics of clinical biochemistry in domestic animals*. Enyovche Publishing House, 94 p. (BG).

Nakai, N., Nawa, K., Maekawa, M., Nagasawa, H. (1992). Age-related changes in hematological and serum biochemical values in cats. *Jikken Dobutso*, 41, 287–294.

Nidl, C., Merlino, J.O., Lopez, E.A., Monreal, A.E., Aquirre, G., Rangel, J.A., Venegas, C. (2017). Effect of Age, Gender, and Season on Hematological Parameters in Quarter Horses. *Journal of Veterinary Science and Medical Diagnosis*, 6:2, 1-4.

Ono, T., Yamada, Y., Hata, A. (2019). Reference values of hematological and blood biochemical parameters for the Noma horse. *Journal of Equine Science*, 30(3), 69-73.

Padilha, F. G. F., Dimache, L. A. G., Almeida, F. Q., Ferreira, A. M. R. (2017). Blood biochemical parameters of Brazilian sport horses under training in tropical climate. *Revista Brasileira de Zootecnia,* 46(8), 678-682.

Popov, V., Yorov, I., Dzhurkov, D., Zhelyazkov, S. (1974). Testing the effect of compound feeding stuffs on purebred Arabian mares feeding during pregnancy and lactation. *Animal Sciences*, XI(8), 135-138.

Qadri, S.I.A., Ganai, I., Dar, P., Yousuf, R., Kawa, M., Habib, R. (2018). Blood Parameters of Horses Maintained Over Pasture under Temperate Climatic Conditions in Kashmir. *International Journal of Current Microbiology and Applied Sciences*, 7(7), 282-282.

Raimundo, A. Barrêto-Júnior, A., Minervino, H., Federico, Rodrigues, A., Enoch, B., Meira, J., Lima, I., Sousa, A., Mori, R., Carolina, C., Araújo, A., Wilson, R.F., Ortolani, E. (2017). Clinical presentation and biochemical profile of horses during induction and treatment of hypocalcemia. *Austral J Vet Sci*., 1, 9-14.

Ramiro, E.T. (2011). Disorders of Calcium and Phosphate Metabolism in Horses. *Veterinary Clinics of North America: Equine Practice*, 27(1), 129-147.

Sabeva, I., Popova, M. (2019). Monitoring of genealogical structure development of East Bulgarian breed horses originating from stallions of other sporting breeds. *Agricultural Science and Technology*, 11 (3), 195-197.

Sabeva, I., Popova, M., Kastchiev, S. (2018). Sources of specific variance and heritability of free jump qualities in 2-years old horses from the East Bulgarian breed. *Bulg. J. Agric. Sci*., 24 (2), 85-89.

Shawaf, T. (2017). Some haematological and serum biochemical parameters in apparently clinically healthy Hassawi donkey. *Alex J Vet Sci*, 53, 125-30.

Shawaf, T., Hussen, J., Al-Zoubi, M., Hamaash, H., Al-Busadah, K. (2018). Impact of season, age and gender on some clinical, haematological and serum parameters in Shetland ponies in east province, Saudi Arabia. *Int. J. Vet. Sci. Med*., 6, 61–64.

Tsang, C.W., Lazarus, R., Smith, W., Mitchell, P., Koutts, J. (1998). Hematological indices in an older population simple: derivation of healthy reference values. *Clin.Chem*., 44, 96-101.

Valchev, G., Popova-Ralcheva, S., Bonovska, M., Zaprianova, I., Gudev, D. (2009). Effect of dietary supplements of herb extracts on performance in growing pigs. *Biotechnology in Animal Husbandry*, 25 (5-6), 859-870.

Winnicka, A. (2011). *Reference values of basic laboratory tests in veterinary medicine*. Warszawa, PL: SGGW Publishing House, 5th ed., 136 pp.

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RESEARCH OF THE INFLUENCE OF ANTIOXIDANTS ON THE RAMS SPERMOGRAM

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Abstract

The study was conducted for the first time on rams of the Karakul Moldavian breed. As an antioxidant, two versions of the ZooBior preparation were used, which contains: BioR, spirulina, zinc and selenium. The preparation was given to rams in teenium for 50 days at the rate of 5 g/head/day. Studies have shown that the ZooBior drug increased the volume of ejaculate by 0.4 ml in the second experimental group, the motility by 11%, the concentration of sperm in the sperm by 0.1 billion/ml and the total number of sperm in the ejaculate with rectilinear movements. With 6.5%, the data are reliable compared with the indicators found at the beginning of the experiment. There have also been some fluctuations in sperm kinetics. Semen advancement rate increased in the experimental groups, but the data are not significant.

Key words*: antioxidants, ejaculated, mobility, ram, sperm.*

INTRODUCTION

Fertility is a process of primary necessity and is the most important in the breeding of animals.Low fertility in animal husbandry is considered a problem with the quality of sperm material obtained from males of zootechnical interest. There are several reasons for low fertility, including the influence of nutritional factors that adversely affect the reproductive success of animals (Sural, 1997). Research by Sural (1997) in the field of optimization of ratios in parallel with their supplement with biologically active drugs, revealed their availability and increased biological activity. Their influence was recorded in reproductive biotechnology and, especially, on the processes of spermatogenesis and improvement of the vital properties of spermatozoa (Sora, 2004; 2008;). At the same time, an imbalance between pro-concentration and antioxidants is known to negatively affect sperm quality (Sikka, 1995). Maintaining the intensity of spermatogenesis as a physiological process, encompassing all the transformations through which spermatozoa pass, is one of the priorities of biotechnology and is achieved through targeted training and regulation of physiological status in changing environmental conditions. (Furdui et al., 2002)

MATERIALS AND METHODS

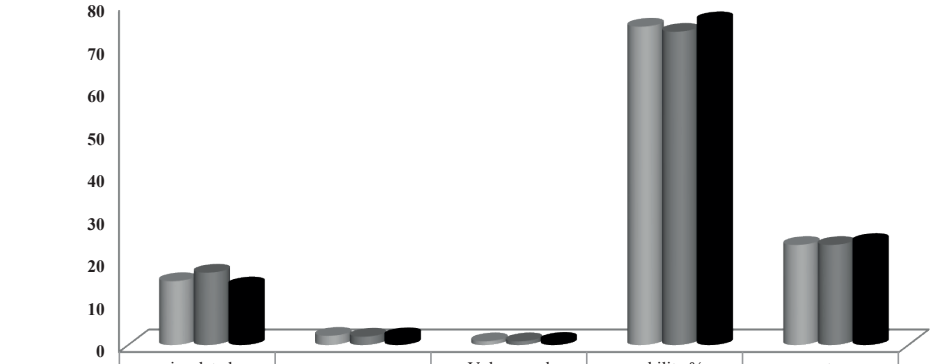
The study was conducted during the breeding season. The purpose of these studies was to determine, and then in a statistical interpretation of the data regarding the sperm material of rams of the Moldavian Karakul breed. The biological material used was a herd of 5 Karakul Moldavian sheep aged three and four years. Harvesting techniques included the preparation of an artificial vagina followed by harvesting. After harvesting, we analyzed the quantitative and qualitative parameters of sperm. A macroscopic analysis of the appearance and volume of sperm was accompanied by a microscopic analysis of sperm motility / ejaculate concentration and sperm velocity (VAP, VSL, VCL) using the CEROS computer method. During the breeding period of rams from the experimental group for 50 days, they were given 5 g per head/day of ZooBior preparation containing BioR spirulina selenium and zinc. Spermogram indices were monitored at the beginning and at the end of the experiment. Statistical analysis of experimental

data was carried out using parametric criteria according to student.

RESULTS AND DISCUSSIONS

The method for stimulating spermatogenesis in rams was based on the use of BDM¹-1plus and BDM²-2plus preparations synthesized from the cyanobacterial biomass Spirulina platensis.

The drugs were administered in the daily diet of sheep in the amount of 5 g per head per day for 50 days. Spermogram indices for rams during the experimental period are shown in Figure 1.

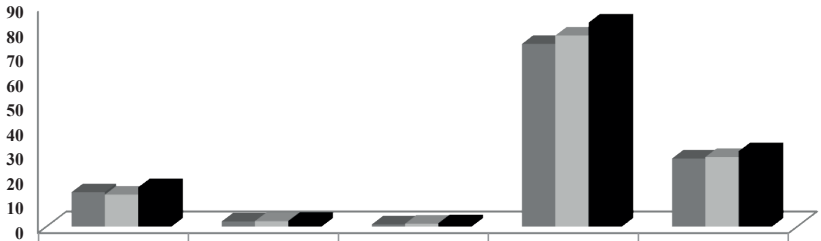


	ejaculated	sperm concentration, mlrd/ml	Volume, ml	mobility,%	spermatozoa with rectilinear movements,%
control group	15	2,19	0,9	74,7	23,5
BDM¹-1plus	17	1,98	0,9	73,5	23,5
BDM¹-2plus	14	2,09	1	76,2	24,2

Figure 1. Spermogram indices in rams at the beginning of the experiment

The data shown in Figure 1 shows that the ejaculate volume averaged 0.9 ml in the control group, 0.9 ml in the first experimental group and 1.0 ml in the second experimental group. The sperm concentration was 2.19 billion/ml in the control group, 1.98 billion/ml in the first experimental group and 2.09 billion/ml in the second experimental group. Mobility had control fluctuations of 74.7% in the control group, 73.5% in the first experimental group and 76.2% in the second experimental group. The proportion of sperm with rectilinear movements ranged from 23.5% to 24.2%. The experimental data obtained between the experimental groups and the statistical control group are insignificant.

Experimental data on the spermogram in sheep after administration of the drug ZooBior 1, in which includes BioR, zinc and selenium, administered to rams in the first experimental group of 5 g/day, and ZooBior 2, consisting of BioR, spirulina, zinc and selenium extract, administered to rams in the second experimental group, 5 g/capita/day is shown in Figure 2.



	ejaculated	sperm concentration, mlrd/ml	Volume, ml	mobility,%	spermatozoa with rectilinear movements,%
control group	14	2,1	0,9	74,3	27,7
BDM¹-1plus	13	2,23	1,2	77,7	28,3
BDM¹-2plus	16	2,3	1,4	83	30,7

Figure 2. Spermogram in rams at the end of the experiment

The experimental data shown in Figure 2 confirm the positive effect of BDM¹-1 plus and BDM¹-2 plus on spermatogenesis in sheep. Thus, the concentration of sperm in the ejaculate increased in experimental group 2 to 2.3 ± 0.02 billion/ml during the growing season, and in the control group to 2.1 ± 0.04 bilion/ml. The difference was statistically significant. The ejaculate volume statistically significantly increased in both experimental groups, amounting to 1.2 ± 0.04 ml and 1.4 ± 0.1 ml, respectively. Mobility was statistically significantly increased in experimental group 2, amounting to 83.0 ± 1.5% at the end of the experiment Computerized semen analysis CEROS is a modern and much more effective method than traditional sperm analysis methods with a large storage space, which are used in measurements to determine sperm quality. Sperm advancement rate data (VAP, VSL, VCL) are shown in Figures 3 and 4.

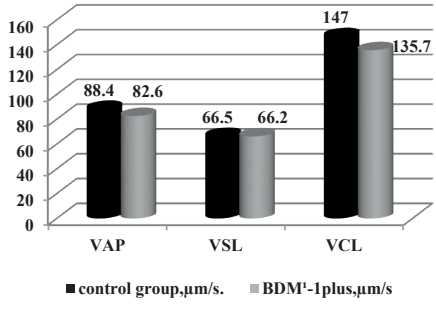


Figure 3. Analysis of VAP, VSL and VCL indices in the first experimental group at the beginning of the experiment

The data presented show that at the beginning of the experiment, in the first experimental group, statistically significant differences between VAP, VSL and VCL were not recorded. Data analysis shows that statistically significant differences in VAP, VSL and VCL have not been recorded, which shows that the rams studied were correctly selected.

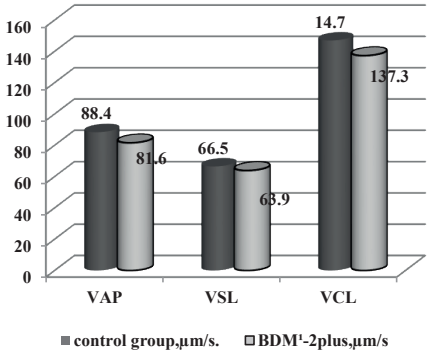


Figure 4. Analysis of VAP, VSL and VCL indices in the second experimental group at the beginning of the experiment

After 50 days of administration of 5 g of ZooBior-1 preparation, the sperm development rate did not show differences between the control and experimental groups compared with the indicators obtained at the beginning of the experiment.

Experimental data on the rate of sperm progression at the end of the experiment after 50 days of introducing 5 g/day of rams into the body from ZooBior 2, which includes Bior, an

extract of spirulina, zinc and selenium, are shown in Figures 5 and 6.

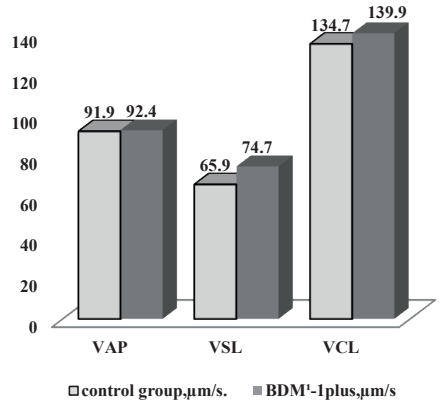


Figure 5. Analysis of VAP, VSL and VCL indices in the first experimental group at the end of the experiment

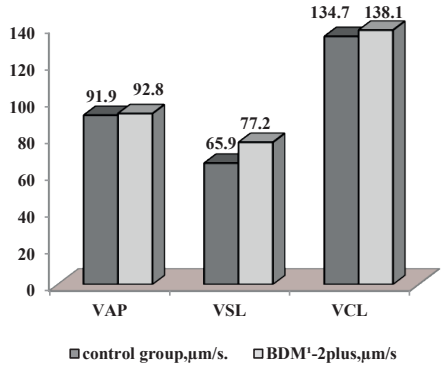


Figure 6. Analysis of VAP, VSL and VCL indices in the second experimental group at the end of the experiment

The data presented show that the sperm velocity was measured in a straight line (VAP), which measures the distance travelled by the sperm in the general direction and the given observation period, while the differences between the control and experimental groups are not determined, the average speed is 91.9 µm/s. Following the distance travelled by sperm (VSL) in a straight line from one point to another during the observation period, deviations between groups were detected. In the experimental group, VSL was 77.2 µm/s, and in the control group it was 65.9 µm/s ($P \leq 0.01$).

The experimental data show that at the end of the experiment in the control group and in the experimental group no statistically significant differences were presented or presented, and the linear velocity (VCL) in the control group was 134.7/91.9 µm/s. and 138.1/91.9 µm/s. in the experimental group.

Experimental data on hematological parameters of blood taken from sheep studied in the main breeding season are presented in Tables 1 and 2.

Analysis of blood parameters in the rams of the control and experimental groups revealed various fluctuations in hematological constants by the end of the study period.

These differences both in the control group and in the experimental groups are within physiological deviations at the lower and upper levels of norms, which shows that the studied drugs do not have a negative effect on the animal organism.

Table 1. Influence of BDM[1]-1 plus and BDM[1]-2 plus preparations pnl on the hematological indices of the blood, the beginning of the experiment

BDM[1]-1 plus (*Experimental group 1*)

Specification	*Control group*	*Experimental group 1*
Erythrocytes (x10^{12}/L)	7.3 ±0.5	8.1 ±0.9
Leukocytes (x10^{9}/L)	10.6 ±3.8	15.2 ±3.0
Hb, g/L	92.5 ±4.3	108.2 ±4.2
VSE, mm/hour	2.8 ±0.5	2.0 ±0.6
E, %	11.0 ±1.2	9.7 ±1.9
segmented, %	20.8 ±4.4	20.7 ±5.2
not segmented, %	11.3 ±3.0	7.3 ±2.4
lymphocytes, %	59.5 ±3.8	62.3 ±5.5

BDM[1]-2 plus *(Experimental group 2*)

Specification	*Control group*	*Experimental group 2*
erythrocytes (x10^{12}/L)	7.3 ±0.5	7.3 ±0.6
leukocytes (x10^{9}/L)	10.6 ±3.8	12.4± 0.5
Hb, g/L	92.5 ±4.3	108.3 ±1.7
VSE, mm/hour	2.8 ±0.5	1.3 ±0.3
E, %	11.0 ±1.2	8.7 ±4.7
segmented, %	20.8 ±4.4	16.0 ±3.1
not segmented, %	11.3 ±3.0	10.0 ±3.2
lymphocytes, %	59.5 ±3.8	65.3 ±3.4

Table 2. Influence of BDM[1]-1 plus and BDM[1]-2 plus preparations pnl on the hematological indices of the blood, the end of the experiment

BDM[1]-1 plus (*Experimental group 1*)

Specification	*Control group*	Experimental group 1
erythrocytes (x10^{12}/L)	10.6 ±1.4	10.5 ±0.5
leukocytes (x10^{9}/L)	7.9 ±0.4	8.7 ±0.4
Hb, g/L	101.8 ±4.4	102.8 ±1.5
VSE, mm/hour	3.3 ±0.3	4.3 ±0.9
E, %	9.0 ±1.0	13.0 ± 0.6
segmented, %	13.7 ±0.3	17.3 ± 1.5
not segmented, %	15.9 ±1.7	12.3± 1.2
lymphocytes, %	60.7 ±0.9	57.3 ±2.0

BDM[1]-2 plus(*Experimental group 2*)

Specification	*Control group*	*Experimental group 2*
erythrocytes (x10^{12}/L)	10.6 ±1.4	9.4 ±0.6
leukocytes (x10^{9}/L)	7.9 ±0.4	8.5 ±0.6
Hb, g/L	101.8 ±4.4	101.0 ±3.1
VSE, mm/hour	3.3 ±0.3	4.7 ±0.3
E, %	9.0 ±1.0	8.0 ± 1.0
segmented, %	13.7 ±0.3	14.7± 0.9
not segmented, %	15.9 ±1.7	7.7 ±0.9
lymphocytes, %	60.7 ±0.9	70.7 ±0.9

CONCLUSIONS

The preparations BDM¹-1 plus and BDM¹-2 plus, introduced into the diets for feeding sheep, have a positive effect on the spermogram. After 50 days of administration in rams, the ejaculate volume increased significantly ($P \leq 0.001$), sperm motility from 74% at the beginning of the experiment and 80% at the end of the experiment in the first experimental group ($P \leq 0.1$).

Various fluctuations in hematological constants were identified, but the differences in the control group, as well as in the experimental groups, are within the physiological changes at the lower and upper levels of norms, which shows that the studied drugs do not adversely affect the animal organism.

REFERENCES

Furdui, F. I, et al. (2002). *Gametogenez kak nacialnii etap zaclodki geneticeskih mehanizmov zdarovia*, Chisinau, 2, 30-39.

Sikka, S.C., et al. (1995). Rol of oxidative stres and antioxidante in male infertility. *Journal of Andrology*, 16, 464-468.

Șora A., Odagiu A. (2008). Disponibilitatea probioticilor și mineralelor în creșterea ecologică a animalelor/Availability of probiotics and minerals in ecological animal husbandry. *ProEnvironmend*, 2, 89-93.

Șora, A., et al. (2004). Efectele utilizării mineralelor organice în nutriția animalelor/The effects of using organic minerals in animal nutrition. *Buletin USAMVCN*, Seria ZB, 60, 100-103.

Sural, P.F., et al. (1997). The relationship between the dietary provision of alpha-tocopherol and the concentration of this vitamin in the semen of chicken effect an lipid composition and susceptibility to peroxidation. *Journal of production and Fertility*, 110, 47-51.

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USE OF THE DIETARY SEA BUCKTHORN MEAL AS PHYTOADITIVE IN HEAT - STRESSED BROILER

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Abstract

The paper aimed to characterize sea buckthorn meal (SBTM) and to assess his effect in the diet of hea t- stressed broiler (32˚C). A total of 60 Cobb 500 broiler chicks were assigned in two groups (C, E) which included in the diet corn and soybean meal as main ingredients. Compared with the C diet, the experimental diet (E diet) included the addition of 1% of SBTM. Samples of SBTM (purchased from a local producer) and samples of compound feed were analysed for their proximate composition, minerals, vitamin E and antioxidant activity. A 5-day balance sheet study was conducted on broilers in the grower stage (week 4) and in the finisher stage (week 6). Performance parameters (0-42 days) were recorded. Results showed that SBTM had an important concentration of polyphenols (11.65 mg/g GAE), lutein and zeaxanthin (91.80 mg/kg), expressing a high antioxidant capacity (99.84 mM ascorbic acid equivalent, 92.1 mM vitamin E equivalent). Dietary SBTM is a valuable by-product which can be used in broiler diet without negatively affecting the coefficients of apparent absorption of nutrients and performance, even in heat stress conditions.

Key words*: broiler, heat stress, sea buckthorn meal, chemical characterization, performance.*

INTRODUCTION

The sea buckthorn (*Hyppophae rhamnoides*) is a fruit-bearing shrub occurring naturally in Romanian flora. Sea buckthorn berries are an excellent source of phytochemicals such as ascorbic acid, tocopherols unsaturated fatty acids, and carotenoids (Yang, et al., 2001; Krejcarová et al., 2015). Not only the fruits, but also the meal (obtained as consequence of oil extraction) has a high antioxidant capacity (Püssa et al., 2007; Geetha et al., 2009) because it is rich in carotenoids, xanthophylls and flavonoids (Jung et al., 2012). Research reports show that buckthorn is beneficial for poultry performance (Hu and Guo, 2006; Biswas et al., 2010; Kaushal and Sharma, 2011), without any potential adverse effect on environment (Vlaicu et al., 2017).

Among the oil extraction industry by-products, meals are vegetable raw materials with low cost that can be used in animal feeding (Panaite et al., 2016). The same authors concluded that sea buckthorn meal as by-product meet the feeding requirements for inclusion as dietary ingredients because of their high level of essential nutrients (protein, amino acids, fatty acids and minerals) and to their high antioxidant capacity. Since the leaves, seeds and fruit residues contain high crude protein, they have advantages as basic materials for feed formulations for poultry (Biswas et al., 2010). Heat stress is a significant cause of economic loss in poultry production and is almost inevitable (Hu et al., 2019). Heat stress impairs the nutrient digestibility (de Souza et al., 2016). Nutrient digestibility plays an important role in ensuring the health and productivity of animals. Heat stress mainly leads to a decrease in growth performance and affect the meat quality of poultry by inducing oxidative stress in the body. Researchers (Criste et al., 2017; Hu et al., 2019) reported that improving the antioxidant capacity of poultry may help mitigate the influence of heat stress. The polyphenols are phytocompounds which have a high antioxidant potential, being considered the most efficient active compounds

(Trifunschi et al., 2017). Polyphenols are natural antioxidants that can reduce oxidative stress and widely exist in plants, they therefore have great potential to be used as a novel feed additive for improving productivity in heat-stressed poultry. In recent years, there is a necessity to use low input forages in poultry feeding as food industry by-products (e.g. meals). However, less attention has been paid to the use of sea buckthorn as a poultry and livestock feed, therefore determining the feed value of sea buckthorn will provide scientific information that can be used to promote its use as livestock and poultry feed in heat-stressed broilers. Thus, the paper aimed to characterize sea buckthorn meal (SBTM) and to assess his effect in the diet of heat - stressed broiler (32°C).

MATERIALS AND METHODS

A six-week feeding trial was conducted on 60 Cobb 500 broiler chicks (1 day of age) evaluated in a completely randomized design, with two groups (30 chicks per group). The chicks were housed randomly in three-tiered digestibility cages, allowing the daily recording of the feed intake and excreta. Throughout the experimental period, the environmental temperature of the experimental hall was kept constant at 32ºC. The humidity parameter was 49%, with 0.45% ventilation/broiler, and 850 ppm CO_2 emission. The light regimen was appropriate to the age of the chicks, 23 hours light/1-hour darkness. From 1 day old, broilers received a corn and soybean meal-based control diet (C). Compared with the C diet (Table 1), the experimental diet (E) included the addition of 1% sea buckthorn meal (SBTM). Sea buckthorn meal was purchased from a local producer (E-Prod SRL, Teleorman, Romania), dried, grounded and packed. Feed and water were provided for *ad libitum* consumption. None of the groups (C, E) had monoensin in the premix. Diet formulations were calculated to meet or exceed the minimum requirements for broiler chicks (NRC, 1994). All diets were fed in mash form.

The coefficients of apparent absorption of the nutrients were determined using the balance technique in weeks 4 and 6. In each of these two weeks, the amounts of ingested feed and of excreted droppings were recorded for 5 consecutive days. The droppings were collected daily, for 5 days, at the same hour, weighed and stored in refrigerator (4°C); average weekly samples (6 samples/group) were formed, homogenized and dried in the drying oven, for 48 h, at 65°C. The compound feeds samples and the average weekly samples of droppings/group, were analysed for the dry matter, at 65-103°C (DM); crude protein (CP); crude fat (EE); crude fibre (CF); Ash (ash). The coefficients of apparent absorption of nutrients were calculated as described by Panaite et al. (2017). Throughout the experimental period (0-42 days, broiler age) the performance (average daily gain, average daily feed intake and feed conversion ratio) was monitored. The chemical proximate composition of feed samples was assayed using the chemical methods from Commission of the European Communities (2009) as described by Olteanu et al. (2016). The calcium concentration in samples was determined according to the titrimetric method SR ISO 6490-1/1996 and P by spectrophotometric method. Trace minerals (Cu, Fe, Zn, Mn) concentrations (expressed as mg/kg) were determined by flame atomic absorption spectrometry after microwave digestion.

The total phenol content of SBTM and feed samples was measured spectrophotometrically according to the Folin-Ciocalteu's method, described by Untea et al. (2018). The results were expressed as mg gallic acid equivalent (GAE)/g DW.

The total antioxidant capacity of the SBTM and feed samples was evaluated by the phosphor-molybdenum method of Prieto et al. (1999). The results were expressed as Mm ascorbic acid equivalent DW and as Mm vitamin E equivalent DW.

Lutein and zeaxanthin were analysed using the method described by Untea et al. (2020) with a high-performance liquid chromatograph. Vitamin E determination (expressed as mg/kg) was performed according to the method described in EC Regulation no. 152/2009, using a high-performance liquid chromatograph and a PDA-UV detector at a wavelength of 292 nm.

Table 1. Diet formulation

Ingredient	Starter (0-14 days)		Grower (15-28 days)		Finisher (29-42 days)	
	C	E	C	E	C	E
	%					
Corn	32.73	31.73	36.47	35.47	40.45	39.45
Wheat	20	20	20	20	20	20
Corn gluten	2	2	4	4	6	6
Soybean meal	36.17	36.17	30.2	30.2	23.95	23.95
Sea buckthorn meal	-	1	-	1	-	1
Sunflower oil	3.85	3.85	4.31	4.31	4.72	4.72
Monocalcium phosphate	1.68	1.68	1.52	1.52	1.43	1.43
Calcium carbonate	1.5	1.5	1.38	1.38	1.31	1.31
Salt	0.39	0.39	0.38	0.38	0.33	0.33
Methionine	0.33	0.33	0.25	0.25	0.21	0.21
Lysine	0.3	0.3	0.29	0.29	0.36	0.36
Threonine	-	-	0.15	0.15	0.19	0.19
Choline	0.05	0.05	0.05	0.05	0.05	0.05
Premix*	1	1	1	1	1	1
Total	100	100	100	100	100	100
Calculated *Metabolisable energy, kcal/kg*	3.039,79	3.039,79	3.128,99	3.128,99	3.217,72	3.217,72
Lysine	1.44	1.44	1.29	1.29	1.19	1.19
Methionine	0.69	0.69	0.61	0.61	0.57	0.57
Threonine	0.97	0.97	0.88	0.88	0.81	0.81
Tryptophan	0.25	0.25	0.22	0.22	0.19	0.19

*1kg premix contains: = 1100000 IU/kg vit. A; 200000 IU/kg vit. D3; 2700 IU/kg vit. E; 300 mg/kg vit. K; 200 mg/kg Vit. B1; 400 mg/kg vit. B2; 1485 mg/kg pantothenic acid; 2700 mg/kg nicotinic acid; 300 mg/kg vit. B6; 4 mg/kg Vit. B7; 100 mg/kg vit. B9; 1.8 mg/kg vit. B12; 2000 mg/kg vit. C; 8000 mg/kg manganese; 8000 mg/kg iron; 500 mg/kg copper; 6000 mg/kg zinc; 37 mg/kg cobalt; 152 mg/kg iodine; 18 mg/kg selenium.
C - conventional diet; E - conventional diet + 1% sea buckthorn meal.

The complete randomized model was used to analyse the data for growth performance. The effects of treatments were tested by analysis of variance (ANOVA and t test) using Stat view for Windows (SAS, version 6.0). The differences between means were considered statistically significant at $P<0.05$.

RESULTS AND DISCUSSIONS

Table 2 shows the chemical composition of SBTM, highlighting a rather high level of fibre (16.11%). The high level of fibre had count on choosing the level of inclusion of SBTM in broiler diet. The SBTM also represents a valuable source of antioxidant compounds as polyphenols, lutein and zeaxanthin (Table 2) and vitamin E which contribute to his antioxidant capacity. The meal used in this study was not as rich in minerals as values in the literature for fruit pulp, but as by-product, contains important amount of Mn and Zn (Table 2).

Table 2. Chemical characterization of sea buckthorn meal

Variable	Sea buckthorn meal
Dry matter, %	88.94
Crude protein, %	12.43
Crude fat, %	13.29
Crude fiber, %	16.11
Ash, %	2.89
Cu, mg/kg	6.69
Fe, mg/kg	1.35
Mn, mg/kg	20.26
Zn, mg/kg	31.86
Vitamin E, mg/kg	143.16
Lutein + zeaxanthin, mg/kg	91.80
Total polyphenols, mg GAE /g DW	11.65
mM ascorbic acid equivalent DW	99.84
mM vitamin E equivalent DW	92.10

Compared with the National Research Council (1994), Biswas et al. (2010) reported that the leaves, seeds and fruit residues of sea buckthorn contain enough crude fat and crude protein for poultry growth. Lu et al. (1991) had characterize the sea buckthorn (seeds, leaves and fruit residues) compared with other feed ingredients (alfalfa, green sweet clover, green Symphytum, leaves of sophora, green reserved maize stalk, carrot, powdered soybean stalk, sorghum seed, maize seed, broad bean, soybean, pea). They showed that the crude protein content of sea buckthorn was higher than that of majority, excepted soybean, broad bean and pea. It contains necessary amount of crude protein and fat and stimulates the growth and productivity of livestock and poultry as well, particularly in dry and cold areas (Biswas et al., 2010; Kaushal & Sharma, 2011). Compared with the polyphenols content of SBTM obtained by Panaite et al. (2016), data from Table 2 show a higher content, by 12.13%. Differences may be originating from the method of oil extraction, method of analyse solvent used for extraction, etc. It was reported that sea buckthorn contains large amounts of carotenoids and vitamin E located mainly in membrane and the fleshy mesocarp (Zeb, 2004). The antioxidant vitamin E content of SBTM makes it a valuable contributor in helping the overall health and scavenging free radicals. In this regard, it has been reported that phytochemicals with antioxidant activity offer great hope as a solution for heat stress in poultry (Hu et al., 2019).

Table 3. Chemical composition of the compound feeds depending on the growth stage

Variable	Starter compound feed (0-14 days)		Grower compound feed (15-28 days)		Finisher compound feed (29-42 days)	
	C	E	C	E	C	E
Dry matter, %	88.52	89.18	88.84	89.41	89.18	89.21
Crude protein, %	23.00	22.79	21.50	21.65	20.00	19.89
Crude fat, %	5.48	5.52	6.01	6.07	6.49	6.52
Crude fibre, %	3.77	4.42	3.57	3.67	3.36	3.83
Ash, %	7.08	7.51	7.01	6.70	6.81	6.53
Ca, %	0.96	0.98	0.87	0.89	0.81	0.90
P, %	0.77	0.77	0.70	0.73	0.65	0.66
Cu, ppm	7.09	6.93	8.77	9.44	9.82	9.47
Fe, ppm	523.71	533.60	534.81	536.77	510.25	566.31
Mn, ppm	143.86	152.45	118.80	125.52	111.14	134.31
Zn, ppm	110.17	104.70	109.21	121.83	105.9	109.39
Vitamin E, ppm	44.01	42.89	45.92	47.41	52.16	52.94
Lutein + zeaxanthin, ppm	8.44	7.24	13.32	13.38	16.15	16.71
Total polyphenols, mg GAE/g	1.67	1.63	1.55	1.77	1.87	1.92
Antioxidant capacity mmol ascorbic acid equivalent/kg DW	29.60	27.25	35.36	38.43	30.69	37.99
Antioxidant capacity mmol vitamin E equivalent/kg DW	30.86	28.28	37.13	40.45	31.94	39.96

C- conventional diet; E- conventional diet + 1% sea buckthorn meal

Besides of people feeding, sea buckthorn, especially its leaves, pomace and press cake, can also be used as an ingredient of animal feed (Kaushal and Sharma, 2011). The results of the chemical analysis of the compound feeds (Table 3) shows that in the starter, grower and finishing stage, the compound feeds were balanced as energy and protein content. Notably, once SBTM was added in the experimental diet, an increase in the crude fiber and crude fat content compared with the control diet was observed (Table 3). Regarding the mineral content (Ca, P, Cu, Fe, Mn, Zn) of compound feeds, there were observed a slightly increase in E diet compared with C diet. Vitamin E concentration was slightly higher in experimental diet during grower and finisher stage compared with control diet. The addition

of SBTM in broiler diet revealed an increase in polyphenol content of experimental diet in grower and finisher stages, resulting in an increase in the antioxidant capacity (Table 3).

Table 4. Effect of SBTM on the coefficients apparent absorption of the nutrients (grower stage)

Variable	C	E	SEM	p-value
Dry matter (DM)				
Ingested (g/chick/day)	70.14	67.84	2.962	0.7467
Excreted (g/chick/day)	17.42	17.67	1.101	0.9157
Absorbed (g/chick/day)	52.71	50.17	2.032	0.5552
Absorption coefficient (%)	74.79	74.23	0.669	0.6962
Organic matter (OM)				
Ingested (g/chick/day)	62.85	61.02	2.655	0.7487
Excreted (g/chick/day)	13.65	13.63	0.856	0.9210
Absorbed (g/chick/day)	49.20	47.19	1.913	0.6235
Absorption coefficient (%)	77.98	77.58	0.588	0.7450
Crude protein (CP)				
Ingested (g/chick/day)	17.45	16.05	0.745	0.3823
Excreted (g/chick/day)	2.21	2.36	0.181	0.7004
Absorbed (g/chick/day)	15.22	13.70	0.641	0.2533
Absorption coefficient (%)	87.18	85.62	0.722	0.3018
Crude fat (EE)				
Ingested (g/chick/day)	4.65	4.72	0.200	0.8713
Excreted (g/chick/day)	0.39	0.38	0.030	0.7991
Absorbed (g/chick/day)	4.25	4.34	0.176	0.8203
Absorption coefficient (%)	91.16	92.14	0.394	0.5085
Crude fiber (CF)				
Ingested (g/chick/day)	2.95	3.41	0.152	0.1304
Excreted (g/chick/day)	2.12	2.21	0.131	0.7518
Absorbed (g/chick/day)	0.82[a]	1.20[b]	0.074	0.0035
Absorption coefficient (%)	26.55[a]	35.75[b]	2.072	0.0173
Ash				
Ingested (g/chick/day)	5.61	5.21	0.239	0.4290
Excreted (g/chick/day)	3.04	3.01	0.194	0.9506
Absorbed (g/chick/day)	2.57	2.20	0.114	0.1031
Absorption coefficient (%)	44.69	42.81	1.658	0.5944

[a, b]Means in the same column with different superscripts differ significantly ($p<0.05$).
SEM = standard error of the means; C - conventional diet; E - conventional diet + 1% sea buckthorn meal.

Table 4 data show the coefficient of apparent absorption of nutrients for broilers in the grower stage. Although SBTM was included in the E diet, the coefficient of apparent absorption of dry matter, organic matter, crude protein, crude fat and ash were not recorded significantly differences ($P>0.05$) compared with C group. Notably is that under heat stress, broilers fed E diet had a significantly ($P<0.05$) higher coefficient of apparent absorption of crude fibre (Table 4). Under thermoneutral conditions, others (Li et al., 2008) reported an increase in the apparent digestibility of dietary crude protein as consequence of broiler diet supplementation with 0.1% and 0.2% flavones of sea buckthorn.

Table 5. Effect of SBTM on the coefficients of apparent absorption of nutrients (finisher stage)

Variable	C	E	SEM	p-value
Dry matter (DM)				
Ingested (g/chick/day)	82.79	74.20	7.550	0.8025
Excreted (g/chick/day)	18.66	17.79	1.607	0.6697
Absorbed (g/chick/day)	64.13	56.40	6.023	0.5468
Absorption coefficient (%)	76.73	75.68	0.545	0.3576
Organic matter (OM)				
Ingested (g/chick/day)	74.82	67.34	6.831	0.6079
Excreted (g/chick/day)	14.84	14.18	1.284	0.6066
Absorbed (g/chick/day)	59.98	53.16	5.619	0.5691
Absorption coefficient (%)	79.54	78.65	0.458	0.3573
Crude protein (CP)				
Ingested (g/chick/day)	17.42	16.85	1.633	0.8692
Excreted (g/chick/day)	2.42	2.35	0.106	0.7465
Absorbed (g/chick/day)	15.00	14.50	1.544	0.8792
Absorption coefficient (%)	84.57	85.25	0.859	0.7125
Crude fat (EE)				
Ingested (g/chick/day)	5.96	5.52	0.549	0.7081
Excreted (g/chick/day)	0.81	0.69	0.073	0.4175
Absorbed (g/chick/day)	5.15	4.83	0.482	0.596
Absorption coefficient (%)	86.39	87.39	0.390	0.2146
Crude fiber (CF)				
Ingested (g/chick/day)	3.63	3.68	0.349	0.9442
Excreted (g/chick/day)	1.99	1.96	0.190	0.9296
Absorbed (g/chick/day)	1.63	1.72	0.177	0.8162
Absorption coefficient (%)	42.84	46.55	1.430	0.2078
Ash				
Ingested (g/chick/day)	6.44	5.53	0.583	0.4597
Excreted (g/chick/day)	3.01	3.03	0.274	0.9710
Absorbed (g/chick/day)	3.44	2.50	0.349	0.1929
Absorption coefficient (%)	51.54[a]	44.52[b]	1.655	0.0252

[a, b]Means in the same column with different superscripts differ significantly ($p<0.05$). SEM = standard error of the means; C - conventional diet; E - conventional diet + 1% sea buckthorn meal.

The coefficients of apparent absorption of nutrients in the finisher stage, can be visualised in the Table 5. Broiler feeding with diet containing 1% SBTM, did not significantly influence ($p>0.05$) the absorption of DM, OM, CP, EE, CF (Table 5). However, it can be observed that the absorption coefficient of Ash was significantly lower ($p<0.05$) in the group fed diet with 1% SBTM compared with the group fed conventional diet. Fewer results are available on nutrients digestibility in hot environments. Many studies support the idea of heat stress can affects nutrient digestibility (Bonnet et al., 1997; de Souza et al., 2016). This affirmation can be explained by the fact that high environmental temperature alters the morphology of small intestine (decrease in villus height and ratio of villus height to crypt

depth) and consequently the absorption of nutrients is affected. It was reported that chronic heat exposure decreases protein digestion, particularly with the summer diet. (Wallis and Balnave, 1984; Zuprizal et al., 1993).

However, in the present study, even if broilers were subjected to heat stress, the absorption of nutrients was not negatively affected. There are studies that have showed the effect of dietary plant materials on digestibility of nutrients in broilers under thermoneutral or heat stress conditions. For example, dietary artichoke extract supplementation improved ($P<0.01$) the digestion coefficients of DM, CP and CF in broilers (Hassan et al., 2015). Significant increases in crude protein (CP) and crude fat (EE) digestibility were achieved by heat-stressed birds fed diets supplemented with cinnamon, turmeric, ginger (0.5 g/kg) or ascorbic acid (200 mg/kg) compared with those of the control group, but those of other nutrients were unaffected (El-Maaty et al., 2014). On the contrary, Cross et al., (2007) showed no effect on the digestibility of nutrients when broilers (7 to 28 days of age) were fed diets with 10 g/kg herb (thyme, oregano, marjoram, rosemary or yarrow) or 1 g/kg of essential oil.

Table 6. Effect of dietary sea buckthorn meal on performance of heat- stressed broiler (0-42 days)

Variable	C	E	SEM	p-value
ADG (g/broiler/day)				
1-14	27.84	27.42	0.519	0.7091
15-28	51.41	48.73	2.205	0.5677
29-42	62.99	57.06	13.063	0.8326
1-42	44.59	40.44	2.632	0.4561
AFI (g feed/broiler/day)				
1-14	37.11	36.53	0.569	0.6352
15-28	77.03	74.04	3.035	0.6452
29-42	97.59	84.36	7.110	0.3769
1-42	72.87	64.98	3.416	0.2671
FCR (g feed/g gain)				
1-14	1.33	1.33	0.014	0.4204
15-28	1.50	1.52	0.147	0.3877
29-42	2.20	1.93	0.119	0.5777
1-42	1.65	1.62	0.029	0.5709

Where: ADG = average daily gain; AFI = average feed intake; FCR = feed conversion ratio; SEM = standard error of the means; C - conventional diet; E - conventional diet + 1% sea buckthorn meal.

The ADG of broilers fed diet with 1% SBTM was not significantly ($p >0.05$) different from that of broilers fed conventional diet (Table 6). Although not statistically significant, the FCR (0-42 days) of broilers from E group was lower compared to those from C group (Table 6). Even if the broilers were exposed to heat stress, there was no effect ($p >0.05$) of diet supplementation with SBTM on AFI (0-42 days). In our study no mortalities were recorded in any of the two experimental groups. Fewer studies were found in the scientific literature on application of sea buckthorn in poultry nutrition. Many researchers reported considerably increase in body weight of livestock and poultry after feeding with leaves, seeds and fruit residues of sea buckthorn (Hu, 2000; Hu and Guo, 2006; Biswas et al., 2010). Zhao et al. (2012) revealed that supplementation of flavones from sea buckthorn leaves significantly decreased AFI without affecting growth performance. Flavones of sea buckthorn play an important role in immunomodulation, antibiosis, and

antioxidant reactions (Suryakumar and Gupta, 2011), resulting in the improvement of growth and feed utilization. Under thermoneutral conditions, Ma et al. (2015) showed that the use of 0.05 to 0.10% flavones from sea buckthorn had a positive influence on growth performance of broilers.

CONCLUSIONS

Sea buckthorn meal represents a valuable by product regarding the chemical composition, which can be included in broiler diet. The present findings showed that dietary SBTM, did not negatively affects the coefficients of apparent absorption of nutrients and broiler performance even under heat stress conditions. These results need to be further evaluated in order to assess the effect of SBTM on the antioxidant status of the heat-stressed broiler.

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REFERENCES

Biswas, A., Bharti, V. K., Charya, S. A., Pawar, D. D. and Singh, S. B. (2010). Sea buckthorn: new feed oportunity for poultry in cold arid Ladakh region of India. *World,s Poultry Science of Journal*, 66(04), 707-714.

Criste, R. D., Panaite, T. D., Tabuc, C., Sărăcilă, M., Șoica, C., Olteanu, M. (2017). Effect of oregano and rosehip supplementson broiler (14-35 days) performance, carcass and internal organs development and gut health. *Agrolife Scientific Journal*, 6(1), 75-83.

Cross, D. E., McDevitt, R. M, Hillman, K. and Acamovic, T. (2007).The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. *British Poultry Science*, 48(4), 496-506.

de Souza, L. F. A., Espinha, L. P., de Almeida, E. A., Lunedo, R., Furlan, R. L., and Macari, M. (2016). How heat stress (continuous or cyclical) interferes with nutrient digestibility, energy and nitrogen balances and performance in broilers. *Livestock Science*, 192, 39-43.

El-Maaty, A., Hayam, M. A., Rabie, M. H., El-Khateeb, A.Y. (2014). Response of heat-stressed broiler chicks to dietary supplementation with some commercial herbs. *Asian Journal of Animal and Veterinary Advances*, 9, 743-755.

Geetha, S., Sai Ram, M., Sharma, S. K., Ilavazhagan, G., Banerjee, P. K., Sawhney, R. C. (2009). Cytoprotective and antioxidant activity of seabuckthorn (*Hippophae rhamnoides* L.) flavones against tert-butyl hydroperoxideinduced cytotoxicity in lymphocytes. *Journal of Medicinal Food*, 12, 151-158.

Hassan, H. M. A., Youssef, A. W., Ali, H. M. and Mohamed, M. A. (2015). Adding phytogenic material and/or organic acids to broiler diets:effect on performance, nutrient digestibility and net profit. *Asian Journal of Poultry Science*, 9(2), 97-105.

Hu, J. Z. (2000). Eco-economic values and comprehensive development techniques of sea buckthorn. Zhengzhou: *The Yellow River Water Conservancy Press* (in Chinese).

Hu, J. Z. and Guo, X. F. (2006). Evaluation of nutrient value of sea buckthorn in north China. *Forestrystudies in China*, 8, 50-52.

Hu, R., He, Y., Arowolo, M. A., Wu, S. and He, J. (2019). Polyphenols as potential attenuators of heat stress in poultry production. *Antioxidants,* 8, 67.

Jung, E. K., Clark, R. M., Park, Y., Lee, J., Fernandez, M. L. (2012). Lutein decreases oxidative stress and inflammation in liver and eyes of guinea pigs fed a hypercholesterolemic diet. *Nutrition Research Practice*, 6, 113-119.

Kaushal, M., Sharma, P. C. (2011). Nutritional and antimicrobial property of sea buckthorn seed oil. *Journal of Scientific and Industrial Research*, 1033-1036.

Krejcarová, J., Straková, E., Suchý, P., Herzig, I., Karásková, K. (2015). Sea buckthorn (Hippophae rhamnoides L.) as a potential source of nutraceutics and its therapeutic possibilities-a review. *Acta Veterinaria Brno,* 84(3), 257-268.

Li, Y., Fu J., Wang, Bao-dong, Wang, Yan-bo, Shan, An-shan. (2008). Effect of flavones of sea buckthorn on carcass characteristics and meat quality of Arbor Acres broilers. *Acta Veterinaria et Zootechnica Sinica*, 39(9), 1217-1223.

Lu, R. S., Lin, X. N., Peng, L. (1991). Leaves nutrition and application aspect of sea buckthorn. *Hippophae*, 4, 43–45.

Olteanu, M., Criste, R. D., Panaite, T. D., Bunduc, V., Panaite, C. V., Ropota, M. and Mitoi, M. (2016). Study on the efficiency of grape seed meals used as antioxidants in layer diets enriched with polyunsaturated fatty acids compared with vitamin E. *Brazilian Journal of Poultry Science* 18(4), 655-662.

Panaite, T. D., Criste, R. D., Ropota, M., Criste, V., Vasile, G., Olteanu, M., Mitoi, M., Socoliuc, R., Vlaicu, P. A. (2016). Determination of the feeding value of food industry by-products. *Scientific Papers-Animal Science Series*, 66, 106-111.

Panaite, T., Criste, R. D., Olteanu, M., Vlaicu, A., Soica, C. (2017). Coefficients of apparent absorption of the dietary nutrients from broiler feeds that include oil industry by-products (rapeseeds, grapes, buckthorn, flax and pumpkin meals), *Scientific Papers: Animal Science and Biotechnologies,* 50(1), 22-28.

Prieto, P., Pineda, M., Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Anal Biochem*, 269(2), 337-341.

Püssa, T., Pällin, R., Raudsepp, P., Soidla, R., Rei, M. (2007). Inhibition of lipid oxidation and dynamics of polyphenol content in mechanically deboned meat supplemented with sea buckthorn (*Hippophae rhamnoides* L.) berry residues. *Journal of Food Chemistry*, 107, 714-721.

Suryakumar, G., Gupta, A. (2011). Medicinal and therapeutic potential of sea buckthorn (*Hippophae rhamnoides* L.). *Journal of Ethnopharmacology*, 138(2), 268-278.

Trifunschi, S., Munteanu, M. F., Pogurschi, E., Gligor, R.(2017). Characterisation of Polyphenolic Compounds in Viscum album L. and Allium sativum L. extracts. *Revista de Chimie (Bucharest)*, 68, 1677-1680.

Untea, A. E., Varzaru, I., Panaite, T. D., Gavris, T., Lupu, A., Ropota, M. (2020). The effects of dietary inclusion of bilberry and walnut leaves in laying hens' diets on the antioxidant properties of eggs. *Animals,* 10, 191.

Untea, A., Lupu, A., Saracila, M., Panaite, T. (2018). Comparison of ABTS, DPPH, phosphomolybdenum assays for estimating antioxidant activity and phenolic compounds in five different plant extracts. *Bulletin UASVM Animal Science and Biotechnologies,* 75(2), 110-113.

Vlaicu, P. A., Panaite, T. D., Olteanu, M., Ropota, M., Criste, V., Vasile, G., Grosu, I. (2017). Production parameters, carcass development and blood parameters of the broiler chicks fed diets which include rapeseed, flax, grape and buckthorn meals. *Scientific Papers: Animal Science and Biotechnologies*, 50(1).

Wallis, I. R., and Balnave D. (1984). The influence of environmental temperature, age and sex on the digestibility of amino acids in growing broiler chickens. *Br. Poult. Sci*., 25, 401-407.

Yang, B., and Kallio, H. P. (2001). Fatty acids composition of lipids in sea buckthorn (Hippophae rhamnoides L.) berries of different origins. *J. Agric. Food Chem*. 49, 1939-1947.

Zeb, A. (2004). Chemical and nutritional constituents of sea buckthorn juice. *Pakistan Journal of Nutrition*, 3(2), 99-106.

Zhao, W., Chen, X., Yan, C., Liu, H., Zhang, Z., Wang, P., Su, J. and Li, Y. (2012). Effect of sea buckthorn leaves on inosine monophosphate and adenylosuccinatelyase gene expression in broilers during heat stress. *Asian-Australasian Journal of Animal Sciences*, 25(1), 92.

Zuprizal, M., Larbier, A., Chagneau M., Geraert P. A. (1993). Influence of ambient temperature on true digestibility of protein and amino acids of rapeseed and soybean meals in broilers. *Poultry Sci.,* 72, 289-295.

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EVALUATION OF THE CHEMICAL COMPOSITION AND NUTRITIONAL QUALITY OF DEHULLED LUPIN SEED MEAL (*Lupinus* spp. L.) AND ITS USE FOR MONOGASTRICS ANIMAL NUTRITION: A REVIEW

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Abstract

The utilization of the lupine seeds (Lupinus spp. L.) from low-alkaloids varieties in monogastric animals feeds is limited due to the presence of antinutritive factors. Studies show that a considerable improvement of the nutritional composition of lupine is achieved by dehulling the seeds. The seed dehulling process increases the crude protein and fat levels, and decreases the content of cellulose, neutral detergent fibers and acid detergent fibers. An improvement of the biological value of lupine protein and the nutritional quality of fat is also obtained by dehulling. Antinutritive factors, such as non-starch polysaccharides and oligosaccharides, are partially reduced by dehulling, because of their storage in high concentrations in the kernel. For poultry and swine feeding, the utilization of whole lupine seeds is limited due to the lack of endogenous enzymes for processing the antinutritional factors. Therefore, numerous studies highlight that the use of dehulled seeds realizes similar bioproductive performances as soybean by-products. This is possible if the optimal inclusion level of lupine in diets and the adequate balance in limited amino acids are realized. Lupine seeds are therefore a valuable alternative source of proteins and fat, which is proven to be able to support productions, while improving the quality of animal products.

Key words*: dehulled, lupine seeds, monogastric animals, protein.*

INTRODUCTION

Currently, concurrency for protein feed between humans and animals, especially monogastrics, determines protein sources to become progressively more limited and expensive (Henchion et al., 2017). The use of soybean seeds as the main plant protein source raises some ethical, ecological and especially economic issues, so it is necessary to find new possibilities to reduce dependence on the imported protein sources in Europe, while guaranteeing the security of origin (Kasprowicz et al., 2016). Lupine (*Lupinus* spp. L.) is considered a valuable vegetal protein source because of its nutritional profile comparable to other vegetal protein sources used in farm animalnutrition, such as soybean meal (De Visser et al., 2014). Lupin is part of the Fabaceae family, and the genus *Lupinus* includes 267 annual and perennial herbaceous species cultivated in different pedo-climatic areas (Drummond, 2008; Drummond et al., 2012).

The most commonly used species of sweet lupine are *Lupinus albus*, *Lupinus luteus* in Europe, *Lupinus angustifolius* in Australia and *Lupinus mutabilis* in South America (Lucas et al., 2015; Sedláková et al., 2016). Lupine has been shown to be successfully used in animal nutrition, thus becoming an integrated part of modern agriculture, also used to increase soil fertility due to nitrogen fixation, being considered a very good precursor plant for other crops (Seymour et al., 2012; Weisskopf et al., 2008). Presently, the use of lupins from low alkaloid varieties, so called sweet lupins, in the nutrition of monogastrics animals is limited due to their content in certain anti-nutritional factors, such as non-starch polysaccharides, oligosaccharides, hemicellulose, cellulose and especially neutral detergent fibers (NDF) and

acid detergent fibers (ADF) that are lowly digestible and reduce the digestibility of nutrients (Olkowski, 2010).
Studies show that the utilization of mechanical removal procedures of hulls from lupine seeds (such as dehulling) contributes to the increase in nutritional value and the degree of feed utilization and implicitly, to the bioproductive performances of the animals (De Vries et al., 2012). Therefore, the aim of this review is to highlight the effect of dehulling lupine seeds on the chemical composition and nutrition value of seeds and its use for monogastric animal nutrition.

MATERIALS AND METHODS

NUTRITIONAL CARACTERIZATION OF LUPINE SEEDS

Current researches about the chemical composition and nutritional quality of lupine seeds show a variable nutritional profile depending on the cultivars, pedoclimatic conditions and applied agrotechnics (Mierlita et al., 2012; Księżak et al., 2018; Tomczak et al., 2018).
In order to improve the nutritional value of lupine seeds, studies show that some procedures may be applied, such as dehulling, crushing, germination (Chilomer et al., 2013), decreasing particle size (Kim et al., 2009) roasting, microwaves, autoclaving (Batterham et al., 1986) and addition of specific enzymes. Among the physical methods, dehulling of seeds and use the kernel has proven to be an effective and economical method that considerably improves the nutritional value of lupine seeds (Embaby, 2010; Laudadio and Tufarelli, 2011a).

Chemical composition of lupine seed

Numerous studies are focused on the chemical composition of whole lupine seeds characterized by high content in crude protein, crude fat and cellulose (Yilmaz et al., 2015; Bartkiene et al., 2016). Values differ according to lupine species, cultivars and pedoclimatic conditions, but are comparable to other vegetal protein sources, e.g. soybean (*Glycine max*) (Bähr et al., 2014) or faba bean (*Vicia faba*) (Mattila et al., 2018). Other studies have also shown that dehulling of lupine seeds determines a significant increase in protein and fat content, as well as a reduction in crude cellulose level, especially in cell wall constituents (NDF, ADF) and carbohydrates (Glencross et al., 2007; Večerek et al., 2008).
According to table 1, whole lupine seeds are characterized by variable protein content, between 25.01 and 42.8% in DM (dry matter). After dehulling the seeds, an increase in the level of crude protein is observed, having values between 31.1 and 54.4% in DM. Bähr et al (2014) mentions that the protein level in dehulled lupine seeds (from three species) is similar to the soybean protein level.

Table 1. Effect of dehulling on the chemical composition (% of dry matter) of low-alkaloid lupine seeds varieties

Nutrients	*L. albus*				*L. luteus*		*L. angustifolius*	
	1		2		3		4	
	WS	DS	WS	DS	WS	DS	WS	DS
Dry matter	92.9	93.1	95.9	95.7	-	-	91.1	91.1
Crude protein	35.5	42.9	38.4	43.5	42.8	54.4	29.0	37.2
Crude ash	3.5	3.6	4.1	4.2	5.3	6.4	2.5	2.5
Crude fat	8.8	9.3	7.9	10.2	3.7	5.7	5.3	6.7
Crude cellulose	11.9	8.0	15.2	3.7	16.9	3.8	13.1	2.1
NDF	20.6	15.0	21.6	8.6	25.6	-	26.0	5.8
ADF	12.9	7.8	17.4	5.4	22.2	13.6	17.2	3.2

Note: 1 - Laudadio and Tufarelli (2011a); 2 - Písaříková et al. (2008); 3 - Mieczkowska et al. (2005); 4 - King et al. (2000); WS - whole seeds; DS - dehulled seeds; NDF - neutral detergent fibers; ADF - acid detergent fibers.

The fat content can be influenced by the genotype of the species as well as the pedoclimatic growth factors of the plant (Boschin et al., 2008). Rybiński et al. (2018) found a variation of the crude fat content from *L. albus* whole seeds, ranging between 6.9% and 14.1% in DM, with an average of 9.81% DM. According to the data from table 1, by dehulling, the crude fat level increases from 3.7-8.81% of DM (whole seeds) to 5.42-10.23% DM (dehulled seeds).
The high content in crude celullose of lupine seeds slows digestion, affects metabolic assimilation of nutrients and reduces the nutritional value of feed for monogastric animals, incapable to efficiently degrade cellulose. Straková et al. (2006) mention that for the lupine species cultivated in Europe, the content of cellulose in whole seeds varies between 94-142 g/kg and between 124-192 g/kg, values higher than in soybeans. The content of crude celluose in lupine seeds is

about 13% in DM, which is double compared with soybean meal (6.2% DM), peas (5.3% DM) or faba beans (8.1% DM) (Van Barneveld, 1999). Dehulling proces is demonstrated to reduce NDF content by 16% and ADF by 13.7% (Mera-Zuñiga et al., 2018).
The crude ash level in lupin seeds is a good indicator of macroelements and microelements content (Grela et al., 2017). According to table 1, there is a slight increase in ash for dehulled seeds, by a species-dependent mode.
The content in N-free extract (NFE) differs in lupine species and contains, in addition to starch, pectin, more water soluble non-starch polisaccharides and oligosaccharides (Sujak et al., 2006). The content in NFE decreases after dehulling, from 47.8% to 38.3% of DM in kernel (Saez et al., 2015).
The high energy value of lupine seeds is due to high fat content, thus Bartkiene et al. (2016) established the metabolisable energy value to be 369 kcal/100 g for *L. luteus* and 378 kcal/100 g for *L. angustifolius* seeds. The content in non-starch polisaccharides (NSP) depreciates the energy value of lupine seeds for monogastrics animals, demonstrating that for each percentage of NSP presence in lupine seeds administrated to broiler chickens, a decrease with 0.288 MJ of metabolisable energy occurs (Sipsas and Glencross, 2005).
Gross energy suffers minor changes after seeds dehulling, increasing by up 3.4 percentage points for *L. albus* (Saez et al., 2015). King et al (2000) highlight that by dehulling *L. angustifolius* seeds, the digestible energy increases from 15.81 to 16.85 MJ/kg DM. According to Nalle et al. (2010), the AME_N (nitrogen-corrected apparent metabolisable energy) improves with 30% by dehulling the *L. angustifolius* seeds and with 52% according to Mera-Zúñiga et al. (2018).

Biological value of lupine seeds protein

The nutritional value of lupine seed protein is reflected by the essential amino acids content. The value of lupine protein is comparable to the values of soybean meal protein, peas or other legume grains (Sujak et al., 2006). Whole sweet lupine seeds are characterized by a variable amino acid profile, being rich in lysine, leucine, valine, threonine, isoleucine, serine, but deficitary in tryptophan and sulfur amino acids such as methionine and cystine (Table 2). Among the non-essential amino acids, the highest content is in glutamine, aspartic acid and arginine (Nalle et al., 2011).

Table 2. Effect of dehulling on the amino acids content (g/16 g N) of lupine seeds from the low alkaloid varieties

AA	*L. albus*				*L. luteus*		*L. angustifolius*	
	1*		2*		3*	4*	5*	
	WS	DS	WS	DS	WS	DS	WS	DS
Essential AA								
Lys	3,63	3,61	5,46	5,19	5,40	3,08	4,69	4,47
Met	0,76	0,77	1,12	1,15	0,20	0,55	0,62	0,62
Thr	3,49	3,49	3,72	3,63	3,80	3,52	3,30	3,39
Ile	3,58	3,56	3,90	3,97	3,10	3,66	3,37	3,46
Val	3,69	3,70	3,74	3,70	3,30	3,41	8,22	8,77
Leu	7,24	7,24	6,26	6,17	8,20	7,80	6,09	6,22
His	2,34	2,35	3,09	2,94	2,80	2,55	2,56	2,47
Phe	3,97	3,98	3,53	3,12	4,00	3,81	3,53	3,60
Non-essential AA								
Arg	9,49	9,46	11,38	11,7	11,5	11,19	8,22	8,77
Cys	1,10	1,12	2,00	2,25	2,00	7,14	9,16	9,42
Asp	10,42	10,41	10,58	10,5	11,6	10,71	3,53	3,56
Ser	5,46	5,45	5,07	5,12	6,50	5,24	3,95	4,13
Tyr	4,08	4,08	2,39	2,13	2,30	3,08	4,31	4,37
Glu	19,92	19,89	15,10	14,7	23,7	24,63	3,92	3,96
Pro	4,17	4,17	4,18	4,20	2,30	6,63	18,45	19,7
Gly	4,31	4,31	3,79	3,76	4,50	3,96	1,29	1,20
Ala	3,41	3,42	3,20	3,17	3,60	3,37	3,82	3,82

Note: 1 - Laudadio and Tufarelli (2011a); 2 - Písaříková et al. (2008); 3 - Pastor-Cavada et al. (2009); 4 - Smith et al. (2007); 5 - Nalle et al. (2010); * - value adapted at g/16g N; AA - Amino Acids; WS - whole seed; DS - dehulled seed.

As resulting from table 2, the effect of dehulling on the amino acid composition of lupine seeds is a slight improvement in their content. The increase of protein content in amino acids as a result of dehulling depends on species and cultivars (Nalle et al., 2010). According to Mera-Zúñiga et al. (2018), the dehulling process of lupine seeds determines an increase in essential amino acid content by 0.7-0.48 percents. Previously, Písaříková et al. (2009) mention in a review that in dehulled lupine seeds the level of protein and amino acids increases by even 13%. Improving the amino acid content in dehulled seeds also determines the improvement of the biological value of proteins (Straková et al., 2006).
However, given the limited content of lupine seed proteins in some essential amino acids (methionine, tryptophan), this may be used in the nutrition of monogastric animals in association with others plant proteins based on their complementarity and/or by the use of synthetic amino acids (Písaříková et al., 2008).

Fatty acids content in lupine seeds

The fatty acid profile of lupine seeds shows the high content in polyunsaturated fatty acids

(especially linoleic and linolenic acids) as well as oleic acid (Table 3), which attributes to lupine seeds the quality for a valuable source of essential fatty acids. Fatty acid content varies with species, cultivars and environmental conditions (Rybiński et al., 2018).

Table 3. Effect of dehulling on the fatty acid content (% of FAME) of lupine seeds from the low alkaloid varieties

Fatty acids (FA)	*L. albus*		*L. luteus*		*L. angustifolius*	
	1	2	3	4	3	4
	WS	DS	WS	WS	WS	WS
Palmitic C16:0	5.86	6.95	7.39	5.6	11.06	12.47
Stearic C18:0	2.98	1.63	2.87	2.70	5.73	6.40
Oleic C18:1 n-9	47.65	57.72	22.62	24.1	36.53	34.94
Linoleic C18:2 n-6	19.97	12.52	49.19	47.7	36.68	37.64
Arachidic C20:0	0.90	-	2.88	2.58	1.79	0.76
α-linolenic C18:3 n-3	10.93	7.89	7.98	6.86	4.32	4.53
Behenic C22:0	3.15	-	-	5.61	-	1.40
Gadoleic C20:1 n-9	6.82	-	1.88	1.55	0.34	0.23
Erucic C22:1 n-9	1.42	-	0.61	0.70	0.17	0.03
Σ SFA	12.89	9.96	5.80	17.5	11.10	21.78
Σ MUFA	56.21	65.54	13.3	26.4	23.70	35.26
Σ PUFA	30.90	24.50	30.70	55.2	27.40	42.3
n-3/n-6 FA	0.55	1.10	-	7.12	-	8.34

Note: 1 - Mierlita et al. (2018); 2 - Volek et al. (2018); 3 - Grela et al. 2017; 4 - Andrzejewska et al. (2016); SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids; FAME - mettyl esterified fatty acids; n-3/n-6 = linolenic/linoleic fatty acids; WS - whole seed; DS - dehulled seed.

The nutritional value of lipids is represented by the content and profile of fatty acids as well as by their report. In *L. angustifolius* and *L. luteus* whole seeds, linoleic acid has the highest level, followed by oleic acid, but oleic acid followed by linoleic have the highest level in *L. albus* (Boschin et al., 2008; Andrzejewska et al., 2016). According to Chiofalo et al. (2012), the high content in gadoleic and oleic acid is specific for *L. albus*.

As shown in the table 3, monounsaturated fatty acids (MUFAs) are well represented in whole seeds of *L. albus* (56.21%) and polyunsaturated acids (PUFAs) in whole seeds of *L. luteus* and *L. angustifolius* (27.4% - 55.24%). According to Musco et al (2017) white lupine is representative for the high MUFA content (54.4% of FAME), yellow lupine is for PUFA (56.9% of FAME) and blue lupine is for SFA (24.8% of FAME). Polyunsaturated fatty acids are essential nutrients and the linolenic/linoleic ratio (ω-3/ω-6) is important for animal nutrition (Suchy et al., 2008).

Uzun et al. (2007) found that the high proportion of oleic fatty acid (47.6% of FAME) from whole white lupin seeds is negatively correlated with linoleic fatty acid (20.3% of FAME) and the latter was positively correlated with the fat content of the seeds.

Dehulling lupine seeds contributes to a slight improvement of some fatty acid levels and table 3 reveald that the MUFA content (65.54% of FAME) is high in dehulled *L. albus* seeds. The eicosenoic acid level in *L. albus* dehulled seeds is 5.21% of FAME and palmitoleic acid represents 0.35% of FAME (Volek et al., 2018). According to Suchy et al. (2008), after dehulling, the content of fatty acids presents an increase with 20.04-25.18% in *L. albus*, *L. luteus*, *L. angustifolius* dehulled seeds.

Content in minerals and vitamins of lupine seed

The vitamin and mineral content in low-alkaloid lupine seeds is significantly influenced by species and cultivars (Bartkiene et al., 2016; Karnpanit et al., 2017). Lupine seeds are characterized by an appreciable content in minerals such as Ca, P and K (Table 4).

Table 4. The content of minerals in whole lupine seeds (g/kg DM) from the low alkaloid varieties

Minerals	*L. albus*		*L. luteus*		*L. angustifolius*	
	1	2	1	3	1	3
Ca	3.6	2.38	3.3	2.95	3.4	3.33
K	11.9	11.0	12.8	12.6	11.0	13.4
P	4.8	5.23	8.1	7.47	4.9	6.84
Mg	1.9	1.35	3.1	3.14	2.0	2.10
Na	0.118	0.17	0.096	0.08	0.094	0.08
Fe	0.059	0.038	0.095	0.13	0.061	0.07
Mn	0.672	0.252	0.065	0.08	0.041	0.13
Zn	0.047	0.043	0.077	0.070	0.0391	0.070
Cu	0.0061	0.0082	0.010	0.020	0.0046	0.040

Note: 1 - Wasilewko (1999); 2 - Grela et al. (2017); 3 - Rutkowski et al. (2015).

According to Grela et al. (2017), P and K are well represented in *L. angustifolius* and *L. luteus*; Ca is found in higher quantities in *L. albus* and the high content in Mg is characteristic for *L. luteus*.

Phytic acid is the main factor that reduces the bioavailability of minerals in leguminous grains, because it is bound in non-degradable

phytates due to chelation with Cu, Fe, Zn, Ca, Mg, K cations (Gupta et al., 2015). The phytic acid content of whole lupine seeds (~ 1%) reduces the bioavailability of phosphorus (Kasprowicz et al., 2016). For *L. angustifolius*, it has been demonstrated that the dehulling of seeds produces a reduction in total phosphorus from 3.5 to 1.0 g/kg DM (Kim et al., 2012).
Beacause of the hulls store the most Ca, the dehulling process of lupine leads to a decrease from 0.36 % to 0.24 % of DM, but the level in P increases from 0.61% to 0.77% of DM (Mera-Zúñiga et al., 2018) due to its presence in the kernel (Večerek et al., 2008). Karnpanit et al. (2017) highlight that although the level of Ca decrease in *L. angustifolius* seeds by dehulling, its bioavailability increases from 6.0 to 10.5 %.
The content in vitamins of whole *L. albus* seeds is 3.9 mg/kg DM for thiamine, 2.3 mg/kg DM for riboflavin and 39.1 mg/kg DM for niacin (Erbaş et al., 2005). The niacin level of lupine seeds is higher compared with that of soybean meal (32.6 mg/kg DM) (Erbaş et al., 2005). Martínez-Villaluenga et al. (2006) show for *L. albus* and *L. luteus* a vitamin E content between 0.19-0.47 mg/100 g DM, a vitamin C content of 2.56-6.48 mg/100 g DM and a riboflavin content of 0.37-0.85 mg/100 g DM.

RESULTS AND DISCUSSIONS

ANTINUTRITIONAL FACTORS OF LUPINE SEEDS FROM LOW-ALKALOID VARIETIES

New varieties of sweet lupine seeds are characterized by a low content of alkaloids (less than 0.02%), but there are certain antinutritional factors such as cellulose (NDF and ADF), non-starch polysaccharides and oligosaccharides that reduce the value of nutrients from lupine seeds, especially for monogastric animals (Musco et al., 2017). These compounds cannot be degraded by the endogenous enzymes of monogastrics due to the absence of specific enzymes.

Non-starch polysaccharides (NSP)

Lupine seeds are characterized by a significant content in antinutritional substances such as non-starch polysaccharides, bioactive compounds that cannot be efficiently metabolized by endogenous enzymes of monogastric animals (Smulikowska et al., 2014; Hejdysz et al., 2018b). In poultry nutrition, NSP have a low digestibility and reduce the efficiency of feed utilization, representing the most important antinutritive factor from lupine seeds (Kaczmarek et al., 2016b). Their content in whole seeds is up to 40% of DM (Petterson, 2000).
Lupine seeds contain 22-24% hulls and 76-78% kernel (Van Barneveld, 1997; Večerek et al., 2008). Polysaccharides such as cellulose, hemicellulose, arabinoxylans and pectins are found in the structure of the hull, and carbohydrates such as non-structural cell wall polysaccharides (arabinose, galactose, uronic acid) are found in the kernel (Table 5). The most important cell wall polysaccharide is cellulose (48.1% of DM, in hulls) (Knudsen, 2014), amounting in whole lupine seeds to 167-192 g/kg DM (Gdala and Buraczewska, 1996).

Table 5. Effect of dehulling on the non-starch polysaccharide content (g/kg DM) of lupine seeds

PNA	*L. albus*		*L. luteus*		*L. angustifolius*	
	1	1	2	3	1	1
	WS	DS	WS	WS	WS	DS
Rhamnose	2.4	1.0	1.1	-	2.8	1.2
Arabinose	44.9	38.1	34.8	39.4	45.7	43.9
Xylose	37.6	17.5	26.3	34.9	27.9	22.3
Mannose	15.2	9.1	3.5	4.5	16.0	12.5
Galactose	154.4	142.6	52.2	58.2	190.1	191.8
Glucose	95.6	36.0	85.0	126	115.8	47.4
Total PNA	-	-	240.2	302.2	-	-

Note: 1 - King et al. (2000); 2 - Olkowski (2011); 3 - Krawczyk et al. (2015b); PNA - non-starch polysaccharides; WS - whole seed; DS - dehulled seed.

In kernel of lupine seeds there are some fractions of soluble NSP and oligosaccharides that favor fermentation processes in the small intestine, thus reducing the efficiency utilisation of feed energy from monogastric animal diets (King et al., 2000, Lee et al., 2016). The soluble fraction is considered to have an antinutritional effect characterized by increasing the intestinal passage time and gives a viscosity consistency to the digesta (Konieczka and Smulikowska, 2017). The content of lupine in soluble NSP is 5-10% (Evans et al., 1993; Kocher et al., 2000). Insoluble fractions (approximately 30%) do not have major negative effects on the utilisation of

nutrients by monogastric animals, with even positive effects on intestinal motility (Petterson, 2016).
According to the results reported by King et al. (2000), the dehulling process reduces the content of NSP from lupine seeds (Table 5). Nalle et al. (2010) found that, by dehulling, a reduction in the content of *L. angustifolius* seeds in soluble NSP (from 31.5 to 19.3 g/kg) and insoluble (from 463 to 240 g/kg) is produced. For poultry, NSP lead to an increase of intestinal digesta viscosity (especially in the ileum) and excretion humidity, reducing the ingestion and the degree of feed utilization and on the other hand, alters the microclimate parameters of poultry breeding shelters (Kocher et al., 2000; Steenfeldt et al., 2003).

Raffinose family oligosaccharides (RFO)
The main oligosaccharides present in lupine are raffinose, stachyose, verbascose and sucrose (Table 6). The content in seeds differs according to species, varieties and year of cultivation (Karnpanit et al., 2016).
Oligosaccharides are found in high proportions in lupine seeds, presenting variations between 5.3-12.3% of DM, and *L. luteus* is the species with highest content of oligosaccharides (9.46-12.3% DM) (Martínez-Villaluenga et al., 2005). According to Stanek et al (2015), the oligosaccharide content in *L. angustifolius* varies between 63.2 and 73.6 g/kg DM.

Table 6. The raffinose family oligosaccharides content (g/kg DM) of lupine seeds from low alkaloid varieties

RFO	*L. albus*		*L. luteus*		*L. angustifolius*	
	WS	DS	WS	WS	WS	WS
	1	1	2	3	4	5
Saccharose	24.9	26.3	19.68	-	-	-
Raffinose	7.02	7.92	10.91	11.0	12.0	13.0
Stachylose	43.4	59.1	74.15	49.4	56.1	53.0
Verbascose	7.47	10.3	45.85	25.3	19.6	19.0
Total RFO	83.5	104.3	130.91	85.7	87.7	85.0

Note: 1 - Písaříková et al. (2008); 2 - Chilomer et al. (2012); 3 - Rutkowski et al. (2016); 4 - Hejdysz et al. (2018a); 5 - Karnpanit et al. (2016). RFO - raffinose family oligosaccharides; WS - whole seed; DS - dehulled seed.

According to the values from Table 6, there is no positive change in the content of oligosaccharides by dehulling lupine seeds. An increase of their level is observable, but it may be due to the method of dehulling (wet or dry method) (Karnpanit et al., 2016). According to Brenes et al. (2003), the *L. albus* dehulled seeds have a higher content in total RFO than whole seeds: 63.4 mg/g vs. 58.8 mg/g. Lupine whole seeds contain more oligosaccharides (8.26%) than soybean seeds (6.96%), sunflower (1.73%) or rape (1.79%) (Geigerová et al., 2017).

Alkaloid content
Currently, sweet lupine varieties of *L. luteus*, *L. albus* and *L. angustifolius* species with low-alkaloids content are cultivated (Frick et al., 2017). Therefore, the inconvenience generated by the presence of a high level of alkaloids has been eliminated, which has led to the progressive use of lupine in animal feeds.
However, the content of alkaloids in lupine seeds is influenced by some factors such as species, cultivars, geographical area or pedo-climatic conditions (Güémes-Vera et al., 2012; Maknickiene et al., 2013; Romeo et al., 2018). In the whole seeds of *L. albus*, the total alkaloid content ranges from 3.63 to 165 mg/100 g (Calabrò et al., 2015). In *L. luteus* the level of alkaloids is 42.6-58.5 mg/kg (Kaczmarek et al., 2016b) and in *L. angustfolius* it is 0.36-0.56 mg/kg (Stanek et al., 2015). The main alkaloids from lupine seeds are lupanine (34.6 mg/100 g in *L. albus*), sparteine (1.01 mg/100 g in *L. luteus* and 1.24 mg/100 g in *L. angustifolius*), angustifoline (1.28 mg/100 g in *L. albus* and 0.45 mg/100 g in *L. angustifolius*), 13-α-hydroxylupanine (2.54 mg/100 g in *L. albus* and 1.34 mg/100 g in *L. angustifolius*) (Musco et al., 2017).

THE UTILIZATION OF SWEET LUPINE SEEDS IN THE MONOGASTRIC ANIMAL FEEDING

Use of lupine seeds in poultry feeding
Attempts to use lupine seeds as the main source of protein in poultry feeding are an objective of researches in the field. Poultry have the ability to efficiently use the amino acids and energy of lupine, but the antinutritive factors minimize their biological potential to valorize their nutrients. Studies are primarily focused to determine the bioproductive effects of lupine as a result of the substitution of protein from soybean meal in different proportions. Soybean

meal is the most important protein source in poultry feeding.

Use of lupine seeds in broiler chicken feeding

Raw lupine seeds can be administered to broiler chicken feed in a proportion of 15% to 25, without exerting negative effects on bioproductive performances (Brenes et al., 2002; Hejdysz et al., 2018b). According to some studies conducted by Straková et al. (2010), the administration of 8.67 to 31.03% lupine seeds in broiler chicken (1-42 days) feeds has been shown to improve the quality of breast and thigh muscles, with an increase in the MUFA content of lipids, especially in oleic acid ($p < 0.01$), compared to chickens that had soybean meal in their diet. Authors conclude that the inclusion of lupine in broiler feeding increases the nutritional value of the meat.

Most of studies show that the use of lupine in broiler chicken feeding over the tolerance limit of the organism mainly affects the bioproductive performance and physiological status. In this respect, Brenes et al. (2002) highlight that the inclusion of lupine (*L. albus*) in 35% and 45% in broiler diet at the whole growing period, determines the depreciation of growth performances compared to the group in which soybean meal was added. Kaczmarek et al. (2016a) observed that the inclusion of yellow lupine meal over 150 g/kg in diets of 1-35 days old chickens negatively affects growth performances (body weight gain, feed consumption) compared to the control group (whith soybean meal) and causes a linear decrease of AME_N and ileal digestibility of protein, fat and starch from the diet. The use of whole blue lupine seeds in quantities up to 250 g/kg combined fodder, leads to an increase in the ileal viscosity directly proportional with the dose of lupine inclusion, for 1-35 days old broiler chickens (Hejdysz et al., 2018c). Also, Smulikowska et al (2014) observed that the inclusion of 25% *L. angustifolius* seeds in combined fodder for 36 days old broiler chickens, determines a significant increase of the ileal viscosity.

In order to diminish the effects caused by the antinutritive factors, it has been shown that some methods such as dehulling the seeds and/or supplementation of feed with specific enzymes increase the organism tolerance and the bioproductive efficiency of lupine (Annison et al., 1996; Orda et al., 2006). The use of dehulled lupine seeds in broiler feeds has been shown to reduce the negative effects of antinutritive substances (Guillamón et al., 2008; Nalle et al., 2010). Brenes et al. (2003) found an improvement with 68 percent for oligosaccharides ileal digestibility in the case of *L. albus* dehulled seed usage in the diet of broiler chickens with the age of 14-18 days, compared to the use of whole seeds. Both dehulling and enzyme addition in the combined feed significantly improved ($p<0.05$) the weight gain of chickens, feed conversion and protein digestibility. Mieczkowska et al. (2005) observed that after dehulling lupine seeds, the ileal viscosity of chickens decreased with 37%.

Diaz et al. (2006) found that the administration of 300 g/kg of dehulled lupine in broiler chicken diets significantly reduced ($p<0.01$) feed consumption throughout the growing period compared to the similar amount in whole form, and to the administration of soybean meal in the control group.

Laudadio and Tufarelli (2011a) established the reduction in fat deposition ($p<0.05$) and the increase of water-holding capacity in breast and drumstick meat (juiciness indicator) for 14-49 days of broiler growing period, in which the feed incorpored dehulled *L. albus* seeds (240 g/kg). Compared to the control group where soybean meal was administered (195 g/kg), the dehulling of lupine did not modify the dressing percentage, or the breast and drumstick percentages.

According to Mera-Zúñiga et al. (2018), the total substitution of soybean meal from broiler feed with *L. angustifolius* dehulled seeds, throughout the whole growing period, can generate a similar weight gain and even a better feed conversion ($p < 0.05$) if the specific enzymes are used in the feed compound.

Use of lupine seeds in laying hens feeding

Numerous studies reveal the bioproductive effects resulted by the different inclusions of lupine seeds in the feed compound of laying hens, highlighting the possibility of improving the degree of seed utilization by applying a subsequent initial physic and/or enzymatic treatment to inactivate or eliminate the major antinutritional factors.

Studies show that whole lupine can be admitted in the laying hens diet up to 20% (Lee et al.,

2016) or even 25% (in feed) if combined fodder is supplemented with methionine (amino acid deficitary in lupine) (Hammershøj and Steenfeldt, 2005). The results obtained by Drażbo et al. (2014) indicate that whole seeds of *L. angustifolius* can be introduced up to 20% in the feed of laying hens in the 18-38 weeks period without affecting the egg production. These authors show that, regarding the quality of eggs, a substitution with 10-20% of soybean meal with whole blue lupine seeds contributes to an increase in C18:3 n-3 and C18:2 n-6 acids and total polyunsaturated fatty acids from the yolk lipids, improving the PUFA n-6/n-3 ratio. Regarding the physical properties of the eggs, the authors report a significant increase in the shell weight and the breaking strength.

Krawczyk et al. (2015a) observed an improvement in PUFA from yolk lipids as well as certain sensory parameters (intensified yolk color) when yellow lupine flour (whole seeds) was added up to 30% in feed compound of laying hens of 32-48 weeks age. Authors demonstrate that the bioproductive performances (feed consumption, feed conversion rate, eggs production, egg weight) and the characteristics of egg components (thickness and strength of the shell, relative weight of yolk, the quality of the egg expressed as Haugh units) are not affected. Park et al (2016) conclude that supplementing the dites based on soybean meal with blue lupine (whole seeds) up to 22% can improve the egg production of hens ($p<0.05$) without affecting daily feed intake .

Zduńczyk et al (2014a) report that 20% blue lupin meal (whole seeds) used in the laying hens feed (18-42 weeks growing period) exercite positive effects on the majority of microbiota from the caecum, consisiting in the significant increases of beneficial bacteria from the genera *Bifidobacterium*, *Lactobacillus* or *Enterococcus* and a decrease in the number of potential pathogenens such as *E. coli*, *Prevotella*, *Bacteroides* and *Porphyromonas*, compared to the hens fed with soybean meal. In addition, there was a significant contribution of lupine in the increases of cecal microbiota enzyme activities such as α-arabinopyranosidase, α- and β-glucosidase, α- and β-galactosidase.

Research conducted by Laudadio and Tufarelli (2011b) indicates that dehulled and micronised lupines (*L. albus*) represent a suitable ingredient for laying hens production (18-28 weeks) and the complete replacement of soybean meal (150 g/kg) with lupine (180 g/kg) in the combined fodder produced similar bioproductive performances.

Use of lupine seeds in feeding other species of poultry

Usage of whole *L. luteus* (Zduńczyk et al., 2014b) and *L. angustifolius* (Mikulski et al., 2014) seeds in the feeding of turkeys (aged 13 to 18 weeks) as a protein substitute for soybean meal in proportions up to 18%, has been shown to not affect negatively the bioproductive performances, carcass properties or sensorial indices of the meat.

In feeding of turkeys in the first 4 weeks of growth, lupine meal (from whole seeds) may be included up to 16% without affecting the bioproductive performances, and in the following growth phases it may be included in the diet up to 24%, thus successfully substituting soybean meal (Krawczyk et al., 2015b). The authors point out that the presence of lupine in compound feed has led to a significant decrease in the concentration of saturated fatty acids from lipids. Compared to the control group (with soybean meal), in groups where lupin seeds was utilized up to 24%, a significant increase ($p<0.001$) of polyunsaturated fatty acids in lipids, including linoleic acid and linolenic acid was found, without altering the PUFA n-6/n-3 ratio, but improving the value of the atherogenic and thrombogenic indices.

In the feeding of 1 to 40 days-old ducks, substitution of soybean meal with *L. albus* whole seeds in a 50% proportion in the diet has not been shown to significantly improve the live weight of the ducks, but has increased the content of Lactobacilli and Bifidobacteria from the caecum ($p <0.05$) (Geigerová et al., 2017).

In the Japanese quail diets for 1 to 42 days-old, the usage of 15% white lupine seeds in combined fodder proved to be more effective in dehulled form than whole, but compared to the group that benefited of soybean meal as a protein source in the diet, the bioproductive effects of lupine were insignificant (Arslan and Seker, 2002).

Use of lupine seeds in swine feeding

Data from the scientific literature on the optimal inclusion and efficiency of using lupine seeds in the diets of swine are characterized by controversial results of bioproductive effects.

In the feeding of young pigs (10-20 kg) lupine is recommended to be used with caution due to the incomplete development of thier digestive system (McNiven and Casteli, 1995). According to Kim et al. (2011), the optimum inclusion level of whole lupine seeds (*L. angustifolius*) in the diet of growing pigs is 5 - 10% to weaners, 20% to growers and up to 35% in the fattening period. The presence of white lupine in the swine feed is associated with changes in the fatty acid structure of intramuscular and storage fat, so the major fatty acid in white lupine (oleic acid) was found in significant amounts in the intramuscular and storage fats, together with the linoleic and linolenic acid (Petterson et al., 2000).

Froidmont et al. (2005) suggest that α-galactosides are the main antinutritive factor in lupine seeds for the growth and finishing phase of the swine, due to the binding of nutrients in non-digestible complexes. Also, α-galactosides cannot be efficiently degraded in the small intestine of pigs due to an α-galactosidase deficiency (Gdala et al., 1997).

Kasprowicz et al. (2016) mention that whole seeds of *L. angustifolius* may partly replace soybean meal in feeds for starter, growth and fattening (20 - 105 kg body weight) phases, without affecting the bioproductive parameters, noting that in the starter phase better results are obtained by substituting soybean meal with 20% lupine. Also, Zralý et al. (2007) mention that soybean meal can be completely replaced with lupine (whole) in diets for fattening pigs with the condition of equilibration the ratio in essential amino acids such as methionine, according to the requirements of the category.

Dehulled white lupine seeds may substitute partially or totally (100%) soybean meal in the starter, growth and finishing phases without significantly affecting the bioproductive performances or nutritional parameters of the meat (Zralý et al., 2008).

Písaříková et al. (2008) mention that by a complete replacement of protein from soybean meal in the basal diet of growing pigs with protein from dehulled white lupin, similar bioproductive performances can be obtained. Also, compared to the control group, the digestibilities of organic matter ($p<0.01$), crude protein ($p<0.05$), crude fat and crude cellulose ($p<0.01$), N-free extract ($p<0.01$) NDF, ADF and cellulose ($p<0.01$) are increasing. In addition, the authors report that the digestibility of threonine ($p<0.01$) and lysine was higher, whereas for methionine it was lower.

According to Prandini et al. (2010), *L. albus* dehulled seeds can be included in weaned piglet (28 - 70 days) diets in a dose of 170 g/kg without significantly affecting the growth performances (body weight gain, feed consumtion and feed conversion index) compared with piglets fed with soybean meal. Kim et al. (2012) report the possibility of using *L. angustifolius* dehulled seeds as a substitute for whey and skim milk powder in weaner pigs, because similar growth performances are achieved up to a replacement of 75% (180 g/kg) lupine in the diet.

CONCLUSIONS

In conclusion, the lupine species from low-alkaloid varieties represent a promising alternative source of protein to soybean meal protein in monogastric animal nutrition. Studies highlight that the process of seed dehulling significantly improves the nutritional value of lupine. Researches on the bioproductive efficiency of whole lupine seeds in poultry and swine feeding highlight the possibility of inclusion of lupine seeds in limited proportions in the combined fodder, as soybean meal replacement. Seed dehulling is a method that greatly improves the bioproductive efficiency of lupine utilisation in monogastric animals, increasing the organism tolerance. Thus, depending on the species, age category and direction of exploitation, the dehulled lupine seeds can substitute in larger proportions the soybean meal from the feed, with the condition of balancing the ration in deficient amino acids.

REFERENCES

Andrzejewska, J., Stanisław, I., Paweł, B. (2016). Oil content and fatty acid profile in seeds of polish breeding lines and cultivars of legumes. *Acta Scientiarum Polonorum Agricultura*, 15(2), 3-13.

Annison, G., Hughes, R.J., Choct, M. (1996). Effects of enzyme supplementation on the nutritive value of dehulled lupins. *British Poultry Science*, 37(1), 157–172.

Arslan, C., Seker, E (2002). Effects of processed white lupin seed (*Lupinus albus* L.) on growth performance of japanese quail. *Revue de Médecine Vétérinaire,* 153(10), 643-646.

Bähr, M., Fechner, A., Hasenkopf, K., Mittermaier, S., Jahreis, G. (2014). Chemical composition of dehulled seeds of selected lupin cultivars in comparison to pea and soya bean. *Food Science and Technology*, 59(1), 587–590.

Bartkiene E., Bartkevics, V., Starkute, V., Krungleviciute, V., Cizeikiene, D., Zadeike, D., Juodeikiene, G., Maknickiene, Z. (2016). Chemical composition and nutritional value of seeds of *Lupinus luteus* L., *L. angustifolius* L. and new hybrid lines of *L. angustifolius* L. *Zemdirbyste-Agriculture*, 103(1), 107–114.

Batterham, E. S., Andersen, L. M., Lowe, R. F., Darnell, R. E. (1986). Nutritional value of lupin (*Lupinus albus*)-seed meal for growing pigs: availability of lysine, effect of autoclaving and net energy content. *British Journal of Nutrition*, 56(03), 645-659.

Boschin, G., D'Agostina, A., Annicchiarico, P., Arnoldi, A. (2008). Effect of genotype and environment on fatty acid composition of *Lupinus albus* L. seed. *Food Chemistry*, 108(2), 600–606.

Brenes, A., Slominski, B., Marquardt, R., Guenter, W., Viveros, A. (2003). Effect of enzyme addition on the digestibilities of cell wall polysaccharides and oligosaccharides from whole, dehulled, and ethanol-extracted white lupins in chickens. *Poultry Science*, 82(11), 1716–1725.

Brenes, A., Marquardt, R. R., Guenter, W., Viveros, A. (2002). Effect of enzyme addition on the performance and gastrointestinal tract size of chicks fed lupin seed and their fractions. *Poultry Science,* 81, 670–678.

Calabrò, S., Cutrignelli, M. I., Lo Presti, V., Tudisco, R., Chiofalo, V., Grossi, M., Infascelli, F., Chiofalo, B. (2015). Characterization and effect of year of harvest on the nutritional properties of three varieties of white lupine (*Lupinus albus* L.). *Journal of the Science of Food and Agriculture*, 95(15), 3127–3136.

Chilomer, K., Kasprowicz, P. M., Gulewicz, P., Frankiewicz, A. (2012). The influence of lupin seed germination on the chemical composition and standardized ileal digestibility of protein and amino acids in pigs. *Journal of Animal Physiology and Animal Nutrition*, 97(4), 639–646.

Chiofalo, B., Lo Presti, V., Chiofalo, V., Gresta, F. (2012). The productive traits, fatty acid profile and nutritional indices of three lupin (*Lupinus spp*.) species cultivated in a Mediterranean environment for the livestock. *Animal Feed Science and Technology*, 171, 230–239.

De Visser, C. L. M., Schreuder, R., Stoddard, F. (2014). The EU's dependency on soya bean import for the animal feed industry and potential for EU produced alternatives. *Oil seeds Fats Crops Lipids,* 21(4), D407.

De Vries, S., Pustjens, A. M., Scols, H. A., Hendriks, W. H., Gerrits, W. J. J. (2012). Improving digestive utilization of fiber-rich feedstuffs in pigs and poultry byprocessing and enzyme technologies: a review. *Animal Feed Science and Technology*, 178, 123–138.

Diaz, D., Morlacchini, M., Masoero, F., Moschini, M., Fusconi, G., Piva, G. (2006). Pea seeds (*Pisum sativum*), faba beans (*Vicia faba* var. minor) and lupin seeds *(Lupinus albus* var. multitalia) as protein sources in broiler diets: effect of extrusion on growth performance. *Italian Journal of Animal Science*, 5(1), 43–53.

Drażbo, A., Mikulski, D., Zduńczyk, Z., Szmatowicz, B., Rutkowski, A., Jankowski, J. (2014). Fatty acid composition, physicochemical and sensory properties of eggs from laying hens fed diets containing blue lupine seeds. *European Poultry Science*, 78, 1-14.

Drummond, C. S. (2008). Diversification of *Lupinus* (Leguminosae) in the western New World: derived evolution of perennial life history and colonization ofmontane habitats. *Molecular Phylogenetics and Evolution*, 4(2), 408–21.

Drummond, C. S., Eastwood, R. J., Miotto, S. T. S., Hughes, C. E. (2012). Multiple continental radiations and correlates of diversification in *Lupinus* (Leguminosae): testing for key innovation with incomplete taxon sampling. *Systematic Biology*. 61, 443–460.

Embaby, H. E. S. (2010). Effect of soaking, dehulling, and cooking methods on certain antinutrients and in vitro protein digestibility of bitter and sweet lupin seeds. *Food Science and Biotechnology*, 19(4), 1055–1062.

Erbaş, M., Certel, M., Uslu, M. K. (2005). Some chemical properties of white lupin seeds (*Lupinus albus* L.). *Food Chemistry*, 89(3), 341–345.

Evans, A. J., Cheung, P. C. K., Cheetham, N. W. H., (1993). The carbohydrate composition of cotyledons and hulls of cultivars of *Lupinus angustifolius* from Western Australia. *Journal of the Science of Food and Agriculture*, 61: 189–194.

Frick, K. M., Kamphuis, L. G., Siddique, K. H. M., Singh, K. B., Foley, R. C. (2017). Quinolizidine alkaloid biosynthesis in *Lupins* and prospects for grain quality improvement. *Frontiers in Plant Science*, 8(87), 1-12.

Froidmont, E., Wathelet, B., Beckers, Y., Romnee, J. M., Dehareng, F. (2005). Improvement of lupin seed valorisation by the pig with the addition of α-galactosidase in the feed and the choice of a suited variety. *Biotechnology, Agronomy, Society and Environment*, 9(4), 225–235.

Gdala, J., Buraczewska, L. (1996). Chemical composition and carbohydrate content of several varieties of faba bean and pea seeds. *Journal of Animal and Feed Sciences*, 6(1), 123–135.

Gdala, J., Jansman, A. J. M., Buraczewska, L., Huisman, J., van Leeuwen, P. (1997). The influence of α-galactosidase supplementation on the ileal digestibility of lupin seed carbohydrates and dietary protein in young pigs. *Animal Feed Science and Technology*, 67(2), 115–125.

Geigerová, M., Švejstil, R., Skřivanová, E., Straková, Ev., Suchý, P. (2017). Effect of dietary lupin (*Lupinus albus*) on the gastrointestinal microbiota composition in broiler chickens and ducks. *Czech Journal of Animal Science,* 62(9), 369–376.

Glencross, B., Hawkins, W., Veitch, C., Dods, K., Mccafferty, P., Hauler, R. (2007). The influence of dehulling efficiency on the digestible value of lupin (*Lupinus angustifolius*) kernel meal when fed to rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition*, 13(6), 462–470.

Grela, E. R., Samolińska, W., Kiczorowska, B., Klebaniuk, R., Kiczorowski, P. (2017). Content of minerals and fatty acids and their correlation with phytochemical compounds and antioxidant activity of leguminous seeds. *Biological Trace Element Research*, 180(2), 338–348.

Güémes-Vera, N., Martinez-H. J., Hernandez-C. J. F., Yanez-F. J., Totosaus, A. (2012). Comparison of chemical composition and protein digestibility, carotenoids, tanins and alkaloids content of wild *Lupinus* varieties flour. *Pakistan Journal of Nutrition*, 11, 774–780.

Guillamón, E., Pedrosa, M. M., Burbano, C., Cuadrado, C., Sánchez, M., Muzquiz, M. (2008). The trypsin inhibitors present in seed of different grain legume species and cultivar. *Food Chemistry*, 107, 68–74.

Gupta, R. K., Gangoliya, S. S., Singh, N. K. (2015). Reduction of phyticacid and enhancement of bioavailable micronutrients in food grains. *Journal of Food Science and Technology-Mysore*, 52, 676–684.

Hammershøj, M., Steenfeldt, S. (2005). Effects of blue lupin (*Lupinus angustifolius*) in organic layer diets and supplementation with foraging material on egg production and some egg quality parameters. Poultry Science, 84, 723–733.

Hejdysz, M., Kaczmarek, S. A., Kubiś, M., Jamroz, D., Kasprowicz-P. M., Zaworska, A., Rutkowski, A. (2018a). Effect of increasing levels of raw and extruded narrow-leafed lupin seeds in broiler diet on performance parameters, nutrient digestibility and AMEN value of diet. Jo*urnal of Animal and Feed Sciences*, 27(1), 55–64.

Hejdysz, M., Kaczmarek, S. A., Rogiewicz, A., Rutkowski, A. (2018b). Influence of graded dietary levels of meals from three lupin species on the excreta dry matter, intestinal viscosity, excretion of total and free sialic acids, and intestinal morphology of broiler chickens. *Animal Feed Science and Technology*, 241, 223–232.

Hejdysz, M., Kaczmarek, S. A., Rogiewicz, A., Rutkowski, A., (2018c). Influence of graded dietary levels of seeds of three especies of lupin on growth performance, nutrient digestibility, and intestinal morphology of broiler chickens. *Animal Feed Science and Technology*. 241, 223–232.

Henchion, M., Hayes, M., Mullen, A., Fenelon, M., Tiwari, B. (2017). Future protein supply and demand: strategies and factors influencing a sustainable equilibrium. *Foods*, 6(7), 53-59.

Kaczmarek, S. A., Hejdysz, M., Kubiś, M., Rutkowski, A. (2016a). Influence of graded inclusion of white lupin (*Lupinus albus*) meal on performance, nutrient digestibility and intestinal morphology of broiler chickens*, British Poultry Science*, 57(3), 364–374.

Kaczmarek, S. A., Hejdysz, M., Kubis, M., Kasprowicz-P. M., Rutkowski, A. (2016b). The nutritional value of yellow lupin (*Lupinus luteus* L.) for broilers. *Animal Feed Science and Technology*, 222, 43–53.

Karnpanit, W., Coorey, R., Clements, J., Nasar-A. S. M., Khan, M. K., Jayasena, V. (2016). Effect of cultivar, cultivation year and dehulling on raffinose family oligosaccharides in Australian sweet lupin (*Lupinus angustifolius* L.). *International Journal of Food Science & Technology*, 51(6), 1386–1392.

Karnpanit, W., Coorey, R., Clements, J., Benjapong, W., Jayasena, V. (2017). Calcium, Iron, and Zinc bioaccessibilities of Australian sweet lupin (*Lupinus angustifolius* L.) cultivars. *Journal of Agricultural and Food Chemistry*, 65(23), 4722–4727.

Kasprowicz-Potocka, M., Zaworska, A., Kaczmarek, S. A., Rutkowski, A. (2016). The nutritional value of narrow-leafed lupine (*Lupinus angustifolius*) for fattening pigs. *Archives of Animal Nutrition*, 70(3), 209–223.

Kim, J. C., Heo, J. M., Mullan, B. P., Pluske, J. R. (2012). Performance and intestinal responses to dehulling and inclusion level of Australian sweet lupins (*Lupinus angustifolius* L.) in diets for weaner pigs. *Animal Feed Science and Technology*, 172(3-4), 201–209.

Kim, J. C., Mullan, B. P., Heo, J. M., Hansen, C. F., Pluske, J. R. (2009). Decreasing dietary particle size of lupins increases apparent ileal amino acid digestibility and alters fermentation characteristics in the gastrointestinal tract of pigs. *British Journal of Nutrition,* 102(03), 350–360.

Kim, J. C., Mullan, B. P., Nicholls, R. R., Pluske, J. R. (2011). Effect of Australian sweet lupin (*Lupinus angustifolius* L.) inclusion levels and enzyme supplementation on the performance, carcass composition and meat quality of grower/finisher pigs. *Animal Production Science*, 51(1), 37–43.

King, R. H., Dunshea, F. R., Morrish, L., Eason, P. J., van Barneveld, R. J., Mullan, B. P., Campbell, R. G. (2000). The energy value of *Lupinus angustifolius* and *Lupinus albus* for growing pigs. *Animal Feed Science and Technology*, 83(1), 17–30.

Knudsen, K. E. B. (2014). Fiber and nonstarch polysaccharide content and variation in common crops used in broiler diets. *Poultry Science*, 93(9), 2380–2393.

Kocher, A., Choct, M., Hughes, R. J., Broz, J. (2000). Effect of food enzymes on utilisation of lupin carbohydrates by broilers. *British Poultry Science*, 41(1), 75–82.

Konieczka, P., Smulikowska S. (2017). Viscosity negatively affects the nutritional value of blue lupin seeds for broilers. *Animal*, 12(06), 1144–1153.

Krawczyk, M., Mikulski, D., Przywitowski, M., Jankowski J., (2015b). The effect of dietary yellow lupine (*L. Luteus* cv. Baryt) on growth performance, carcass characteristics, meat quality and selected serum parameters of turkeys. *Journal of Animal and Feed Sciences*, 24, 61–70.

Krawczyk, M., Przywitowski, M., Mikulski, D. (2015a). Effect of yellow lupine (*L. luteus*) on the egg yolk fatty acid profile, the physicochemical and sensory properties of eggs, and laying hen performance. *Poultry Science*, 94(6), 1360–1367.

Księżak, J., Staniak, M., Bojarszczuk, J. (2018). Nutrient Contents in yellow lupine (*Lupinus luteus* L.) and blue lupine (*Lupinus angustifolius* L.) cultivars depending on habitat conditions. *Polish Journal of Environmental Studies*, 27(3), 1145-1153.

Laudadio, V., Tufarelli, V. (2011a). Dehulled-micronised lupin (*Lupinus albus* L. cv. Multitalia) as the main protein source for broilers: influence on growth performance, carcass traits and meat fatty acid composition. *Journal of the Science of Food and Agriculture*, 91(11), 2081–2087.

Laudadio, V., Tufarelli, V. (2011b). Influence of substituting dietary soybean meal for dehulled-micronized lupin (*Lupinus albus* cv. Multitalia) on early phase laying hens production and egg quality. *Livestock Science,* 140, 184–188.

Lee, M. R. F., Parkinson, S., Fleming, H. R., Theobald, V. J., Leemans, D. K., Burgess, T. (2016). The potential of blue lupins as a protein source, in the diets of laying hens. *Veterinary and Animal Science*, 1(2), 29–35.

Lucas, M. M., Stoddard, F. L., Annicchiarico, P., Frías, J., Martínez-Villaluenga, C., Sussmann, D., Duranti, M., Seger A., Peter, M. Z., Pueyo, J. J. (2015). The future of lupin as a protein crop in Europe. Frontiers in Plant Science, 6(705), 1-6.

Maknickiene, Z., Asakaviciute, R., Baksiene E., Razukaś A. (2013). Alkaloid content variations in *Lupinus luteus* L. and *Lupinus angustifolius* L. *Archives of Biological Sciences*, 65(1), 107-112.

Martínez-Villaluenga, C., Frías, J., Vidal-Valverde, C. (2005). Raffinose family oligosaccharides and sucrose contents in 13 Spanish lupin cultivars. *Food Chemistry* 91, 645–649.

Martínez-Villaluenga, C., Frías J., Vidal-Valverde, C. (2006). Functional lupin seeds (*Lupinus albus* L. and *Lupinus luteus* L.) after extraction of α-galactosides. *Food Chemistry*, 98(2), 291–299.

Mattila, P., Mäkinen, S., Eurola, M., Jalava, T., Pihlava, J.-M., Hellström, J., Pihlanto, A. (2018). Nutritional value of commercial protein-rich plant products. *Plant foods for human nutrition*, 73(2), 108–115.

McNiven, M. A., Casteli, A. G. (1995). Replacement of soybean meal with lupin seed (*Lupinus albus*) in pig starter diets. *Animal Feed Science and Technology* 52, 333–338.

Mera-Zúñiga, F., Pro-Martínez, A., Zamora-N., J. F., Sosa-M., E., Guerrero-R., J. D., Mendoza-P., S. I., Cuca-G., J. M., López-R., R. M., Chan-D., D., Becerril-P., C. M., Vargas-G., A. J., Bautista-O., J. (2018). Soybean meal substitution by dehulled lupine (*Lupinus angustifolius*) with enzymes in broiler diets. *Asian-Australasian Journal of Animal Sciences*. 32(4), 564-573

Mieczkowska, A., Jansman, A. J. M., Kwakkel, R. P., Smulikowska, S. (2005). Effect of dehulling and α-galactosidase supplement on the ileal digestibility of yellow lupin based diets in broiler chickens and adult roosters. *Journal of Animal and Feed Sciences*, 14(2), 297–304.

Mierlita, D. (2012). Studies on cultivation suitability and nutritional characterization of lupine alkaloid-free varieties. Annals University of Oradea: *Ecotoxicology, Animal Husbandry and Food Industry Technologies*, 11, 501–508.

Mierlita, D., Simeanu, D., Pop, I. M., Criste, F., Pop, C., Simeanu, C., Lup, F. (2018). Chemical composition and nutritional evaluation of the lupine seeds (*Lupinus albus* L.) from low-alkaloid varieties. *Revista de Chimie*, 69(2), 453–458.

Mikulski, D., Zduńczyk, Z., Juśkiewicz, J., Rogiewicz A., Jankowski, J. (2014). The effect of different blue lupine (*L. angustifolius*) inclusion levels on gastrointestinal function, growth performance and meat quality in growing-finishing turkeys. *Animal Feed Science and Technology*, 198, 347–352.

Musco, N., Cutrignelli, M. I., Calabrò, S., Tudisco, R., Infascelli, F., Grazioli, R., Lo Presti, V., Gresta, F., Chiofalo, B. (2017). Comparison of nutritional and antinutritional traits among different species (*Lupinus albus* L., *Lupinus luteus* L., *Lupinus angustifolius* L.) and varieties of lupin seeds. *Journal of Animal Physiology and Animal Nutrition*, 101(6), 1227–1241.

Nalle, C. L., Ravindran, G., Ravindran, V. (2010). Influence of dehulling on the apparent metabolisable energy and ileal amino acid digestibility of grain legumes for broilers. *Journal of the Science of Food and Agriculture*, 90(7), 1227–1231.

Nalle, C. L., Ravindran, V., Ravindran, G. (2011). Nutritional value of narrow-leafed lupin (*Lupinus angustifolius*) for broilers. *British Poultry Science,* 52(6), 775–781.

Olkowski, B. (2011). Lupin as primary protein source in young broiler chicken diets: Effect of enzymes preparations catalyzing degradation of non-starch polysaccharides or phytates. *World Journal of Microbiology and Biotechnology*, 27(2), 341–347.

Olkowski, B. I., Janiuk, I., Jakubczak, A. (2010). Effect of enzyme preparation with activity directed towards degradation of non-starch polysaccharides on yellow lupine seed based diet for young broilers. *Acta Veterinaria Brno*, 79(3), 395–402.

Orda, J., Jamroz, D., Wiliczkiewicz, A., Wertelecki, T., Skorupinska, J., Broz, J. (2006). Effects of increased dietary inclusion of yellow lupins and enzyme supplementation on performance, ileal digestibility of nutrients and microbial status of large intestine in broiler chickens. *Archiv Geflugelkunde*, 70(1), 14-21.

Park, J. H., Lee, S. I., Kim, I. H. (2016). Effects of lupin seed supplementation on egg production performance, and qualitative egg traits in laying hens. *Veterinární medicína*, 61(12), 701–709.

Pastor-Cavada, E., Juan, R., Pastor, J. E., Alaiz, M., Vioque, J. (2009). Analytical nutritional

characteristics of seed proteins in six wild *Lupinus* species from Southern Spain. *Food Chemistry*, 117(3), 466–469.

Petterson, D. S. (2016). Lupin: overview, 2nd edn. *Elsevier Ltd*, Amsterdam

Petterson, D. S. (2000). The use of lupins in feeding systems. Review, *Asian-Australasian Journal of Animal Sciences*, 13, 861-882.

Písaříková, B., Zraly, Z., Bunka, F., Trckova, M. (2008). Nutritional value of white lupine cultivar Butan in diets for fattening pigs. *Veterinární medicína*, 53, 124–134.

Písaříková, B., Zralý, Z. (2009). Nutritional value of lupine in the diets for pigs (a review). *Acta Veterinaria Brno*, 78(3), 399–409.

Prandini, A., Morlacchini, M., Moschini, M., Fusconi, G., Masoero, F., Piva, G. (2005). Raw and extruded pea (*Pisum sativum*) and lupin (*Lupinus albusvar*. Multitalia) seeds as protein sources in weaned piglets' diets: effect on growth rate and blood parameters. *Italian Journal of Animal Science*, 4(4), 385–394.

Romeo, F., Fabroni, S., Ballistreri, G., Muccilli, S., Spina, A., Rapisarda, P. (2018). Characterization and antimicrobial activity of alkaloid extracts from seeds of different genotypes of *Lupinus* spp., S*ustainability*, 10(788), 1–12.

Rutkowski, A., Kaczmarek, S. A., Hejdysz, M., Jamroz, D. (2016). Effect of extrusion on nutrients digestibility, metabolizable energy and nutritional value of yellow lupine seeds for broiler chickens. *Annals of Animal Science*, 16(4), 1059–1072.

Rutkowski, A., Kaczmarek, S. A., Hejdysz, M., Nowaczewski, S., Jamroz, D. (2015). Concentrates made from legume seeds (*Lupinus angustifolius*, *Lupinus luteus* and *Pisum sativum*) and rapeseed meal as protein sources in laying hen diets. *Annals of Animal Science*, 15(1), 129–142.

Rybiński, W., Święcicki, W., Bocianowski, J., Börner, A., Starzycka-K., E., Starzycki, M. (2018). Variability of fat content and fatty acids profiles in seeds of a Polish white lupin (*Lupinus albus* L.) collection. *Genetic Resources and Crop Evolution*, 65(2), 417–431.

Saez, P., Borquez, A., Dantagnan, P., & Hernández, A. (2015). Effects of dehulling, steam-cooking and microwave-irradiation on digestive value of white lupin (*Lupinus albus*) seed meal for rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). *Archives of Animal Nutrition*, 69(2), 143–157.

Sedláková, K., Straková, E., Suchý P., Krejcarová, J., Herzig, I. (2016). Lupin as a perspective protein plant for animal and human nutrition – a review. *Acta Veterinaria Brno*, 85, 165-175.

Seymour, M., Kirkegaard, J. A., Peoples, M. B., White, P. F., French, R. J. (2012). Break-crop benefits to wheat in Western Australia - insights from over three decades of research. *Crop and Pasture Science*, 63, 1-16.

Sipsas, S, Gleneross, B. (2005). Implications of variability amongst Lupin cultivars in aquaculture feeds. *Proeessing Proceedings of the Third Workshop for Seeding a Future for Grains*, 14 April, Fremantle, Western Australia, 7-13.

Smulikowska, S., Konieczka, P., Czerwinski, J., Mieczkowska, A., Jankowiak, J. (2014). Feeding broiler chickens with practical diets containing lupin seeds (*L. angustifolius* or *L. luteus*): effects of incorporation level and mannanase supplementation on growth performance, digesta viscosity, microbial fermentation and gut morphology. *Journal of Animal and Feed Sciences*. 23(1), 64–72.

Smith, D. M., Tabrett, S. J., Glencross, B. D., Irvin, S. J., Barclay, M. C. (2007). Digestibility of lupin kernel meals in feeds for the black tiger shrimp, *Penaeus monodon*. *Aquaculture*, 264(1), 353–362.

Stanek, M., Rotkiewicz, T., Sobotka, W., Bogusz, J., Otrocka-D., I., Rotkiewicz, A. (2015). The effect of alkaloids present in blue lupine (*Lupinus angustifolius*) seeds on the growth rate, selected biochemical blood indicators and histopathological changes in the liver of rats. *Acta Veterinaria Brno*, 84(1), 55–62.

Steenfeldt, S., González, E., Knudsen, N. K. (2003). Effects of inclusion with blue lupins (*Lupinus angustifolius*) in broiler diets and enzyme supplementation on production performance, digestibility and dietary AME content. *Animal Feed Science and Technology*, 110(1), 185–200.

Straková, E., Suchý, P., Herzig, I., Hudečková, P., Ivanko, Š. (2010). Variation in fatty acids in chicken meat as a result of a lupin-containing diet. *Czech Journal of Animal Science*, 55(2), 75–82.

Straková, E., Suchý, P., Večerek, V., Šerman, V., Mas, N., Jůzl, M. (2006). Nutritional composition of seeds of the genus Lupinus. *Acta Veterinaria Brno*, 75, 489–493.

Suchy, P., Straková, E., Kroupa, L., Vecerek, V. (2008). The fatty acid content of oil from seeds of somelupin varieties. In: Palta, J.A., Berger, J.B. (Eds.), *Lupins for Health and Wealth, Proceedings of the12th International Lupin Conference*. Canterbury, New Zealand, 188-191.

Sujak A., KotlarzA., Strobel W., 2006. Compositional and nutritional evaluation of several lupin seeds. Food Chemistry, 98(4), 711–719.

Tomczak, A., Zielińska-D., M., Piasecka-K., D., Lampart-S., E. (2018). Blue lupine seeds protein content and amino acids composition. *Plant, Soil and Environment*, 64, 147–155.

Uzun, B., Arslan, C., Karhan, M., Toker, C., (2007). Fat and fatty acids of white lupin (*Lupinus albus* L.) in comparison to sesame (*Sesamum indicum* L.). *Food Chemistry*, 102(1), 45–49.

Van Barneveld, R. J. (1997). Understanding the nutritional value of ASL. *Pig Research and Development Corporation Final Report*, DAS 33P, October, Canberra.

Van Barneveld, R. J. (1999). Understanding the nutritional chemistry of lupin (*Lupinus* spp.) seed to improve livestock production efficiency. *Nutrition Research Reviews*, 12(02), 203-220.

Večerek, V., Suchý, P., Straková, E., Machácek, M., (2008). Nutritive Composition of seeds of the lupin varieties registered in the Czech Republic. In: JA

Palta and JB Berger (eds). "*Lupins for Health and Wealth". Proceedings of the 12th International Lupin Conference*, 14-18 September., Fremantle, Western Australia, 123-126.

Volek, Z., Bureš, D., Uhlířová, L. (2018). Effect of dietary dehulled white lupine seed supplementation on the growth, carcass traits and chemical, physical and sensory meat quality parameters of growing-fattening rabbits. *Meat Science*, 141, 50–56.

Wasilewko, J., Buraczewska, L. (1999). Chemical composition including content of amino acids, minerals and alkaloids in seeds of three lupin species cultivated in Poland. *Journal of Animal and Feed Sciences*. 8, 1–12.

Weisskopf, L., Akello, P., Milleret, R., Khan, Z. R., Schulthess, F., Gobat, J. M., Le Bayon, R. C. (2008). White lupin leads to increased maize yield through a soil fertility-independent mechanism: a new candidate for fighting *Striga hermonthica* infestation? *Plant and Soil*, 319(1-2), 101–114.

Yilmaz, Ş., Mehmet, A., Mustafa, E. (2015). Comparison of chemical composition in Lupin (*Lupinus spp*.) species *Revista de Chimie*, 66(3), 324–327.

Zduńczyk, Z., Jankowski, J., Rutkowski, A., Sosnowska, E., Drazbo, A., Zdunczyk, P., Juskiewicz, J. (2014a). The composition and enzymatic activity of gut microbiota in laying hens fed diets supplemented with blue lupine seeds. *Animal Feed Science and Technology*, 191, 57–66.

Zduńczyk, Z. Jankowski, J., Mikulski, D., Mikulska, M., Lamparski, G., Slominski, B. A., Juśkiewicz, J. (2014b). Growth performance, gastrointestinal function and meat quality in growing-finishing turkeys fed diets with different levels of yellow lupine (*L. luteus*) seeds. *Archives of Animal Nutrition*, 68(3), 211–226.

Zralý, Z., Písaříková, B., Trčková, M., Doležal, M., Thiemel, J., Simeonovová, J., Jůzl, M. (2008). Replacement of soya in pig diets with white lupine cv. Butan. Czech Journal of Animal Science, 53(10), 418–430.

Zralý, Z., Písaříková, B., Trckova, M., Herzig, I., Juzl, M., Simeonovova, J. (2007). The effect of white lupine on the performance, health, carcass characteristics and meat quality of market pigs. *Veterinární medicína*, 52(1), 29–41.

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NATURAL HONEY AS A POTENTIAL NUTRACEUTICAL SOURCE (REVIEW)

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Abstract

Honey, the sweet natural substance produced by honeybees is currently considered one of the nature's most powerful products. Natural honey can be regarded as a nutraceutical product due to its nutritional benefits and therapeutic promises. In addition to this, the use of honey as food and medicine has been embraced by different civilizations, from ancient times to the present, transcending the barriers of cultural and religious beliefs. The aim of the present review was to highlight and summarize some of the numerous medicinal attributes of honey, apart from its nutritional profile, that can contribute to its framing as nutraceutical agent. In this regard, it was proved that honey can promote metabolic and cardiovascular benefits, oral and bone health, haematological beneficial effects, anticancer activity. Moreover, evidence has been found for the use of honey as an alternative cure in several skin disorders.

Key words*: honey, medicinal properties, nutraceutical product.*

INTRODUCTION

In recent years, nutraceuticals have represented a growing field of interest for scientific research, due to both nutritive and therapeutic potentials. The term "nutraceutical" was originally coined by Stephen De Felice in 1989 and it derived from "nutrition" (a nourishing food or food component) and "pharmaceutics" (a medical drug) (De Felice, 1995; Brower, 1998; Mannion, 1998; Maddi et al., 2007). According to De Felice, a nutraceutical was described as, "a food that provides medical or health benefits, including the prevention and/ or treatment of a disease". The original definition has been reformulated throughout time, a nutraceutical being designated to possess physiological benefits, that provide protection against several disorders (Kalra, 2003; Sarin, 2012; Golla, 2018).

Natural honey can be considered a nutraceutical agent due to the fact that besides its nutritional value, it is widely appreciated for its therapeutic properties (Chua et al., 2014).

Honey represents a significant source of sugars, with a high nutritive value. It also consists of a minor class of compounds, such as enzymes, amino acids, proteins, polyphenols, organic acids, vitamins and minerals (Alvarez-Suarez et al., 2010; Da Silva et al., 2016).

A plethora of research documented honey's beneficial roles for medicinal purposes, namely metabolic and cardiovascular benefits (Busserolles et al., 2002; Agrawal et al., 2007; Ahmad et al., 2008; Bahrami et al., 2009), bone and oral health (Molan, 2001; English et al., 2004; Farid, 2009; Zaid et al., 2012; Hajizadeh et al., 2018), haematological beneficial effects (Chepulis, 2007; Beloor et al., 2010; Abioja et al., 2013), anticancer activity (Fernandez-Cabezudo et al., 2013; Aliyu et al., 2013; Spilioti et al., 2014). In addition to this, honey has been widely used in the treatment of wounds and numerous skin pathologies (e.g., eczema, dermatitis, burns, ulcers) due to strong antimicrobial and anti-inflammatory activities (Song and Salcido, 2011; Mayer et al., 2014; Oguz et al., 2018; Lukanc et al., 2018).

The present review aimed to summarize the numerous attributes of honey as food and as medicine, highlighting the need for its framing in the field of nutraceuticals.

HONEY AND NUTRITION

The wide array of compounds present in honey enhances its nutritional profile and promotes its use as food.

From a nutritional point of view, honey is mainly composed of sugars and water (White

and Doner, 1980; Bogdanov et al., 2008; Bradbear, 2009; Solayman et al., 2016).

The monosaccharides (fructose and glucose) represent the majority of the sugars detected in honey (75%), while disaccharides, trisaccharides and oligosaccharides make up the remaining 10 to 15% (Bogdanov et al., 2008; Da Silva et al., 2016).

Fructose is the prevalent sugar in acacia honey (Persano Oddo and Piro, 2004; Escuredo et al., 2014), while other monofloral honeys, such as rape honey (*Brassica napus*) or dandelion honey (*Taraxacum officinale*) recorded higher concentrations of glucose (Crane, 1990; Persano-Oddo and Piro, 2004).

These findings indicate that honey can be considered a genuine source of energy due to its high content of sugars. Furthermore, even though both fructose and glucose are monosaccharides, they possess distinct metabolism mechanisms. In this regard, Ajibola et al. (2012) showed that glucose is quickly absorbed into the blood system in order to provide energy, while the absorption of fructose takes place slower; but even so, fructose ensures energy for the individual, for a longer period of time than glucose does.

According to Santos-Buelga and González-Paramás (2017), the most abundant disaccharides detected in nectar honeys were maltose, isomaltose, kojibiose and turanose. Additionally, the reseach conducted by De la Fuente et al. (2011) revealed that sucrose derivates represent the majority of trisaccharides found in honey. Different studies emphasized increased amounts of the trisaccharides melezitose, erlose and raffinose in honeydew honey (Bogdanov et al., 2008; Rybak et al., 2013).

In addition, the oligosaccharides present in honey can display prebiotic effects, by stimulating both the *in vivo* and *in vitro* growth of bifidobacteria and lactobacilli populations in the gastrointestinal tract (Kajiwara et al., 2002; Sanz et al., 2005). Besides the sugars, honey is also constituted by other minor components, such as proteins, enzymes, amino acids, vitamins, minerals, organic acids, volatile and phenolic compounds (Viuda-Martos et al., 2008; Solayman et al., 2016). All these substances bring more value to the nutritive profile of honey and play a crucial role in the daily diet.

The honey's proteins originate predominantly from secretions of cephalic glands of honey bees, but they also derive from the nectar and pollen of flowers (White and Doner, 1980; Lee et al., 1998; Santos-Buelga and González-Paramás, 2017). Several factors such as, honey bee species, honeydew-producing insects, nectar and pollen source are thought to influence the mean protein content of honey (Lee et al., 1998). Furthermore, Escuredo et al. (2013) demonstrated that European honeydew and chestnut honeys recorded superior values concerning the protein content (1 g of proteins/100 g of honey) compared to eucalyptus (0.6%), blackberry or polyfloral honeys (0.7%). The major royal jelly protein 1 (MRJP1) represents the main protein found in honey and its average amount was 23.4% as compared with the total protein content (Bilikova and Simuth, 2010). Moreover, other evidences revealed that honey samples from different botanical and geographical origins, contained up to nine major royal jelly proteins (Simuth et al., 2004; Won et al., 2009; Bilikova and Simuth, 2010; Rossano et al., 2012).

The presence of enzymes in honey was reported a long time ago by White (1978). The α-glucosidase (invertase or saccharase) is found in the hypopharyngeal gland of the forager bee and it represents about 50% of the total protein of the gland, while amylase and glucose oxidase, each is estimated to represent 2-3% (Ohashi et al., 1999).

Other enzymes described in honey were catalase, acid phosphatase, proteases and esterases (Belitz et al., 2009). In general, enzyme activities are associated with the intensity of the nectar flow, such as the amount of nectar provided by the flowers or the concentration and composition of the nectar. Therefore, honeys obtained fom rich nectar sources, like acacia, frequently display decreased natural enzyme activities (Wehling et al., 2006).

Honey also contains amino acids (all of the nine essential amino acids and all of the non-essential amino acids, except asparagine and glutamine) (Iglesias et al., 2004). Proline is the predominant amino acid, representing 50-85% of this category, followed by phenylalanine

(Belitz et al., 2009). Several studies revealed that the sources which may provide the amino acids in honey are represented by nectar (Baker and Baker, 1986; Wunnachit et al., 1992), pollen (Marshall and Williams, 1987; Sing and Sing, 1996), or even the bees themselves, when discussing about proline (Ball, 2007; Truzzi et al., 2014).

The mineral content is variable, depending on the honey assortment. Therefore, it recorded lower values in light honeys (0.04%) than in the dark ones (0.2%) (Da Silva et al., 2016). Among the minerals described in honey, potassium is the most abundant one (Terrab et al., 2004; Da Silva et al., 2016; Kadri et al., 2017), followed by calcium, sodium or magnesium, depending on the honey type (González-Paramás et al., 2000; Atanassova et al., 2012; Mondragón-Cortez et al., 2013). Other macroelements and microelements identified in honey were iron, phosphorus, manganese, iodine, zinc, lithium, cobalt (Da Silva et al., 2016).

Honey contains low quantities of vitamins, most of them originating from the pollen grains (Ciulu et al., 2011). The predominant vitamin detected in honey is ascorbic acid, with mean amounts around 2 mg/100 g (Alvarez-Suarez et al., 2010a). Other vitamins discovered in honey include thiamine (B1), riboflavin (B2), nicotinic acid (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B8 or H) and folic acid (B9) (León-Ruiz et al., 2013; Da Silva et al., 2016).

Organic acids are another category of minor compounds that were reported in honey. They are mainly represented by gluconic, aspartic, butyric, citric, acetic, formic, fumaric, galacturonic, glutamic, glutaric, butyric, glyoxylic, 2-hydroxybutyric, α-hydroxyglutaric, isocitric, α-ketoglutaric, lactic, malic, malonic, methylmalonic, 2-oxopentanoic, propionic, pyruvic, quinic, succinic, tartaric, oxalic, levulinic, formic acids (Mato et al., 2007; Da Silva et al., 2016). Among them, the gluconic acid is the prevailing one in most honeys, excepting fir honey, where galacturonic acid has been mentioned as the predominant organic acid (Daniele et al., 2012).

The 600 volatile compounds found in honey provide its aroma and flavour qualities. They include hydrocarbons, aldehydes, ketones, fatty acids and other carboxylic acids, superior alcohols, esters, benzene and its derivatives, norisoprenoids, sesquiterpenes and its derivatives, sulphur and cyclic compounds (Manyi-Loh et al., 2011a; 2011b).

The phenolic compounds detected in honey can be classified into phenolic acids (e.g., vanillic, caffeic, syringic, p-coumaric, ferulic, ellagic, 3-hydroxybenzoic, chlorogenic, 4-hydroxybenzoic, rosmarinic, gallic and benzoic acids) and flavonoids (e.g., quercetin, kaempferol, myricetin, pinobanksin, pinocembrin, chrysin, galangin, hesperetin, apigenin, C and O-glycosyl derivatives and others) (Tomás-Barberán et al., 2001; Dimitrova et al., 2007; Trautvetter et al., 2009; Truchado et al., 2011; Da Silva et al., 2016). Moreover, evidences confirm that these polyphenols exert strong antioxidant activity by neutralizing free radicals (Rice-Evans, 1996; Gheldof and Engeseth, 2002; Khalil et al., 2012).

Overall, the above mentioned components identified in honey are responsible for its unique chemical composition, allowing honey to become one of the most important bee products, with high nutritive value and suitable for long-term storage.

HONEY AND MEDICINE

Haematology. In poultry, the red blood cell indices, such as haematocrit, erythrocyte and haemoglobin indices often record decreased values as a result of stress factors (Yahav and Hurwitz, 1996; Yahav, 1999).

In this regard, the basic immunosuppressive factors affecting broiler chickens on a daily basis, are considered to be the following ones: extreme environmental temperature, humidity, vaccination, feed and/or water deprivation, mycotoxins in feed, dust, ammonia, radiation, bacterial or viral exposure, overcrowding, failure to comply with the provisions related to transportation (Beloor et al., 2010; Abioja et al., 2013; Chikumba and Chimonyo, 2014; Olukomaiya et al., 2015).

In addition, Abioja et al. (2019) revealed that supplementing the drinking water with honey, in stressed broiler chickens, resulted in the improvement of the haematocrit (Ht), red blood

cell (RBC) count and haemoglobin (Hb) concentration. In a previous research, Obun et al. (2008) attested the efficiency of implementing a honey-based diet in broiler chickens and the amelioration of the haematological parameters, such as Ht, RBC and Hb concentration.

Furthermore, Adekunle et al. (2017) reported that the heterophyl/lymphocyte rate was not modified by a honey-based diet in chickens.

Other research highlighted the benefits of a honey-based diet on blood indices of adult rats. Thus, they observed an improved haemoglobin concentration, increased erythrocyte count and elevated haematocrit in the investigated animals (Ajibola et al., 2007).

An improved hematological profile in rats was also confirmed by Chepulis (2007), following a honeydew honey-based diet.

Metabolic and cardiovascular effects. Many studies have shown that honey can prevent metabolic and cardiovascular pathologies. For instance, it was demonstrated that honey decreases total cholesterol, low-density lipoprotein cholesterol (LDL-C), triglycerides (TG) and enhances high-density lipoprotein cholesterol (HDL-C), in both healthy individuals and patients with high risk (Yaghoobi et al., 2008). Additionally, Al-Waili (2004a) emphasized that honey lowers triglycerides in patients with hypertriglyceridaemia and decreases low-density lipoprotein cholesterol (LDL-C) in patients with hyperlipidaemia.

Moreover, among the metabolic conditions that are thought to be involved in cardiovascular diseases is diabetes. Different studies highlight positive effects of honey in both healthy and diabetic patients (Agrawal et al., 2007; Ahmad et al., 2008; Bahrami et al. 2009). The study conducted by Erejuwa et al. (2011; 2012a) evaluated the effect of honey in diabetic rats. Their findings outlined a decrease of hyperglycaemia and dyslipidaemia in the investigated animals, probably due to the oligosaccharides detected in honey. In addition, Nemoseck et al. (2011) fed healthy rats with a diet containing 20% honey and observed significant decrease of triglycerides, body weight, food/energy intake, and epididymal fat weight, but not significantly glucose decrease, total cholesterol decrease, adiponectin, and C-reactive proteins.

In another research, Aluko et al. (2014) revealed the ability of honey to lower blood pressure in healthy male subjects. Hypertension represents one of the most important risk factors for premature cardiovascular diseases. Notably, Erejuwa et al. (2012b) underlined the antihypertensive effect of honey in experimental rats.

Some studies have shown that the metabolic and cardiovascular effects of honey could be associated with its valuable compounds, particularly flavonoids (Shen et al., 2012; Panchal et al., 2012).

Oral care. Scientific evidences suggest that honey is able to support oral health, due to its strong antibacterial properties. In this regard, there are studies which confirm the inhibitory effect of honey on the growth of some pathogens responsible for the development of dental caries (Steinberg et al., 1996; Molan, 2001a).

Furthermore, English et al. (2004) indicated that Manuka honey, a honey that gained a lot of attention due to its high antibacterial activity, could prevent the evolution of some inflammatory conditions, such as gingivitis and periodontal disease. Additionally, Manuka honey proved to combat the malodor of oral squamous cell carcinomas (Drain and Fleming, 2015).

Several studies emphasized the major role of honey in lowering the incidence of radio/chemotherapy-induced oral mucositis (Biswal et al., 2003; Samdariya et al., 2015; Rao et al., 2017).

A recent research also demonstrated the ability of honey to prevent radiation-induced xerostomia (Charalambous et al., 2017).

Bone health. Nowadays, osteoporosis is regarded as one of the most important threats for the population, being characterized by a decreased bone mass and a bone architectural degradation thus, compromising the bone vitality (Christiansen, 1991). Lately, honey has become an alternative cure in bone disorders. In this regard, Zaid et al. (2010) confirm that Tualang honey was able to produce an increase in bone density and to restore osteoporotic bone in female ovariectomized rats; this effect was probably due to honey's high content of

phenolic and flavonoid compounds (Jaganathan and Mandal, 2009) and due to its strong anti-inflammatory activity (Owoyele et al., 2011).

Another research conducted by Zaid et al. (2012) revealed that Tualang honey displayed better effects on the trabecular bone structure in the ovariectomized rats than calcium supplementation. Moreover, Farid (2009) discovered that the anti-osteoporotic effect of Tualang honey could be associated with the presence of calcium and gluconic acid in its composition, the latter one increasing the calcium absorption in bone and thereby, providing the bone mass strength (Ariefdjohan et al., 2008).

In a recent study, Hajizadeh et al. (2018) highlighted the efficacy of topical application of honey in mandibular bone defect healing in rats. Moreover, Sahin et al. (2018) discovered that grayanotoxin-containing honey, derived from *Rhododendron* spp. and *Kalmia* spp. enhanced the healing mechanisms of an artificial transverse fracture.

Anticancer activity. The potential anticancer activity of honey was evaluated in both *in vitro* and *in vivo* studies.

Hence, honey samples of different floral origins were tested against distinct human cancer cell lines (e.g., prostate, breast, endometrial, lung, skin, cervical, liver, bladder, kidney, oral squamous cell carcinoma, osteosarcoma) (Ghashm et al., 2010; Samarghandian et al., 2011; Morales and Haza, 2013; Aliyu et al., 2013; Spilioti et al., 2014).

Some *in vivo* investigations were performed on induced-cancer rats/mice (Mabrouk et al., 2002) or transplanted-cancer rats/mice (Oršolić et al., 2005). Moreover, Erejuwa et al. (2014) stated that honey supresses the evolution of cancer by stopping the most important stages of carcinogenesis, such as initiation, proliferation and progression.

According to Tomasin and Cintra Gomes-Marcondes (2011), honey is able to decrease cell proliferation in Walker 256 carcinoma. Likewise, the flavonoid and phenolic content of honey inhibits the cell cycle of colon (Jaganathan and Mandal, 2009), glioma (Lee et al., 2003) and melanoma (Pichichero et al., 2010) cancer cell lines in G0/G1 phase.

The overexpression of Bcl-xL in breast cancer patients is generally correlated with metastasis (Espana et al., 2004). Ahmed and Othman (2017) reported that Bcl-xL expression was stopped by Tualang honey at its intrinsic mitochondrial pathway.

Dermatological properties. The beneficial effects of honey on skin care have been documented since earliest civilizations. Therefore, honey has been widely used in several skin pathologies due to its antimicrobial, antioxidant, immuno-stimulatory and anti-inflammatory properties (Kwakman et al., 2008; Vandamme et al., 2013; McLoone et al., 2016; Pereira and Bártolo, 2016).

The main components of honey involved in the wound healing process are represented by hydrogen peroxide, glucose oxidase, gluconic acid, methylgloxal, polyphenols, as well as hygroscopicity, hypertonicity and lower pH (Al-Waili et al., 2011; Hadagali and Chua, 2014; Majtan, 2014; Devasvaran and Yong, 2016). In addition, available literature indicates the ability of honey in stimulating angiogenesis, granulation and epithelialization (Molan, 2001b), lymphocytes and phagocytes (Al-Waili et al., 2011), and in initiating the expression of tissue repair markers (Barui et al., 2011). It was reported a very low toxic effect of honey on keratinocytes and fibroblasts (Ranzato et al., 2012).

Mayer et al. (2014) showed that topical application of honey improved chronic venous leg ulcers within six weeks, in 25 patients. The healing effects of honey on experimental and traumatic wounds were also revealed by latest studies (Oguz et al, 2018; Lukanc et al., 2018).

Natural honey also manifested antifungal activity against *Malassezia* yeasts, proving its efficacy in seborrheic dermatitis treatment (Al-Waili, 2001; Gupta et al., 2004). Furthermore, a mixture consisting of honey, beeswax and olive oil revealed its beneficial effects in pityriasis versicolor, tinea cruris, tinea corporis, and tinea faciei and diaper dermatitis (Al-Waili, 2004b).

In general, honey can be applied alone in the treatment of wounds (Osuagwu et al., 2004; Khoo et al., 2010) or in combination with other products, such as aloe vera and milk or ascorbic acid (Farzadinia et al., 2016; Schencke et al., 2016).

CONCLUSIONS

Nowadays, the permanent change of modern society has led to the imminent need for natural products and health-promoting foods. Therefore, nutraceuticals have received extensive attention from the population due to both high nutritional value and ability to counteract the negative effects of many pathologies that threaten the quality of life.
Moreover, numerous evidences attested the enhanced nutritive profile and multiple therapeutic properties of honey. As a consequence, honey could easily be classified as a nutraceutical product due to its valuable characteristics and its worlwide recognition.

REFERENCES

Abioja, M.O., Akinjute, O.F., Balogun, S.I., Oguntimehin, M.S., Oluwasola, T.O. (2019). Effect of honey on haematology, plasma biochemistry and liver enzymes in broiler chickens administered dietary corticosterone. *Journal of Stress Physiology & Biochemistry*, 15(3), 5–13.

Abioja, M.O., Osinowo, O.A., Smith, O.F., Eruvbetine, D. (2013). Physiological and haematological responses of broiler chickens offered cold water and vitamin C during hot-dry season. *Nigerian Journal of Animal Production*, 40, 24–36.

Agrawal, O.P., Pachauri, A., Yadav, H., et al. (2007). Subjects with impaired glucose tolerance exhibit a high degree of tolerance to honey. *Journal of Medicinal Food*, 10(3), 473–478.

Ahmad, A., Azim, M.K., Mesaik, M.A., Khan, R.A. (2008). Natural honey modulates physiological glycemic response compared to simulated honey and D-glucose. *Journal of Food Science*, 73(7), H165–H167.

Ahmed, S., Othman, N.H. (2017). The anti-cancer effects of Tualang honey in modulating breast carcinogenesis: an experimental animal study. *BMC Complement Altern Med*, 17, 208.

Ajibola, A., Chamunorwa, J., Erlwanger, K. (2012). Nutraceutical values of natural honey and its contribution to human health and wealth. *Nutrition & metabolism*, 9, 61.

Ajibola, A., Idowu, G.O., Amballi, A.A., Oyefuga, O.H., Iquot, I.S. (2007). Improvement of some haematological parameters in albino rats with pure natural honey. *J Biol Sci Res*, 2, 67–69.

Aliyu, M., Odunolam, O.A., Farooq, A.D., et al. (2013). Molecular mechanism of antiproliferation potential of Acacia honey on NCI-H460 cell line. *Nutr Cancer*., 65, 296–304.

Aluko, E., Olubobokun, T., Atang, D., Nna, V. (2014). Honey's ability to reduce blood pressure and heart rate in healthy male subjects. *Frontiers in Science*, 4, 8–11.

Alvarez-Suarez, J.M., González-Paramás, A.M., Santos-Buelga, C., Battino, M. (2010a). Antioxidant characterization of native monofloral Cuban honeys. *J Agric Food Chem*, 58, 9817–9824.

Alvarez-Suarez, J.M., Tulipani, S., Romandini, S., Bertoli, E., Battino, M. (2010b). Contribution of honey in nutrition and human health: a review. *Mediterranean Journal of Nutrition and Metabolism*, 3(1), 15–23.

Al-Waili, N.S. (2001). Therapeutic and prophylactic effects of crude honey on chronic seborrheic dermatitis and dandruff. *Eur J Med Res*., 6(7), 306–8.

Al-Waili, N.S. (2004a). Natural honey lowers plasma glucose, C-reactive protein, homocysteine, and blood lipids in healthy, diabetic, and hyperlipidemic subjects: comparison with dextrose and sucrose. J Med Food, 7, 100–107.

Al-Waili, N.S. (2004b). An alternative treatment for pityriasis versicolor, tinea cruris, tinea corporis and tinea faciei with topical application of honey, olive oil and beeswax mixture: an open pilot study. *Complement Ther Med*, 12, 45–7.

Al-Waili, N.S. (2005). Clinical and mycological benefits of topical application of honey, olive oil and beeswax in diaper dermatitis. *Clin Microbiol Infect*, 11, 160–3.

Al-Waili, N.S., Salom, K., Al-Ghamdi, A. (2011). Honey for wound healing, ulcers, and burns; data supporting its use in chemical practice. *Sci World J.*, 11:766–787.

Ariefdjohan, M.W. Martin, B.R. Lachcik, P.J., Weaver, C.M. (2008). Acute and chronic effects of honey and its carbohydrate constituents on calcium absorption in rats. *Journal of Agricultural and Food Chemistry,* 56, (8), 2649–2654.

Atanassova, J., Yurukova, L., Lazarova, M. (2012). Pollen and inorganic characteristics of Bulgarian unifloral honeys. *Czech J Food Sci,* 30, 520–526.

Bahrami, M., Ataie-Jafari, A., Hosseini, S., Foruzanfar, M.H., Rahmani, M., Pajouhi, M. (2009). Effects of natural honey consumption in diabetic patients: an 8-week randomized clinical trial. International *Journal of Food Science and Nutrition*, 60(7), 618–626.

Baker, H.G., Baker, I. (1986) The occurrence and significance of amino acids in floral nectar. *Plant Syst Evol,* 151, 175–186.

Ball, D.W. (2007). The chemical composition of honey. *J Chem Educ,* 84, 1643–1646.

Barui, A., Banerjee, P., Das, R.K., et al. (2011). Immunohistochemical evaluation of p63, e-cadherin, collagen I and III expression in lower limb wound healing under honey. *Evid Based Complement Alternat Med*, 2011: 239864.

Belitz, H.D., Grosch, W., Schieberle, P. (2009). Sugars, sugar alcohols and honey. In: Food *chemistry*, 4th edn., Berlin,GE: Springer Publishing House, 883−890.

Beloor, J., Kang, H.K., Kim, Y.J., Subramani, V.K., Jang, I.S., Sohn, S.H. and Moon, Y.S. (2010). The effect of stocking density on stress related gens and telomeric length in broiler chickens. *Asian-Australian Journal of Animal Science*, 23, 437–443.

Bilikova, E.K., Simuth, J. (2010). New criterion for evaluation of honey:quantification of royal jelly

protein Apalbumin 1 in honey. *J Agric Food Chem,* 58, 8776–8781.

Biswal, B.M., Zakaria, A., Ahmad, N.M. (2003).Topical application of honey in the management of radiation mucositis. A Preliminary study. *Support Care Cancer* , 11, 242–248.

Bogdanov, S., Jurendic, T., Sieber, R., Gallmann, P. (2008). Honey for Nutrition and Health: A Review. *J Am Coll Nutr*, 27(6), 677–689.

Bradbear, N. (2009). Definition and uses of honey. In: *Bees and their role in forest livelihoods*, FAO, Rome, 81–88.

Brower, V. (1998). Nutraceuticals: poised for a healthy slice of the health care market? *Nat Biotechnol.,* 16, 728–731.

Busserolles, J., Gueux, E., Rock, E., Mazur, A., Rayssiquier, Y. (2002). Substituting honey for refined carbohydrates protects rats from hypertriglyceridemic and prooxidative effects of fructose. *J. Nutr.*, 132(11), 3379–3382.

Charalambous, A., Lambrinou, E., Katodritis, N., Vomvas, D., Raftopoulos, V., Georgiou, M., Paikousis, L., Charalambous, M. (2017). The effectiveness of thyme honey for the management of treatment-induced xerostomia in head and neck cancer patients: A feasibility randomized control trial. *European Journal of Oncology Nursing*, 27, 1–8.

Chepulis, L.M. (2007). The effects of honey compared with sucrose and a sugarfree diet on neutrophil phagocytosis and lymphocyte numbers after long-term feeding in rats. *J. Compl. Integrat. Med.*, 4(1), 1–7.

Chepulis, L.M. (2007). The effects of honey compared with sucrose and a sugarfree diet on neutrophil phagocytosis and lymphocyte numbers after long term feeding in rats. *J. Compl. Integrat. Med.,* 4(1), 1–7.

Chikumba, N., Chmonyo, M. (2014). Effects of water restriction on growth performance, carcass characteristics and organ weights of naked neck and Ovambo chickens of southern Africa. *Asian Australian Journal of Animal Science*, 27, 974–980.

Christiansen, C. (1991). Consensus Development Conference: prophylaxis and treatment of osteoporosis. *American Journal of Medicine*, 90, 1, 107–110.

Chua, L.S., Adnan, N.A. (2014). Biochemical and nutritional components of selected honey samples. *Acta Sci. Pol. Technol. Aliment*., 13, 169–179.

Ciulu, M., Solinas, S., Floris, I., Panzanellia, A., Pilo, M.I., Piu, P.C., Spanoa, N., Sanna, G. (2011). RPHPLC determination of water-soluble vitamins in honey. *Talanta*, 83, 924–929.

Crane, E. (1990). The traditional hive products: honey and beeswax. In: Crane E (ed) *Bees and beekeeping: science, practice and world resources*, *Heinemann Newnes, Oxford*, 388–413.

Da Silva, P.M., Gauche, C., Gonzaga, L.V., Costa, A. C. O., Fett, R. (2016). Honey: chemical composition, stability and authenticity. *Food Chemistry*, 196, 309–323.

Daniele, G., Maitre, D., Casabianca, H. (2012). Identification, quantification and carbon stable isotopes determinations of organics acids in monofloral honeys. A powerful tool for botanical and authenticity control. *Rapid Commun Mass Spectrom.*, 26, 1993–1998.

De Felice, S.L. (1995). The nutraceutical revolution, its impact on food industry. *Trends in Food Sci and Tech.,* 6(2), 59–61.

De la Fuente, E., Ruiz-Matute, A.I., Valencia-Barrera, R.M., Sanz, J., Martínez-Castro, I. (2011). Carbohydrate composition of Spanish unifloral honeys. *Food Chem.,* 129, 1483–1489.

Devasvaran, K., Yong, Y.K. (2016). Anti-inflammatory and wound healing properties of Malaysia Tualang Honey. *Curr. Sci.,* 110, 47–51.

Dimitrova, B., Gevrenova, R., Anklam, E. (2007). Analysis of phenolic acids in honeys of different floral origin by solid-phase extraction and high-performance liquid chromatography. *Phytochem. Anal.*, 18, 24–32.

Drain, J., Fleming, M.O. (2015). Palliative management of malodorous squamous cell carcinoma of the oral cavity with manuka honey. *J. Wound Ostomy Cont. Nurs.,* 42(2), 190–192.

English, H.K., Pack, A.R., Molan, P.C. (2004). The effects of manuka honey on plaque and gingivitis: a pilot study. *J. Int. Acad. Periodontol.*, 6, 63–67.

Erejuwa, O.O., Sulaiman, S.A., Ab Wahab, M.S., Sirajudeen, K.N., Salleh, S., Gurtu, S. (2012b). Honey supplementation in spontaneously hypertensive rats elicits antihypertensive effect via amelioration of renal oxidative stress. *Oxid. Med. Cell Longev*., 374037.

Erejuwa, O.O., Sulaiman, S.A., Wahab, M.S. (2011). Oligosaccharides might contribute to the antidiabetic effect of honey: a review of the literature. *Molecules*, 17, 248–266.

Erejuwa, O.O., Sulaiman, S.A., Wahab, M.S. (2012a). Honey-a novel antidiabetic agent. *Int. J. Biol. Sci*., 8, 913–934.

Erejuwa, O.O., Sulaiman, S.A., Wahab, M.S. (2014). Effects of honey and its mechanisms of action on the development and progression of cancer. *Molecules*, 19, 2497–2522.

Escuredo, O., Dobre, I., Fernández-González, M., Seijo, M.C. (2014). Contribution of botanical origin and sugar composition of honeys on the crystallization phenomenon. *Food Chem.*, 149, 84–90.

Escuredo, O., Míguez, M., Fernández-González, M., Seijo, M.C. (2013). Nutritional value and antioxidant activity of honeys produced in a European Atlantic area. *Food Chem.*, 138, 851–856.

Espana, L., Fernandez, Y., Rubio, N., Torregrosa, A., Blanco, J., Sierra, A. (2004). Overexpression of Bcl-xL in human breast cancer cells enhances organ-selective lymph node metastasis. *Breast Cancer Res. Treat.*, 87(1), 33–44.

Farzadinia, P., Jofreh, N., Khatamsaz, S., et al. (2016). Anti-inflammatory and wound healing activities of Aloe vera, honey and milk ointment on second-degree burns in rats. *Int. J. Low Extrem Wounds*, 15, 241–247.

Fernandez-Cabezudo, M.J., El-Kharrag, R., Torab, F., et al. (2013). Intravenous administration of manuka honey inhibits tumor growth and improves host survival when used in combination with chemotherapy in a melanoma mouse model. PLoS ONE, 8, e55993.

Ghashm, A.A., Othman, N.H., Khattak, M.N., Ismail, N.M., Saini, R. (2010). Antiproliferative effect of tualang honey on oral squamous cell carcinoma and osteosarcoma cell lines. *BMC Complement Altern. Med*., 10, 49.

Gheldof, N., Engeseth, N.J. (2002). Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. *J. Agric. Food Chem*., 50, 3050.

Golla, U. (2018). Emergence of nutraceuticals as the alternative medications for pharmaceuticals. *Int. J. Complement Alt. Med.,* 11(3), 155–158.

González-Paramás, A.M., Gómez-Bárez, J.A., García-Villanova, R.J., Rivas Palá, T., Ardanuy Albajar, R., Sánchez, J. (2000). Geographical discrimination of honeys by using mineral composition and common chemical quality parameters. *J. Sci. Food Agric.,* 80, 157–165.

Gupta, A.K., Nicol, K., Batra, R. (2004). Role of antifungal agents in the treatment of seborrheic dermatitis. *Am. J. Clin. Dermatol*., 5, 417–22.

Hadagali, M.D., Chua, L.S. (2014). The anti-inflammatory and wound healing properties of honey. *Eur. Food Res. Technol*., 239, 1003–1014.

Hajizadeh, F., Derakhshan, B., Peimani, A., Abbasi, Z. (2018). Effect of topical honey on mandibular bone defect healing in rats. *9e Journal of Contemporary Dental Practice,* 19(1), 47–51.

Iglesias, M.T., De Lorenzo, C., Del Carmen Polo, M., Martin-Alvarez, P.J., Pueyo, E. (2004). Usefulness of amino acid composition to discriminate between honeydew and floral honeys, Application to honeys from a small geographic area. *J. Agric. Food Chem.*, 52, 84–89.

Jaganathan, S.K., Mandal, M. (2009). Antiproliferative effects of honey and of its polyphenols: a review, *Journal of Biomedicine and Biotechnology*, vol. 2009, Article ID 830616, 13 pages.

Kadri, S.M., Zaluski, R., de Oliveira Orsi, R. (2017). Nutritional and mineral contents of honey extracted by centrifugation and pressed processes. *Food Chem.,* 218, 237–241.

Kajiwara, S., Gandhi, H., Ustunol, Z. (2002). Effect of honey on the growth of and acid production by human intestinal *Bifidobacterium spp*. An in vitro comparison with commercial oligosaccharides and inulin. *J. Food Prot.*, 65, 214–218.

Kalra, E.K. (2003). Nutraceutical-Definition and Introduction. *AAPS Pharm. Sci.,* 5(3), 25.

Khalil, I., Moniruzzaman, M., Boukraa, L., Benhanifia, M., Islam, A., Islam, N., Sulaiman, S.A., Gan, S.H. (2012). Physicochemical and antioxidant properties of Algerian honey. *Molecules*, 17, 11199–11215.

Khoo, Y.T., Halim A.S., Singh K.K.B, Mohamad N.A. (2010). Wound contraction effects and antibacterial properties of Tualang honey on full-thickness burn wounds in rats in comparison to hydrofibre. *BMC Complement Altern. Med.,* 10, 48.

Kwakman, P.H., Van den Akker, J.P., Güçlü, A. et al. (2008). Medicalgrade honey kills antibiotic-resistant bacteria *in vitro* and eradicates skin colonization. *Clin. Infect Dis.,* 46, 1677–82.

Lee, D.C., Lee, S.Y., Cha, S.H., Choi, Y.S., Rhee, H.I. (1998). Discrimination of native bee-honey and foreign bee-honey by SDS–PAGE. *Korean J. Food Sci.,* 30, 1–5.

Lee, Y.J., Kuo, H., Chu, C., Wang, C., Lin, W., Tseng, T. (2003). Involvement of tumor suppressor protein p53 and p38 MAPK in caffeic acid phenethyl ester-induced apoptosis of C6 glioma cells. *Biochemical Pharmacology*, 66(12), 2281–2289.

León-Ruiz, V., Vera, S., González-Porto, A.V., Andrés, M.P.S. (2013). Analysis of water-soluble vitamins in honey by isocratic RP-HPLC. *Food Anal. Methods,* 6, 488–496.

Lukanc, B., Potokar, T., Erjavec, V. (2018). Observational study of the effect of L-Mesitran® medical honey on wound healing in cats. *Vet. Arhiv.,* 88, 59–74.

Mabrouk, G.M., Moselhy, S.S., Zohny, S.F., et al. (2002). Inhibition of methylnitrosourea (MNU) induced oxidative stress and carcinogenesis by orally administered bee honey and Nigella grains in Sprague Dawely rats. *J. Exp. Clin. Cancer Res*., 21, 341–346.

Maddi, V.S., Aragade, P.D., Digge ,V.G., Nitaliker, M.N. (2007). Importance of nutraceuticals in health management. *Phcog. Rev.,* 1, 377–379.

Majtan, J. (2014). Honey: an immunomodulator in wound healing. *Wound Repair Regen*, 22, 187–192.

Mannion, M. (1998). Nutraceutical revolution continues at Foundation for Innovation in Medicine Conference. *Am. J. Nat. Med.,* 5, 30–33.

Manyi-Loh, C., Ndip, R.N., Clarke, A.M. (2011b) Volatile compounds in honey: a review on their involvement in aroma, botanical origin determination and potential biomedical activities. *Int. J. Mol. Sci.,* 12, 9514–9532.

Manyi-Loh, C.E., Clarke, A.M., Ndip, R.N. (2011a). Identification of volatile compounds in solvent extracts of honeys produced in South Africa. *Afr. J. Agric. Res.,* 6, 4327–4334.

Marshall, T., Williams, K.M. (1987). Electrophoresis of honey: characterization of trace proteins from a complex biological matrix by silver staining. *Anal. Biochem.*, 167, 301–303.

Mato, I., Huidobro, J.F., Simal-Lozano, J., Sancho, M.T. (2007). Analytical methods for the determination of organic acids in honey. *Crit. Rev. Anal. Chem.,* 36, 3–11.

Mayer, A., Slezak, V., Takac, P., Olejnik, J., Majtan, J. (2014). Treatment of non-healing leg ulcers with honeydew honey. *J. Tissue Viability*, 23, 94–97.

McLoone, P., Warnock, M., Fyfe, L. (2016). Honey: an immunomodulatory agent for disorders of the skin. *Food Agric. Immunol*., 27, 338–349.

Molan, P.C. (2001a). The potential of honey to promote oral wellness. *Gen. Den*., 49, 584–589

Molan, P.C. (2001b). Potential of honey in the treatment of wounds and burns. *Am. J. Clin. Dermatol.,* 2, 13–9.

Mondragón-Cortez, P., Ulloa, J., Rosas-Ulloa, P., Rodríguez-Rodríguez, R., Resendiz Vázquez, J. (2013). Physicochemical characterization of honey from the West region of México. *CyTA J. Food*, 11, 7–13.

Morales, P., Haza, A.I. (2013). Antiproliferative and apoptotic effects of Spanish honeys. *Pharmacogn. Mag*., 9, 231–237.

Nemoseck, T.M., Carmody, E.G., Furchner-Evanson, A. (2011). Honey promotes lower weight gain, adiposity, and tryglycerides than sucrose in rats. *Nutrition Research*, 31(1), 55–60.

Obun, C.O., Yahaya, M.S., Olafadehan, O.A., Kehinde, A.S., Allison, D.S., Yusuf, A.M., Farouk, I.U. (2008). Dietary value of honey and its effects on abdominal fat deposit, blood and serum profile of finisher broiler chicks. *Journal of Agriculture, Forestry and Social Science*, 6, 1–7.

Oguz, E., Turgut, D., Ucler, K., Faruk, M. C., Sedat, K., Orhan, M. K. (2018). The effect of different types of honey on healing infected wounds. *Journal of Wound Care*, 27, S18–S25.

Ohashi, K., Natori, S., Kubo, T. (1999). Expression of amylase and glucose oxidase in the hypopharyngeal gland with an age-dependent role change of the worker honeybee (*Apis mellifera* L.). *Eur. J. Biochem.,* 265, 127–133.

Olukomaiya, O.O., Adeyemi, O.A., Sogunle, O.M., Abioja, M.O. and Ogunsola, I.A. (2015). Effect of feed restriction and ascorbic acid supplementation on growth performance, rectal temperature and respiratory rate of broiler chicken. *Journal of Animal and Plant Sciences*, 25, 65–71.

Oršolić, N., Terzić, S., Šver, L., Bašić, I. (2005). Honey-bee products in prevention and/or therapy of murine transplantable tumours. *J. Sci. Food Agric*., 85, 363–370.

Osuagwu, F.C., Oladejo, O.W., Imosemi, I.O., et al. (2004). Enhanced wound contraction in fresh wounds dressed with honey in wistar rats (*Rattus novergicus*). *West Afr. J. Med*., 23, 114–118.

Owoyele, B.V. , Adenekan, O.T., Soladoye, A.O. (2011). Effects of honey on inflammation and nitric oxide production in Wistar rats. *Journal of Chinese Integrative Medicine*, 9, (4), 447–452.

Panchal, S.K., Poudyal, H., Brown, L. (2012). Quercetin ameliorates cardiovascular, hepatic, and metabolic changes in diet-induced metabolic syndrome in rats. *J. Nutr*., 42, 1026–1032.

Pereira, R.F., Bártolo, P.J. (2016). Traditional therapies for skin wound healing. *Adv. Wound Care*, 5, 208–229.

Persano Oddo, L., Piro, R. (2004). Main European unifloral honeys: descriptive sheets. *Apidologie*, 35, S38–S81.

Pichichero, E., Cicconi, R., Mattei, M., Muzi, M.G., Canini, A. (2010). Acacia honey and chrysin reduce proliferation of melanoma cells through alterations in cell cycle progression. *International Journal of Oncology*, 37(4), 973–981.

Ranzato, E., Martinotti, S., Burlando, B. (2012). Epithelial mesenchymal transition traits in honey-driven keratinocyte wound healing: comparison among different honeys. *Wound Repair Regen*, 20, 778–85.

Rao, S., Hegde, S.K., Rao, P., Dinkar, C., Thilakchand, K.R., George, T., Baliga-Rao, M.P., Palatty, P.L., Baliga, M.S. (2017). Honey Mitigates Radiation-Induced Oral Mucositis in Head and Neck Cancer Patients without Affecting the Tumor Response. *Foods*, 6, 77.

Rice-Evans, C.A., Miller, N.J., Paganga, G. (1996). Structure-antioxidant activity relationship of flavonoids and phenolic acids. *Free Radic. Biol. Med.*, 20, 933–56.

Rossano, R., Larocca, M., Polito, T., Perna, A.M., Padula, M.C., Martelli, G., Riccio, P. (2012). What are the proteolytic enzymes of honey and what they do tell us? A fingerprint analysis by 2-d zymography of unifloral honeys. PLoS One 7:e49164.

Rybak, C.H., Szczesna, T., Waś, E., Jaśkiewicz, K., Teper, D. (2013). Characteristics of Polish Unifloral Honeys IV. Honeydew Honey, Mainly Abies Alba L. *Journal of Apicultural Science*, 57.

Sahin, A., Turkmen, S., Guzel, N., et al. (2018). A comparison of the effects of grayanotoxin-containing honey (mad honey), normal honey, and propolis on fracture healing. *Medical Principles and Practice*, 27(2), 99–106.

Samarghandian, S., Afshari, J.T., Davoodi, S. (2011). Honey induces apoptosis in renal cell carcinoma. *Pharmacogn Mag*., 7, 46–52.

Samdariya, S., Lewis, S., Kauser, H., Ahmed, I., Kumar, D. (2015). A randomized controlled trial evaluating the role of honey in reducing pain due to radiation induced mucositis in head and neck cancer patients. *Indian J. Palliat Care*, 21, 268–73.

Santos-Buelga, C., González-Paramás, A.M. (2017) Chemical Composition of Honey. In: Alvarez-Suarez J. (eds), *Bee Products - Chemical and Biological Properties*. Berlin, GE: Springer Publishing House.

Sanz, M.L., Polemis, N., Morales, V., Corzo, N., Drakoularakou, A., Gibson, G.R., Rastall, R.A. (2005). *In vitro* investigation into the potential prebiotic activity of honey oligosaccharides. *J. Agric. Food Chem.*, 53, 2914–2921.

Sanz, M.L., Polenis, N., Morales, V., et al. (2005). In vitro investigation into the potential prebiotic activity of honey oligosaccharides. *J. Agric. Food Chem*., 53, 2914–2921.

Sarin, R. (2012). Nutraceuticals: A review. *International Research Journal of Pharmacy,* 3(4), 95–99.

Schencke, C., Vásquez, B., Sandoval, C., Del Sol, M. (2016). El rol de la miel en los procesos morfofisiologicos de reparación de heridas. *Int. J. Morphol*., 34, 385–395.

Shen, Y., Croft, K.D., Hodgson, J.M., et al. (2012). Quercetin and its metabolites improve vessel function by inducing eNOS activity via phosphorylation of AMPK. *Biochem Pharmacol*., 15, 1036–1044.

Simuth, J., Bilikova, K., Kovacova, E., Kuzmova, Z., Schroder, W. (2004). Immunochemical approach to detection of adulteration in honey: physiologically

active royal jelly protein stimulating TNF-alpha release is a regular component of honey. *J. Agric. Food Chem.,* 52, 2154–2158.

Sing, R.P., Sing, P.N. (1996). Amino acid and lipid spectra of larvae of honey bee (*Apis cerana* Fabr) feeding on mustard pollen. *Apidologie,* 27, 21–28.

Solayman, M., Islam, M. A., Paul, S., Ali, Y., Khalil, M. I., Alam, N., Gan, S. H. (2016). Physicochemical properties, minerals, trace elements, and heavy metals in honey of different origins: a comprehensive review. *Compr. Rev. Food Sci. Food Saf.*, 15, 219–233.

Song, J. J., Salcido, R. (2011). Use of honey in wound care: an update. *Adv Skin Wound Care*, 24, 40–44.

Spilioti, E., Jaakkola, M., Tolonen, T., et al. (2014). Phenolic acid composition, antiatherogenic and anticancer potential of honeys derived from various regions in Greece. PLoS ONE, 9:e94860.

Steinberg, D., Kaine, G., Gedalia, I. (1996). Antibacterial effect of propolis and honey on oral bacteria. *Am. J. Dent.*, 9, 236–239.

Terrab, A., Hernanz, D., Heredia, F.J. (2004) Inductively coupled plasma optical emission spectrometric determination of minerals in thyme honeys and their contribution to geographical discrimination. *J. Agric. Food Chem*., 52, 3441–3445.

Tomás-Barberán, F.A., Martos, I., Ferreres, F., Radovic, B.S., Anklam, E. (2001). HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. *J. Sci. Food Agric.*, 81, 485–496.

Tomasin, R., Cintra Gomes-Marcondes, M.C. (2011). Oral administration of Aloe vera and honey reduces walker tumour growth by decreasing cell proliferation and increasing apoptosis in tumour tissue. *Phytotherapy Research*, 25(4), 619–623.

Trautvetter, S., Koelling-Speer, I., Speer, K. (2009). Confirmation of phenolic acids and flavonoids in honeys by UPLC–MS. *Apidologie*, 40, 140–150.

Truchado, P., Vit, P., Ferreres, F., Tomas-Barberan, F. (2011). Liquid chromatography–tandem mass spectrometry analysis allows the simultaneous characterization of C-glycosyl and O- glycosyl flavonoids in stingless bee honeys. *J. Chromatogr. A.,* 1218, 7601–7607.

Truzzi, C., Annibaldi, A., Illuminati, S., Finale, C., Scarponi, G. (2014). Determination of proline in honey: comparison between official methods, optimization and validation of the analytical methodology. *Food. Chem*., 150, 477–481.

Vandamme, L., Heyneman, A., Hoeksema, H., Verbelen, J., Moustrey, S. (2013). Honey in modern wound care: a systematic review. *Burns*, 39, 1514–1525.

Viuda-Martos, M., Navajas, Y., Fernández-López, J., Pérez-Álvarez, J.A. (2008). Functional properties of honey, propolis, and royal jelly. *J. Food Sci.,*73 R117–R124.

Wehling, M., von der Ohe, K., von der Ohe, W. (2006). Problemhonig robinie: Zu wenig invertase-aktivitä. *Deutsch Bienen J.,* 13, 18–19.

White, J.W. Jr. (1978). Honey. In: *Advances in food research*, New York, USA: Academic Publishing House, 287–364.

White, J.W., Doner, L.W. (1980). Honey composition and properties: Beekeeping in the United States. *Agric. Handbook*, 335, 82–91.

Won, S.A., Li, C., Kim, J., Rhee, H. (2009). Immunological characterization of honey major protein and its application. *Food Chem.,* 113, 1334–1338.

Wunnachit, W., Jenner, F., Sedgley, M. (1992). Floral and extrafloral nectar production in Anacardium occidentale l. (anacardiaceae): an andromonoecious species. *Int. J. Plant Sci.,* 153, 413–420.

Yaghoobi, N., Al-Waili, N., Ghayour-Mobarhan, M., et al. (2008). Natural honey and cardiovascular risk factors; effects on blood glucose, cholesterol, triacylglycerole,CRP, and body weight compared with sucrose. *Sci. World J.,* 8, 463–469.

Yahav, S. (1999). The effect of constant and diurnal cyclic temperatures on performance and blood system of young turkeys. *Journal of Thermal Biology*, 24, 71–78.

Yahav, S., Hurwitz, S. (1996). Induction of thermotolerance in male broiler chickens by temperature conditioning at an early age. *Poultry Science*, 75, 402–406.

Zaid, S.S.M., Sulaiman, S.A., Sirajudeen, K.N.M, Othman, N.H. (2010). The effects of tualang honey on female reproductive organs, tibia bone and hormonal profile in ovariectomised rats animal model for menopause. *BMC Complementary and Alternative Medicine*, 10, article 82.

Zaid, S.S.M, Sulaiman, S.A., Othman, N.H., et al. (2012). Protective effects of Tualang honey on bone structure in experimental postmenopausal rats. *Clinics*, 67(7), 779–784.

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BIOCHEMICAL FEATURES OF PROTEIN NUTRITION OF HONEY BEES

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Abstract

The features of protein nutrition and protein requirements of honey bees must be taken into account, given that bees are social insects, and should be examined at three levels: bee colony nutrition; adult bee nutrition; larva nutrition, since disturbances in protein nutrition at the previous stages of development affect the subsequent stages and vice versa. The content of free amino acids in the main protein sources - pollen, bee bread royal jelly, as well as the content of amino acids in some bee protein additives and in whey was examined. The quality of the natural bee protein food, must be evaluated, depends on the essential amino acid content, especially leucine, isoleucine and valine. When replacing the natural protein food of bee colonies, with other protein additives and substitutes, it is necessary to consider the protein and essential amino content, and also the lysine/arginine ratio, as an index of the quality of the protein and the protein additive respectively.

Key words*: amino acids, honey bee, proteins.*

INTRODUCTION

It is known that for normal growth and development, honey bees need basic nutrients: proteins, carbohydrates and lipids, as well as vitamins and minerals. It has also been proven that adequate nutrition is the basis for the growth and development of bee colonies. Nutritional deficiencies and starvation are likely causes of bee colony loss worldwide (VanEngelsdorp et al., 2009*).*

Proteins play the most important role in the health and vital functions of honey bees and honey bee colonies, respectively (Crailsheim, 1990):

• From a biological point of view, proteins are the main substances of a living cell, since the formation and growth of new organisms depends on them.

• From a physiological point of view, proteins take part in most of the vital processes of the body, as they form the basis of enzymes, hormones and many other biologically active substances.

The protein requirement of bees must be taking into account based on the fact that bees are social insects, often considered superorganisms (Seeley, 1989). Thus, protein nutrition should be regarded at three levels: features of protein nutrition of the bee colony as a whole; features of protein nutrition of an adult bee; features of protein nutrition of the larva, since, disturbance in protein nutrition at previous stages of development affect subsequent stages and vice versa (Brodschneider and Crailsheim, 2010). Proteins are necessary for young bees to form wing muscles (Hersch et al., 1978). A suboptimal protein diet slows down the time needed to reach the maximum thoracic mass of a working bee (Hagedorn and Möller, 1968). Proteins are also necessary for the development of hypopharyngeal glands (Alqarni, 2006) and ovaries (Hoover et al., 2006). It has been shown that glucose oxidase activity (an indicator of social immunity) and fat body mass (an indirect indicator of the immunocompetence of the individual bees) depend on protein nutrition (Alaux et al., 2010). Protein-rich food is absolutely essential in early spring for the growth and development of brood, to ensure the duration and quality of life of working bees (Algarni, 2006; Brodschneider and Crailsheim, 2010). In addition, protein-rich feed during this period are beneficial for bee health and their ability to resist infections and parasites (Alaux et al., 2010).

First of all, proteins are necessary as a source of amino acids that are reused in the biosynthetic processes of the bee's body.

The main source of amino acids and proteins for bees are pollen and bee bread. Royal jelly is a source of proteins for larvae and queen.

The nutritional value of pollen primarily depends on the protein content. It has been proven that the content of proteins and amino acids in pollen depends on the type of plant and can vary from 3 to 61%, but an average make up about 25% (Roulston et al., 2000). Relatively protein-rich pollen is observed in plants most often pollinated by bees or other insects (dandelions, fruit trees, clover, alfalfa and other plants). Low protein pollen is the majority of herbs, sedges, conifers, ragweed, and other plants that are pollinated by the wind.

Thus, pollen collected from different flower sources has different nutritional values for honey bees. It has also been proven that pollen quality directly affects the bee health (immunity, metabolism, brood, lifespan and fertility). The life span of working bees increases significantly when the pollen with a high percentage of protein (25-30%) is consumed - compared with lower quality pollen (less than 20% protein) (Schimdt L.S et al., 1995). Brood growth also depends on the quality of pollen (amount of protein): increases - when only pollen with an optimal content of protein and essential amino acids is available; decreases - when only low protein pollen is available. Pollen with a high nutritional value contributes to the resistance of bee colonies to: parasites, pathogens, stress factors.

The amount of pollen consumed by the bee colony largely depends on the percentage of protein. In his studies, Anderson et al. (2014) found that in order to receive 10 g of bioavailable protein, the bee colony must process 48 g of pollen with 30% protein or 72 g of pollen with 20% protein. That is 2 kg of pollen with 30% protein is equal to 3 kg of pollen with 20% protein. During the year, the bee colony consumes from 25 to 55 kg of pollen.

In addition, many other factors influence the amount of consumed pollen (Anderson et al., 2014). Eating only one type of pollen, which has a small amount of protein, does not cover the bee's body needs for essential amino acids. Schimdt et al. (1995) showed that only a mixture of various types of pollen has a beneficial effect on the development and productivity of bee colonies.

Thus, the nutritional value of pollen also depends on the content of essential amino acids. According to the results obtained by De Groot (1953), honey bees need 10 (ten) essential amino acids to support vital processes - arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. The absence or low content of one of 10 essential amino acids in pollen significantly reduces the development of the bee colony. Most of all, honey bees need leucine, isoleucine and valine, or the so-called BCAA (Branced Chain Amino Acids).

For example, bees need 4% isoleucine from their protein intake. In one type of pollen, only about 2% of isoleucine is contained, which leads to the consumption of twice amount of pollen to meet the requirements of the bee colony or to the mixing of different types of pollen containing a large amount of essential amino acids. In addition, these poly flower pollen mixtures enhance certain immune functions and provide antiseptic protection for honey bees (Alaux et al., 2010).

It was determined that the lysine/arginine ratio, as well as, the high lysine content, is the factor that determines the quality of pollen proteins and bee preferences in some types of pollen (Szezęsna, 2006). In pollen collected from various flowers, this ratio ranges from 1.24 to 3. For acacia pollen, the Liz/Arg ratio is 1.38, and for rape pollen - 1.84. It is considered that those plants that contain more essential amino acids in pollen are more beneficial for honey bees and are more often visited by them.

Amino acids also play an important role in the formation of food motivation and the selective collection of pollen from various plants. It has been established that pollen amino acid composition affects the sensitivity of special bee receptors, thereby contributing to the formation of conditioned reflexes between the quality of food and its aroma and determining the selective preference of honey bees to certain plant species. Some researchers believe that glutamic acid is the main amino acid for the formation of the olfactory memory of bees (Hendriksma and Shafir, 2014).

Thus, the one aim of this work was the comparative study of free amino acid content in

the main sources of protein for the bee colony: pollen, bee bread and royal jelly.
The other objective of this study was the comparative analysis of amino acid content in dried yeasts and whey in order to argue the utilization of whey (whey proteins) as alternative supplemental protein feeding.

MATERIALS AND METHODS

The amino acid content was determined in three types of pollen - acacia, poly flower and sunflower, in bee bread, royal jelly and in whey and dried yeast - as alternative protein feed for bee colonies. Pollen samples were collected from the central zone of the Republic of Moldova.
Amino acid analysis was performed on an AAA 339 M amino acid analyzer by ion exchange chromatography (Moore et al., 1958) at the Institute of Physiology and Sanocreatology, Republic of Moldova.
The analysis is performed in the standard procedure for the determination of free amino acids using lithium buffer solutions, pH 2.90, 2.95, 3.20, 3.80 and 5.00, with a flow rate of 12.0 ml/hr. On the basis of the qualitative calculation of amino acid content in the liquid studied it is stated that the amount of an amino acid in the sample is proportional to the surface of the pick of the chromatogram. The calculation consists in the fact, that sample and standard mixture of amino acids with the same content is analyzed. The amount of amino acids dosed on the ionic column in the test sample is given by the formula below:

$$C_{i(doz.)}=k \cdot n \cdot S_{i(prob.)}/S_{i(st.)} \cdot M_i \cdot 10^{-6} \text{ (mg)},$$

where: $C_{i(doz.)}$ - the ionic concentration of amino acids in the volume of the dosed node; n - the amount of the amino acids in the analyzed mixture; $S_{i(prob.)}$ - the tip(pick) surface of the amino acids in analyzed mixture; $S_{i(st.)}$ - the tip(pick) surface of the amino acids in standard mixture; k - correction coefficient considered to be changing the detector sensitivity; M_i - the ionic molecular weight of the amino acid. The automatical analyzer AAA-339M detects ninhydrin positive components within 1-100 nanomoles concentration. The duration of the analysis of the physiological fluids is 3.5 hours.

RESULTS AND DISCUSSIONS

The amino acid analysis in the pollen samples taken in the study according to the ion exchange liquid chromatography method revealed 17 amino acids. Tryptophan in pollen samples was identified in extremely small amounts, which did not allow its comparative analysis with other amino acids in the samples. Aspartic acid includes both aspartic acid and asparagine and glutamic acid includes both glutamic acid and glutamine (in the process of detection asparagine is combined with aspartate and glutamine with glutamate and so they have the identical picks that reflect the quantity of extraction).
In the investigation of the content of free amino acids in acacia pollen, poly flower pollen and sunflower pollen, a higher amount was determined in acacia pollen, namely 13.2 mg/100 mg. In poly flower pollen this value is 11.95 mg/100 mg, and in sunflower pollen - 8.35 mg/ 100 mg (Figure 1).

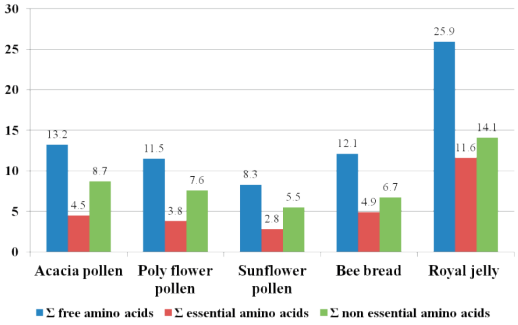

Figure 1. The total content of free amino acids, essential and non-essential amino acids in pollen, bee bread and royal jelly (mg/100 mg d.w.)

In bee bread, the total content of free amino acids, as well as of the essential and non-essential amino acids, corresponds to that in pollen. In royal jelly the level of free amino acids, especially is much higher compared to pollen and bee bread (Figure 1).
In order to identify the value of a protein or protein product, a comparison of the proportion of essential amino acids to the total content of free amino acids is used (Table 1).

Table 1. The proportion of essential and non-essential amino acids to the total content of free amino acids in pollen, bee bread and royal jelly

Samples	Proportion, %	
	Essential amino acids	Non-essential amino acids
Acacia pollen	34.0	65.0
Poly flower pollen	33.0	65.2
Sunflower pollen	33.7	65.0
Bee bread	40.0	55.0
Royal jelly	44.0	54.0

Thus, in all types of pollen studied, the proportion of essential and non-essential amino acids is the same: 34.0% and 65.0% in acacia pollen; 33.0% and 65.2% in poly flower pollen; 33.7% and 65.0% in sunflower pollen, respectively. In bee bread, the ratio of essential amino acids is higher (40.0%) than in pollen. In royal jelly, the highest percentage (proportion) of essential amino acids (44.0%) and the lowest percentage of non essential amino acids (54.0%) were detected.

These data indicate that royal jelly is more valuable in terms of the content of essential amino acids of all of the studied protein sources for the bee colony (Postolati et al., 2012).

The other aim of this work was a study of amino acid content in alternative protein sources for bee colonies.

In some periods of the year, pollen (or bee bread) may be in insufficient quantities, which can cause a protein deficiency in the bee's diet, and, in turn, affect their health and resistance to infections and parasites. Most often, lack of pollen and/or low-quality pollen is caused by intensive cultivation of monocultures in agriculture. Since pollinating services for honey bees are often provided within a human-defined ecosystem, the basic nutrient needs of bees cannot be adequately provided (Naug, 2009).

To compensate for the lack of protein in bee nutrition, additional protein feeding of bees is widely used, depending on the seasonal specifics of the functional state of the honey bee and the requirements of the bee colony in protein: in spring - for feeding and brood growth; and in the fall - for the accumulation of a fat body, which ensures better survival of colonies during the winter (Brodschneider and Crailsheim, 2010; Eremia, 2009).

There are various methods and technologies for producing protein supplements based on soy, peas, corn, oats, barley flour, egg powder and egg whites, whole or dried milk, fish flour, dry yeast etc. These protein additives are introduced in the form of “candy”, which is a mixture of honey, powdered sugar and protein flour or in the form of sugar syrup with the addition of proteins or amino acids (Malaiu, 1976; Standifer et al., 1977; Brodschneider and Crailsheim, 2010; Fleming et al., 2015).

When replacing pollen with other protein-rich feeds, it is recommended to take into account the nutritional value of these substitutes, based on the spectrum of amino acids, as well as of their amount, especially of essential ones. Also, Herbert et al. (1977) showed that the optimal protein content in feed for bees should be 20-30%. At the same time, a 50% level should be avoided.

In 2011, studies of whey as an alternative source of protein and amino acids for bee colonies were started at the Institute of Physiology and Sanocreatology.

The choice of whey as the basis for syrup for honey bee feeding is due to the extremely important value of whey as a source of bioavailable protein with a high biological index (Barth and Behnke, 1997).

Whey proteins make up 20% of milk proteins (the remaining 80% is casein) and are characterized by high nutritional and biological value, having a nutritional index of 1 (casein has an index of 0.8), exceeding egg albumin, which long time has been considered protein No. 1 in terms of biological values (Krissansen, 2007; Gupta and Prakash, 2017). Thus, whey is valuable not only in terms of the amount of protein, but also its quality.

A comparative analysis of the amino acids from whey and pollen showed that whey contains the entire spectrum of amino acids present in pollen, bee bread and royal jelly. However, their amount in whey is higher than in samples taking into study (Figures 1, 2).

It was detected a higher level of essential amino acids especially of BCAA - valine, leucine, isoleucine, and also lysine, methionine, threonine, histidine and tryptophan - amino acids that determine the quality of protein feed for bees (Figure 2).

These data denote the possibility of using whey for supplemental protein feeding of bee colonies.

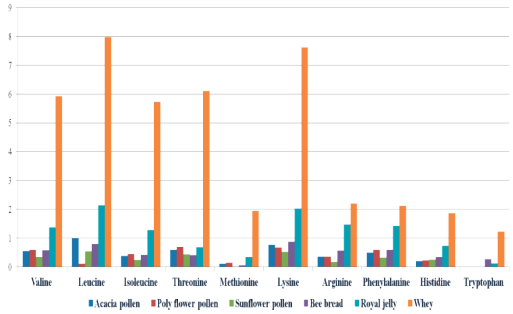

Figure 2. The comparative content of essential amino acids in pollen, bee bread, royal jelly and in whey (mg/100 mg d.w.).

Also, in whey there is a high content of non-essential amino acids, some of which have a significant role in physiological and biochemical processes and are called functional amino acids. Those are: glutamic acid and glutamine, aspartic acid and aspargine, proline, cysteine (Figure 3).

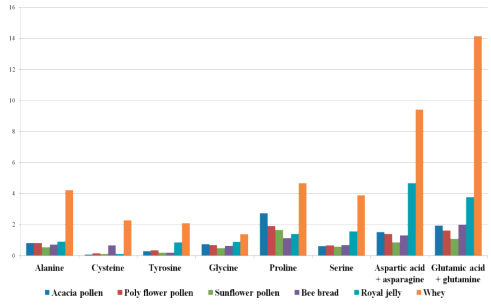

Figure 3. The comparative content of non-essential amino acids in pollen, bee bread, royal jelly and in whey (mg/100 mg d.w.).

As mentioned above, in order to compensate the lack of protein in the diet of bee colony, protein additive feeding is widely used, most often based on soy flour or dried yeast. In order to identify the possibility of using of whey as a source of amino acids and biologically valuable proteins for the bee colony, we compared the content of essential amino acids in whey and dried yeast.

Thus, in dried yeast, the total content of essential amino acids is 4.91 g/100 g d.w., and in whey - 4.85 g/100 g d.w. In comparison with dried yeast, whey contains a higher content of BCAA amino acids – valine, leucine and isoleucine, that is, those amino acids that are most needed for vital processes (Figure 4).

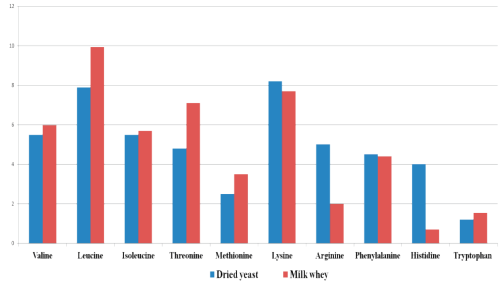

Figure 4. Comparative content of essential amino acids in whey and dry yeast (g/100 g d.w.)

The lysine/arginine ratio as an indicator of the quality of the protein for the bee was also higher in milk whey compared to pollen, bee bread, royal jelly and also dried yeast (Table 2).

Table 2. The ratio of lysine/arginine in pollen, bee bread, royal jelly and whey

Samples	The lysine/arginine ratio
Acacia pollen	2.22
Poly flower pollen	1.87
Sunflower pollen	3.0
Bee bread	1.57
Royal jelly	1.37
Dried yeast	1.64
Whey	**3.45**

Thus, this index (lysine/arginine ratio) can be used to argue the quality of protein feed for bees, and whey, in this regard, has the best characteristics.

Based on whey properties, it was obtained a patent MD 4284 “Method for feeding bee colonies” (Derjanschi et al., 2014) and a patent MD 1312 “Process for producing protein-carbohydrate food for bee colonies” (Vrabie et al., 2019).

CONCLUSIONS

Protein nutrition of the bee colony needs to be considered at three levels: features of protein nutrition of the bee colony as a whole; features of protein nutrition of an adult bee; features of protein nutrition of the larva, since, disturbances in protein nutrition at the previous stages of development affect the subsequent stages and vice versa.

Protein nutrition of bee colony is seasonal: in spring, protein-rich foods are needed primarily for brood rearing; the end of summer and early autumn - for the accumulation of a fat body,

which ensures better survival of colonies during the winter.

The quality of the main source of protein for bee colonies - pollen, depends on the protein content and essential amino acids, especially leucine, isoleucine and valine and the lysine/arginine ratio.

According to the invention, "A method for bee colonies feeding" Patent MD 4284 (2014) and "Process for producing protein-carbohydrate food for bee colonies", Patent Md 1312 (2019), whey can be used as protein additive for bee colony feeding in early spring to intensify brood rearing and bee colony development.

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REFERENCES

Alaux, C., Ducloz, F., Crauser, D., Le Conte, Y. (2010). Diet effects on honeybee immunocompetence. *Biol. Lett.* DOI: 101098/rsbl 2009.0986.

Algarni, A.S. (2006). Influence of some protein diets on the longevity and some physiological conditions of honeybee *Apis mellifera* L. workers. *J. Biol. Sci*., 6, 734-737.

Anderson, K.E., Carroll, M.J., Sheehan, T., Mott, B.M., Maes, P., Corby-Harris, V. (2014). Hive-stored pollen of honey bees: many lines of evidence are consistent with pollen preservation, not nutrient conversion. *Mol Ecol.,* 23, 5904-5917.

Barth, C.A., Behnke, U. (1997). Nutritional physiology of whey and whey components. *Nahrung*, 41(1), 2-12. (in German).

Brodschneider, R., Crailsheim, K. (2010). Nutrition and health of honey bees. *Apidologie*, 41, 278-294.

Crailsheim, K. (1990). The protein balance of the honey bee worker. *Apidologie*, 21, 417-429.

De Groot, A.P. (1953). Protein and amino acid requirements of honeybee (*Apis mellifera* L.). *Physiol. Comp. et d'Ecol*., 3, 197-285.

Derjanschi, V., Vrabie, E., Ciochină, V., Furdui, T. (2014). Process for bee families feeding. Patent MD 4284. *BOPI*, 5, 21.

Eremia, N. (2009). *Apicultura*. Chișinău „IEFS". - 330 p.

Fleming, J.C., Schmehl, D.R., Ellis, J.D. (2015). Characterizing the impact of commercial pollen substitute diets on the level of *Nosema* spp. in honey bees (*Apis mellifera* L.). PLoS One, 10(7), e0132014.

Gupta, C., Prakash, D. (2017). Terapeutic potential of milk whey. Beverages, 3, 31, 14 p.

Hagedorn, H.H., Moeller, F.E. (1968). Effect of age of pollen used in pollen supplements on their nutritive value for the honeybee. I. Effect on thoracic weight, development of hypopharyngeal glands, and brood rearing. *J. Apic. Res.,* 7, 89-95.

Hendriksma, H. P., Shafir, K. L. (2014). Amino acid and carbohydrate tradeoffs by honey bee nectar foragers and their implications for plant-pollinator interactions. *Journal of Insect Physiology*, 69, 56-64.

Herbert, E.W., Shimanuki, H., Caron, D. (1977). Optimum protein levels required by honey bees (Hymenoptera, Apidae) to initiate and maintain brood rearing. *Apidologie*, 8, 141-146.

Hersch, M.I., Crewe, R.M., Hepburn, H.R., Thompson, P.R., Savage, N. (1978). Sequential development of glycolitic competence in muscles of worker noneybees. *Comp.Biochem. Physiol*., B 61, 427-431.

Hoover, S.E., Higo, H.A., Winston, M. L. (2006) Worker honey bee ovary development: seasonal variation and the influence of larval and adult nutrition. *J.Comp. Physiol*., B 176, 55-63.

Hrassnigg, N., Crailsheim, K. (2005) Differences in drone and worker physiology in honeybees (*Apis mellifera* L.). *Apidologie*, 36, 255-277.

Krissansen, G.W. (2007). Emerging health properties of whey proteins and their clinical implication. *J. Amer. Coll. Nutr.*, 26(6), 713-723.

Malaiu, A. (1976). *Increasing honey production / Sporirea producției de miere*. Bucharest, RO: Ceres Publishing House, 262 p.

Moore, S., Spackman, D.H., Stein, W. (1958). Chromatography of amino acids on sulfonated polystyrene resins. *Anal. Chem.*, 30 (1), 1185-1190.

Naug, D. (2009) Nutritional stress due to habitat loss may explain recent honeybee colony collapses. *Biol. Conserv*., 142, 2369-2372.

Postolati, G., Pavalic, G., Mantoptin, A., Furdui, V., Leorda, A., Garaeva, G., Redcozubova, G. (2012) Bee product as source of amino acid detoxifiers. In: Materials of the VI Congress of Physiologists of the Republic of Moldova "Physiology and Health"", Chișinău, 43-51 (in Russian).

Roulston, T.H., Cane, J.H. (2000). Pollen nutritional content and digestibility for animals. *Plant Syst. Evol*., 222, 187-209.

Schimdt, L.S., Schimdt, J.O., Rao, H., Wang, W., Xu, L. (1995) Feeding preference of young worker honey bees (Hymenoptera: Apidae) fed rape, sesame, and sunflower pollen. *J.Econ. Entomol*., 88, 1591-1595.

Seeley, T.D. (1989) The honey bee colony as a super-organism. *Am. Sci*., 77, 546-553.

Standifer, L.N., Moeller, F.E., Kauffeld, N.M., Herbert, E.W., Shimanuki, H. (1977). Supplemental feeding of honey bee colonies. *USDA Agr. Inform. Bull*., 413, 8 p.

Szezęsna, T. (2006). Protein content and amino acid composition of bee-collected pollen from selected botanical origins. *J. Apicultural Science*, 5(2), 81-90.

VanEngelsdorp, D., Evans, J.D., Saegermann, C., Mullin, C., Haubrugge, E., Nguyen, B.K., Frazier, V. et al. (2009). Colony collapse disorder: a descriptive study. *PLosONE* 4, e6481.

Vrabie, V., Derjanschi, V., Ciochină, V., Furdui, T. (2019). Process for producing protein-carbohydrate food for bee families. Patent MD 1312. *BOPI*, 2, 52.

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THE BIOCHEMICAL COMPOSITION AND THE FEED VALUE OF GREEN MASS AND SILAGE FROM *Cynara cardunculus* AND *Helianthus tuberosus* IN THE REPUBLIC OF MOLDOVA

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Abstract

The objective of this research was to evaluate the quality of green mass and silage prepared from cardoon - Cynara cardunculus var. altilis and Jerusalem artichoke - Helianthus tuberosus cv. 'Solar' growing in the experimental field of NBGI, Chişinău, Republic of Moldova. Some assessments of the main biochemical parameters: crude protein (CP), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL), total soluble sugars (TSS), digestible dry matter (DDM) have been determined by near infrared spectroscopy (NIRS), the sensorial characteristics of prepared silages – in accordance with the Moldavian standard SM 108, the concentration of hemicellulose (HC), cellulose (Cel), digestible energy (DE), metabolizable energy (ME), net energy for lactation (NEl) and relative feed value (RFV) were calculated according to standard procedures. It was determined that dry matter content in the harvested green mass varied from 19.8% in Cynara cardunculus to 28.9% in Helianthus tuberosus, its biochemical composition and feed value were: 9.4-12.0% CP, 6.0-7.1% ash, 30.2-40.0% ADF, 54.5-60.3% NDF, 3.1-5.1% ADL, 14.6-27.8% TSS, 27.1-34.9% Cel and 20.3-24.3% HC, 57.7-65.3% DDM, RFV = 89-111, 11.87-12.82 MJ/kg DE, 9.41-10.53 MJ/kg ME and 5.43-6.55 MJ/kg NEl. It was found that the prepared silages had pleasant smell, somehow similar to pickled vegetables, the colour of the cardoon silage was homogeneous olive, but Jerusalem artichoke silage contained dark-green leaves with brownish hues and yellow-green stems. The pH of the silage was 3.77-4.17, it contained 9.7-12.5% CP, 8.4-10.3% ash, 30.6-33.4% ADF, 49.6-53.1% NDF, 2.6-5.0% ADL, 19.2-28.0% Cel, 22.5-28.4% HC with feed value 62.9-65.1% DDM, RFV= 114-118, 12.39-12.78 MJ/kg DE, 10.17-10.49 MJ/kg ME and 6.19-6.51 MJ/kg NEl. The studied perennial Asteraceae species were characterized by high green mass productivity with optimal nutrient contents; good silage quality can be used as alternative feed for ruminant animals.

Key words*: biochemical composition, Cynara cardunculus, feed value, green mass, Helianthus tuberosus, silage.*

INTRODUCTION

The identification, mobilization and cultivation of new species, the use of a wider range of forage crops, providing a stable and balanced diet for farm animals, play an important role in the agricultural economy and food security (Marin et al., 2017).

Cardoon, *Cynara cardunculus* L.*, Asteraceae* family, native to the Mediterranean region, is a perennial C_3 plant species, usually growing 75-150 cm tall, but occasionally reaching up to 2 m in height. The root system is very well developed, consists of the main taproot, which can grow to the depth of 2 m, with variable number of secondary fibrous roots. The leaves form a basal rosette that can be very large – up to 120 cm long and 30 cm wide. Alternate leaves, green-greyish coloured and more or less incised, present on the main and other order stems. The flower heads are almost

round in shape and grow to be 4-5 cm across, usually blue-violet coloured. The fruit is a tetragon-shaped or flattened achene, dark-coloured. This species reproduces by seed, asexually from pieces of cut root, and also re-grows each year from a long-lived underground crown and taproot. It is well-adapted to the xerothermic conditions of southern Europe, is quite tolerant to salinity and intolerant to prolonged waterlogging and prefers slightly acidic soils to the alkaline pH 6.5-8.2. *Cynara cardunculus*, characterized by large yields, offers a wide spectrum of economic uses: food source, raw material for pharmaceutical industry, for biorefineries and renewable energy production; the aerial parts of the plants

- as green or ensiled forage; fresh flowers are used as a vegetable rennet for milk clotting; achenes - to produce oil that can be used by humans, and cake could be used for animal feed (Ferndndez et al., 1996; Pesce et al., 2017; Gominho et al., 2018).

Jerusalem artichoke, otherwise known as topinambour, *Helianthus tuberosus* L., *Asteraceae* family, native to North America, was brought from France in 1605. It is herbaceous perennial C_4 photosynthetic pathway plant, with strong, vigorous stems, sometimes branched at the base, grows 2.5-3.0 m tall, its large leaves reach the length of 20 cm and are arranged on the opposite sides of the stem, alternately to one another. In the underground part, there are unevenly shaped tubers, round or elongated, with bumps, ranging in size between 2 and 10 cm, their color can vary from brown to white, red and even purple.

The analysis of literature sources have revealed that Jerusalem artichoke dry matter yield of aboveground parts ranges from 4 to 30 t/ha and tubers - from 4 to 15 t/ha, depending on the genotype, climatic conditions, soil type and plantation age. It is an economically important plant that can be useful in many ways, such as: functional food and bioactive ingredient source, sustainable feedstock for biorefineries and the production of renewable energy etc. (Duke, 1983; Kays and Nottingham 2008; Ţîţei et al., 2013; Heuzé et al., 2015; Johansson et al. 2016; Herrmann et al., 2016). Jerusalem artichoke has been used as a suitable livestock feed since the mid-1600s, has the potential to replace or be a substitute for other annual and perennial forages for ruminants. The substitution of alfalfa by up to 30% of Jerusalem artichoke foliage, in full bloom stage, did not affect the *in vitro* digestibility of the diet (Cosgrove et al., 2000; Fazaeli et al., 2009; Heuzé et al., 2015).

The melliferous potential of *Cynara scolymus* is 100-150 kg honey/ha, *Helianthus annus* and *Helianthus tuberosus* is 30-60 kg honey/ha (Ion et al., 2018).

The aim of the current study was to evaluate some agrobiological peculiarities and the quality of green mass and silage prepared from *Cynara cardunculus* var. *altilis* and *Helianthus tuberosus* grown under the conditions of the Republic of Moldova.

MATERIALS AND METHODS

The *Asteraceae* species: the cultivar 'Solar' of Jerusalem artichoke*, Helianthus tuberosus,* created in the National Botanical Garden (Institute), registered in 2014, in the Catalogue of Plant Varieties** and patented in 2016, by the State Agency on Intellectual Property (AGEPI) of the Republic of Moldova, patent nr. 205/31.05.2016*, and cardoon, *Cynara cardunculus* var. *altilis*, which were cultivated in the experimental plot of the National Botanical Garden (Institute), Chişinău, served as subjects of the research, and the traditional crop sunflower, *Helianthus annuus*, was used as control.

The green mass of *Cynara cardunculus* was mowed in early flowering stage (early July), *Helianthus tuberosus* - in budding stage (middle August)**,** but the control - *Helianthus annuus* - in the full flowering stage (end of July).

Green mass productivity was determined by weighing the yield obtained from a harvested area of 10 m^2, which was afterwards transformed per hectare.

The leaves/stems ratio was determined by separating the leaves, buds and flowers from the stem, weighing them separately and establishing the ratios for these quantities (leaves/stems). For ensiling, the green mass was shredded and compressed in well-sealed containers.

After 45 days, the containers were opened, and the sensorial and chemical characteristics of prepared silages were determined in accordance with standard laboratory procedures and Moldavian standard SM 108*** for forage quality analysis. Some assessments of the main biochemical parameters: crude protein (CP), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL), total soluble sugars (TSS), digestible dry matter (DDM) have been determined by near infrared spectroscopy (NIRS) technique PERTEN DA 7200.

The concentration of hemicellulose (HC), cellulose (Cel), digestible energy (DE), metabolizable energy (ME), net energy for lactation (NEl) and relative feed value (RFV) were calculated according to standard procedures

RESULTS AND DISCUSSIONS

We could mention that, the studied *Asteraceae* species differed in the growth and development rate, ratio of stems to leaves, content of nutrients. Cardoon, *Cynara cardunculus* var. *altilis*, in the first growing season, developed rosettes with 30-35 dark green, pinnate leaves, in the underground part - deep taproot over 100 cm, the yield reached 3.40 kg/m^2 green mass or 0.50 kg/m^2 dry matter, its biochemical composition: 23.6% crude protein, 9.2% ash, 32.6% NDF, 18.3% ADF, 1.6% ADL, 22.0% TSS with 96% DMD. In the second year, after the winter, the development of cardoon plants restarted, an apical bud appeared on the stem apex, the leaf rosette developed and spread in April, the stalk began to elongate in May and flower heads appeared in June.

The Jerusalem artichoke, *Helianthus tuberosus*, in the first year of development, went through all the ontogenetic stages. In the second growing season, the plants started vegetating at the end of April, the fastest growth and development were observed at the end of May and in July.

Plant height, stem thickness and leaves/stems ratio have significant impact on the yield, but also affect the quality of the phytomass. Results regarding some bio-morphological characteristics of the studied *Asteraceae* species and the structure of the harvested phytomass are presented in Table 1. At the time of harvesting, plant height varied from 201 cm (*Cynara cardunculus*) to 326 cm (*Helianthus tuberosus*), stem thickness - from 24-36 mm (*Cynara cardunculus*) to 12-22 mm (*Helianthus tuberosus*), but the control, sunflower (*Helianthus annuus*) - 201 cm and 20-24 mm, respectively. The productivity of *Cynara cardunculus*, in the second year, was 7.12 kg/m^2 green mass or 1.41 kg/m^2 dry matter, with 45% leaves, 42% stalks and 13% heads; *Helianthus tuberosus* - 10.5 kg/m^2 green mass or 3.03 kg/m^2 dry matter, with 34% leaves and 66% stalks, but the control reached 5.03 kg/m^2 green mass or 0.94 kg/m^2 dry matter, with 43% stalks, 26% leaves and 31% heads.

According to Pesce et al. (2017) the green mass productivity of cv. 'Altilis 41' of *Cynara cardunculus* reached 19.1 t/ha dry mater, with 37.7% leaves, 27.7% stalks and 34.6% inflorescence material. Liu et al., 2011 mentioned that Chinese clones of Jerusalem artichoke cultivated in a semiarid region in the northwest China had a biological yield ranging from 25 to 35 t ha dry matter. Seiler (1993) mentioned that the aboveground biomass of Jerusalem artichoke contained 68% stems, 23% leaves and 9% heads.

Table 1. Some agrobiological peculiarities and the structure of the green mass of the investigated *Asteraceae* species

Plant species	Plant height, cm	Stem, g		Leaf, g		Head, g		Yield, kg/m^2	
		green mass	dry matter	green mass	dry matter	green mass	dry matter	green mass	dry matter
Cynara cardunculus	201	410	81	522	25	142	87	7.12	1.41
Helianthus tuberosus	326	243	73	141	38	-	-	10.5	3.03
Helianthus annuus	178	343	60	169	43	254	38	5.03	0.94

Table 2. Biochemical composition and nutritive value of the harvested green mass of the investigated *Asteraceae* species

Indices	*Cynara cardunculus*	*Helianthus tuberosus*	*Helianthus annuus*
Crude protein, g/kg DM	120	94	102
Minerals, g/kg DM	60	71	83
Acid detergent fibre, g/kg DM ,	302	400	267
Neutral detergent fibre, g/kg DM	545	603	401
Acid detergent lignin, g/kg DM	31	51	41
Total soluble sugars, g/kg DM	278	146	243
Cellulose, g/kg DM	271	349	226
Hemicellulose, g/kg DM	243	203	226
Digestible dry matter, %	65.3	57.7	68.1
Dry matter intake, % BW	2.20	1.99	2.99
Relative feed value	111	89	158
Digestible energy, MJ/ kg	12.82	11.47	13.32
Metabolizable energy, MJ/ kg	10.53	9.41	10.94
Net energy for lactation, MJ/ kg	6.55	5.43	6.96

Analyzing the results of the green mass quality of the *Cynara cardunculus* and *Helianthus tuberosus*, Table 2, we found that dry matter content of the harvested green mass contained

94-120 g/kg CP, 71-60 g/kg ash, 302-400 g/kg ADF, 545-603 g/kg NDF, 31-51 g/kg ADL, 146-278 g/kg TSS, 271-349g/kg Cel and 203-243 g/kg HC, 57.7-65.3% dry matter digestibility. The natural fodder of the studied species have RFV = 89-111, 11.47-12.82 MJ/kg DE, 9.41-10.53 MJ/kg ME and 5.43-6.55 MJ/kg NEl, but the control - *Helianthus annuus* - RFV 158, 13.52 MJ/kg DE, 10.94 MJ/kg ME and 5.43-6.96 MJ/kg NEl, respectively. The natural fodder of *Cynara cardunculus* was characterised by higher concentration of total soluble sugars and optimal protein, as compared with *Helianthus tuberosus* and *Helianthus annuus*. *Helianthus tuberosus* had higher concentration of ADF, NDF, ADL and lower concentration of hemicellulose, which had a negative effect on digestibility, relative feed value and net energy for lactation.

Some authors mentioned various findings about the green mass quality of the studied *Asteraceae* species. Ferndndez et al. (1996), Romero et al. (1997) reported that the green mass of *Cynara cardunculus*, contained 14.3-18.4% CP, 36.3-38.4% NDF; 25.1-28.9% ADF, and 9.3-13.3% ADL, therefore suitable to be used as green forage. Cajarville et al. (1999) mentioned that green forage had high nutritive value, low levels of fibre and lignin, and very high digestibility for organic matter (86%), while ensiling is the most appropriate way for preserving it for long periods. According to Cosgrove et al. (2000), the crude protein (5%) and digestible protein (3%) concentration are low in Jerusalem artichoke tops as compared with alfalfa (14% and 10%), but it was superior in total digestible nutrients (67%) as compared with alfalfa (50%) and the perennial grass smooth brome (46%). Heuzé et al. (2015), revealed that the aerial part of the *Helianthus tuberosus* contained 32.3% dry matter, 15.3% CP, 15.3% CF, 40.6% NDF, 34.5% ADF, 2.2% ether extract, 11.5% lignin, 14.4 % ash, 63.0% organic matter digestibility, 10.1 MJ/kg DE, 8.2 MJ/kg ME and 5.0-6.0 MJ/kg NEl, for ruminants. Ersahince & Kara (2017) have found that the chemical composition of Jerusalem artichoke herbage harvested in early flowering stage was 7.37% protein, 1.70% fats, 40.15% NFC, 39.03% aNDFom, 31.7% ADFom, 6.78% ADL. Seiler (1993) mentioned that *in vitro* digestibility of dry matter of the Jerusalem artichoke cultivars varied from 542 to 715 g/kg in whole plants in the flowering stage.

Silage is the main conserved green succulent roughage fed to domestic animals. When opening the glass vessels with silages made from green mass of *Cynara cardunculus* and *Helianthus tuberosus*, there was no gas or juice leakage from the preserved mass, but from the vessels with *Helianthus annuus* silage, carbon dioxide - a by-product of fermentation - it was moderately eliminated. The prepared silages were of agreeable colour and had specific aroma, the consistency was retained in comparison with the initial green mass, without mould and mucus. During the organoleptic assessment, it was found that the colour of the *Cynara cardunculus* silage was homogeneous olive, with pleasant smell, similar to pickled vegetables, *Helianthus tuberosus* silage - dark-green leaves with brownish hues and yellow-green stems, with pleasant smell, somewhat like the smell of pickled vegetables, but in the *Helianthus annuus* silage, the stems were yellow and the leaves - dark green, its scent was similar to the smell of fresh coniferous wood.

The fermentation quality of silage from the studied *Asteraceae* species is illustrated in Table 3. The materials consolidated well and the fermentation was complete with similar acidic pH values 3.77-4.17. It has been determined that the amounts of organic acids, in the silages prepared from *Asteraceae* species, differed essentially. *Cynara cardunculus* silage was characterised by a very low content of organic acids (43.1 g/kg), in comparison with *Helianthus* species silages (69.5-76.4 g/kg). Most organic acids in tested silages were in fixed form. The butyric acid was detected in fixed form (1.4 g/kg DM) in the *Cynara cardunculus* silage. In the silage from *Helianthus tuberosus*, the content of acetic acid reached 27% of organic acids.

The dry matter content in the prepared silages from *Helianthus annuus* and *Cynara cardunculus* were very low (19.1-19.7%) in comparison with *Helianthus tuberosus* silage (30.5%). The biochemical composition of dry matter and nutritive value of the prepared silages are shown in Table 4. It was determined that the dry matter of the tested silages contained 97-125 g/kg CP, 84-103 g/kg ash, 306-334 g/kg ADF, 496-531 g/kg NDF, 26-50 g/kg ADL, 192-280 g/kg

Cel and 225-284 g/kg HC. The nutritive and energy value of silages from *Helianthus tuberosus* and *Cynara cardunculus*: RFV = 114-118, 12.39-12.78 MJ/kg DE, 10.17-10.49 MJ/kg ME and 6.19-6.51 MJ/kg NEl, but the control - *Helianthus annuus* silage - RFV 161, 14.98 MJ/kg DE, 12.30 MJ/kg ME and 7.12 MJ/kg NEl, respectively.

Table 3. The fermentation quality of the silage prepared from the investigated *Asteraceae* species

Indices	*Cynara cardunculus*	*Helianthus tuberosus*	*Helianthus annuus*
pH index	3.77	4.17	3.82
Content of organic acids, g/kg	43.1	69.5	76.4
Free acetic acid, g/kg	4.0	8.9	9.3
Free butyric acid, g/kg	0.0	0.0	0.0
Free lactic acid, g/kg	12.6	10.4	20.3
Fixed acetic acid, g/kg	3.5	9.7	10.2
Fixed butyric acid, g/kg	1.4	0.0	0.1
Fixed lactic acid, g/kg	21.6	40.5	36.5
Total acetic acid, g/kg	7.5	18.6	19.5
Total butyric acid, g/kg	1.4	0.0	0.1
Total lactic acid, g/kg	34.2	50.9	56.8
Acetic acid, % of organic acids	18	27	24
Butyric acid, % of organic acids	3	-	1
Lactic acid, % of organic acids	79	73	75

Table 4. Biochemical composition and nutritive value of the silage prepared from the investigated *Asteraceae* species

Indices	*Cynara cardunculus*	*Helianthus tuberosus*	*Helianthus annuus*
Raw protein, g/kg DM	125	97	102
Minerals, g/kg DM	84	103	99
Acid detergent fibre, g/kg DM Neutral	306	334	252
Detergent fibre, g/kg DM	531	496	401
Acid detergent lignin, g/kg DM	26	50	35
Cellulose, g/kg DM	280	192	217
Hemicellulose , g/kg DM	225	284	149
Digestible dry matter, %	65.1	62.9	69.29
Dry matter intake, % BW	2.26	2.42	2.99
Relative feed value	114	118	161
Digestible energy, MJ/ kg	12.78	12.39	14.98
Metabolizable energy, MJ/ kg	10.49	10.17	12.30
Net energy for lactation, MJ/ kg	6.51	6.19	7.12

During the process of ensiling of *Helianthus tuberosus* plants, we observed a significant reduction in the neutral detergent fibre content and an obvious increase in the hemicellulose content, and these factors had a positive impact on digestibility, nutritive and energy value of the preserved feed and also on the methane yield.

Some authors mentioned various findings about the quality of *Asteraceae* silages.

According to Pesce et al. (2017), *Cynara cardunculus* produced silage with 32.8% DM, pH 3.3, 1.3% lactic acid, 1.4% acetic acid, 0.2% butyric acid, 14.6% CP, 11.9% ash, 48% NDF, 28.1% ADF. The nutritive value and the fermentation characteristics of artichoke, *Cynara scolymus*, by-products were 150.1 g/kg crude protein, 524.1 g/kg NDF, 411.7 g/kg ADF, the highest matter digestibility at 96 h incubation *in vitro*: 786 g/kg DMD and 804 g/kg OMD (Sallam et al., 2018). The *Cynara cardunculus* silage produced from whole cordon plants mowed in full bloom stage was characterized by 20.6% DM, 8.79% ash, pH 4.13, 72.3 g/kg lactic acid, 21.5 g/kg acetic acid and 49.5 g/kg ethanol (Ferrero et al., 2018).

Herrmann et al. (2016) studied the nutrient and fibre composition of crop silages in Germany and remarked that the Jerusalem artichoke silage contained 28.2% dry matter and 89.7% organic matter, pH 3.9, 7.2% lactic acid, 1.6% acetic acid, 9.8% protein, 1.9% fat, 44.1% NDF, 39.6% ADF and 11.7% ADL; in sunflower silage there was 23.0% dry matter and 87.5% organic matter, pH 4.2, 7.4% lactic acid, 2.0% acetic acid, 9.4% protein, 11.1% fat, 39.9% NDF, 37.6% ADF, 9.5% ADL.

CONCLUSIONS

The nutritive and energy value of green mass from *Cynara cardunculus:* 120 g/kg CP, RFV = 111, 12.82 MJ/kg DE, 10.53 MJ/kg ME and 6.55

MJ/kg NEl, but the prepared silage - 34.2 g/kg lactic acid, 125 g/kg CP, RFV = 114, 12.78 MJ/kg DE, 10.49 MJ/kg ME and 6.51 MJ/kg NEl.

The nutritive and energy value of *Helianthus tuberosus* aerial part: 94 g/kg CP, RFV = 89, 11.47 MJ/kg DE, 9.41 MJ/kg ME and 5.43 MJ/kg NEl, but the prepared silage - 50.9 g/kg lactic acid, 97 g/kg CP, RFV = 118, 12.39 MJ/kg DE, 10.17 MJ/kg ME and 6.19 MJ/kg NEl.

The studied species *Cynara cardunculus* and *Helianthus tuberosus* can be used as alternative feed for ruminant animals.

REFERENCES

Cajarville, C., Gonzalez, J., Repetto, J.L., Rodriguez, C.A., Martinez A. (1999). Nutritive value of green forage and crop by-products of *C. cardunculus*. *Annales De Zootechnie*, 48, 353-365.

Duke, J.A. (1983). Handbook of Energy Crop. https://hort.purdue.edu/newcrop/duke_energy/Helianthus_tuberosus.html

Ersahince, A.C., Kara, K. (2017). Nutrient composition and in vitro digestion parameters of Jerusalem artichoke (*Helianthus tuberosus* L.) herbage at different maturity stages in horse and ruminant. *Journal of Animal and Feed Sciences*, 26, 213-225.

Fazaeli, H., Nosratabadi, M. A., Karkoodi, K., Mirhadi, S. A. (2009). *In vitro* and *in vivo* analysis of Jerusalem artichoke (*Helianthus tuberosus*) and alfafa nutritive value. Journal of Science and *Technology of Agriculture and Natural Resources*, 13, 48(B), 163-174.

Ferndndez, J., Hidalgo, M., del Monte J.P., Curt, M.D. (1996). Aprovechamiento del cardo (*Cynara cardunculus* L.) para la produccion de biomasa lignocelulosica, aceite y forraje verde. *Informacion Tecnica Economica Agraria*, Suppl. 17, 49-56.

Ferrero, F., Dinuccio, E., Rollé, L., Tabacco, E., Borreani G. (2018). Biogas production from thistle (*Cynara cardunculus* L.) silages. https://iris.unito.it/retrieve/.../92018_Ferrero_et_al_Biogas-production-from-thistle.p.

Gominho, J., Curt, M.D., Lourenço, A., Fernandez, J., Pereira, H. (2018). *Cynara cardunculus* L. as a biomass and multi-purpose crop: A review of 30 years of research. *Biomass and Bioenergy*, 109, 257-275.

Hernández, F., Madrid, M.J., Martínez-Teruel, A., Megías, M.D., Meneses, M. (2005). Ensiling capacity, chemical composition and multiresidue evaluation of fresh artichoke (*Cynara scolymus* L.) by-product to be used in ruminant feeding. http://om.ciheam.org/article.php?IDPDF=6600062

Herrmann, C., Idler, C., Heiermann, M. (2016). Biogas crops grown in energy crop rotations: Linking chemical composition and methane production characteristics. *Bioresource Technology*, 206, 23-35.

Heuzé, V., Tran, G., Chapoutot, P., Bastianelli, D., Lebas F. (2015). Jerusalem artichoke (*Helianthus tuberosus*). Feedipedia, aprogramme by INRA, CIRAD, AFZ and FAO. http://www.feedipedia.org/node/544.

Ion, N., Odoux, J.F., Vaissière, B.E. (2018). Melliferous potential of weedy herbaceous plants in the crop fields of Romania from 1949 to 2012. *Journal of Apicultural Science*, 62(2), 1-17.

Johansson, E., Prade, T., Angelidaki, I., Svensson, S.E., Newson, W.R., Gunnarsson, I.B., Hovmalm, H.P. (2015). Economically viable components from jerusalem artichoke (*Helianthus tuberosus* L.) in a biorefinery concept. *International Journal of Molecular Sciences*, 16, 8997-9016.

Kays, S. J., Nottingham, S. F. (2008). Biology and chemistry of Jerusalem artichoke *Helianthus tuberosus* L. CRC Press, Taylor and Francis Group, Boca Raton, USA.

Liu, Z.X., Han, L.P., Yosef, S., Xie, G.H. (2011). Genetic variation and yield performance of Jerusalem artichoke germplasm collected in China. *Agricultural Sciences in China*, 10, 668-678.

Marin, M., Hodoşan, C., Diniţă, G., Nicolae, C.G. (2017). The influence of the chemical composition of maize, barley and peas hybrids on the digestibility of compound feed for pigs. *Agrolife Scientific Journal*, 6(2), 127-132.

Pesce, G.R., Negri, M., Bacenetti, J., Mauromicale, G. (2017). The biomethane, silage and biomass yield obtainable from three accessions of Cynara cardunculus. *Industrial Crops & Products*, 103, 233-239.

Romero, M.J., Otal, J., Lorenzo, I., Pdrer, J.I. (1997). Utilizacion del cardo (*Cynara cardunculus*) como forraje de invierno en ganado ovino. Composicion quimica y digcstibilidad in vivo. Resultados preliminares. *Informacion Tecnica Economica Agraria,* Suppl., 18, 46-48.

Sallam, S.M.A., Bueno, I.C.S., Godoy, P.B., Nozella E.F., Vitti D.M.S.S., Abdalla A.L. (2008). Nutritive value assessment of the artichoke (*Cynara scolymus*) by-product as an alternative feed resource for ruminants. *Tropical and subtropical agro ecosystems*, 8, 181-189.

Seiler, G.J. (1993). Forage and tuber yields and digestibility of selected wild and cultivated genotypes of Jerusalem artichoke. *Agronomy Journal*, 85. 29-33.

Ţiţei, V., Teleuţă, A., Muntean, A. (2013). The perspective of cultivation and utilization of the species *Silphium perfoliatum* L. and *Helianthus tuberosus* L. in Moldova. *Bulletin of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Agriculture*, 70(1), 160-166.

*Buletinul Oficial de Proprietate Intelectuală (BOPI). http://agepi.gov.md/ro/bopi/9-2016

**Catalogul Soiurilor de Plante al Republicii Moldova. http://date.gov.md/ro/system/files/resources/2014-06/Catalog_2014.pdf

***SM 108:1995 (1996). Siloz din plante verzi. Condiţii tehnice. Moldovastandart. 10.

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EFFECTS OF THE EXTRUDED LINSEED AND WALNUT MEAL ON SOME QUALITY CHARACTERISTICS OF *LONGISSIMUS DORSI* AND *SEMITENDINOSUS* MUSCLE OF PIGS

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Abstract

The trial was conducted to evaluate effects of the addition of extruded linseed and walnut meal (ELW) on meat quality parameters. For a 30-days period, 40 TOPIGS pigs (body weight 13.58±0.37 kg) were allotted in two dietary treatments: control, M and experimental E (with 8% mix of ELW). At slaughter (32 ± 4.5 kg final body weight), meat samples from Longissimus dorsi (LD) and Semitendinosus (ST) were collected (n = 3 pigs/group), and analysed for pH, colour and texture profile. The pH of LD muscle was lower (-2.33%, P<0.001) in E diet. LD muscle from pigs fed E diet, registered a lower (-4.20%, P<0.001) value of Lightens (L) and a higher value of redness (a) by 3.07 (P<0.001) compared to M. The ST muscle have registered lower (P<0.001) values of L (-7.30%) and a* (-22.84%) in E diet compared to M diet. Addition of extruded linseed and walnut meal in pigs diet have no negative influence on meat quality parameters.*

Key words*: linseed, meat quality, pork, walnut.*

INTRODUCTION

In past years, consumer interest in the relationship between diet and health has increased the demand for a high quality of the foods. Since 2010, in our country it has been observed that consumer preference towards meat quality have started to increase, even if this means paying a higher price (Food Report Romania, 2016). According to FAO (2012), pig meat is the most consumed meat in the world, due to the nutritional characteristics (proteins with high biological value and optimal amino acid composition) and completely usable in human metabolism (Karlovic et al., 2009).

In Romania, pork accounts for more than half of the total annual meat consumption per capita. In the last recorded, the National Institute of Statistics (2018) reported that in Romania, the average annual pork consumption increased to 38.3 kg per capita, compared to pork consumption in 2017 (36.1 kg/capita). In a recent study, Pogurschi et al. (2018) noticed that there are no significant differences in meat consumption between urban and rural areas in Romania.

It is known that animal nutrition can have a major impact on meat quality. Thus, with the help of diet, we can beneficially modulate the quality of the animal product, so that it has a positive impact on the health of consumers (Habeanu et al., 2019).

The meat quality is complex and multivariate, which is influenced by factors as the breed, genotype, feeding, pre-slaughter handling, stunning, and slaughter method, chilling and storage conditions (Rosenvold and Andersen, 2003; Andres et al., 2007). The physical measurements used to determine meat quality are pH, colour, and texture (hardness, springiness, gumminess etc.).

Given the importance of the pork quality, to meet consumer requirements, the producers are looking for different ingredients with low costs and high nutritional value, to introduce them into the pig's diet, to obtain high quality meat without affecting the animal's health (Habeanu et al., 2018).

In present, alternative nutritional sources are constantly being search, since feed price make up more than 65% from production costs, which will likely persist due to increasing global demand for the grains rather than to the pig sector.

Linseed (*Linum usitatissimum*) is one of the oldest cultivated plants, and primarily it was used for fibre (linen) and oil production. Starting with the 90s, it was discovered that linseeds oil is rich in polyunsaturated fatty acids (35%), notably alpha-linolenic acid and conjugated linoleic acid. Thus, the linseeds are used in animal nutrition to alter the fatty acid profile of meat (Habeanu et al., 2017; Heuzé et al., 2018; Habeanu et al., 2019).

Linseed meal is also rich in protein (30-39% protein, but with a low content in lysine), and can be used in pigs diet as long as the diets are correctly balanced (Leterme et al., 2015). An inconvenience is the fiber content and antinutrients such as linamarin. According to McDonald et al. (2002), if linseed is not processed correctly, this can be toxic to animals (especially monogastric). According to Delgado-Licon et al. (2009), there is a strong correlation between the extrusion procedure and the content of bioactive compounds and antioxidant capacity in the end product. Thus, it has been shown that the extrusion process can lead to a reduction in the contents of bioactive compounds, including antioxidant activity (Giannico et al., 2009; Czech et al., 2017).

Walnut (*Juglans regia*) is a rich source of polyphenols, omega fatty acids, dietary fiber and it is known that are a potential source of antioxidants with a beneficial effect on pigs health (Ghasemi et al., 2011; Gheorghe et al., 2018, Habeanu et al., 2019). An inconvenience is that the walnut by-products have high-fat content and become rancid in a very short time (Brunschwig, 2003). According to Heuzé et al. (2017), in the 19th century, the walnut meal was used commonly in pigs diet.

Therefore, the purpose of this study was to evaluate the effect of the addition of 8% (5:3 wt/wt) extruded linseed and walnut meal (ELW) on some quality characteristics of pigs muscle.

MATERIALS AND METHODS

The animals were treated in accordance with the Romanian Law 305/2006 for handling and protection of animals used for experimental purposes.

The trial was conducted on the experimental farm of INCDBNA Balotesti, according to Law 43/2014/Romania, and all the experimental procedures were approved by the Ethical Committee.

Animals, diets, and sampling

The trial was conducted on 40 growing pigs, of commercial hybrid TOPIGS, maternal line format, from the crossing of two breeds Large White × Hybrid (Large White × Pietrain) and the paternal, of Talent, terminal boar mostly Duroc, initial body weight (BW) 13.58 ± 0.37 kg and age of 81 ± 3 days.

During a period of 30 days, the animals were allotted randomly into two dietary treatments (20 pigs/group, 2 replicate/group): control group (M) was fed with a conventional diet based on corn, triticale and soybean meal, and experimental group (E) with an addition of 8% (5:3 kg/kg) mix of ELW into the basal diet (Table 1). The feed was given in the pelletized form. The water and feed were given *ad libitum* throughout the entire experiment.

At the end of the experimental period, the pigs (n = 3/treatment) were slaughtered at final BW of 32 ± 4.5 kg, for sample collection. The samples (about 200 g) were collected from the *Longissimus dorsi* (LD) and *Semitendinosus* (ST) muscles. All the samples were individually vacuum-packed, labeled and frozen at −18°C until analyzed.

Physical properties determination

All the muscle samples were analysed for pH, colour, and texture, at the Food Engineering Faculty, "Ștefan cel Mare" University, from Suceava, Romania.

Before analyse, the muscle samples were removed from the freezer and thawed overnight (approximately 15 h) at 4 ± 1°C.

Table 1. Ingredient and nutrient composition of pigs diet

Ingredients, %	M diet	ELW diet
Corn	35.83	32.33
Triticale	25.00	25.00
Rice meal	15.00	15.00
Soybean meal	10.00	5.00
Extruded linseed: walnut meal (5:3 wt:wt)	0	8.00
Sunflower meal	5.00	5.00
Corn gluten	2.00	3.00
Milk replacer	3.00	3.00
Vegetable oil	0.70	0
DL-Methionine	0.00	0.06
L-Lysine	0.34	0.52
Calcium carbonate	1.78	1.76
Monocalcium phophate	0.04	0.02
Phytase	0.01	0.01
Salt	0.20	0.20
Choline premix	0.10	0.10
Vitamin-mineral premix[1]	1.00	1.00
Analysed composition, %		
Dry matter	88.43	88.18
Crude protein	17.10	17.30
Lysine	1.05	1.05
Methionine+Cystine	0.67	0.67
Calcium	0.90	0.90
Phosphorus	0.70	0.70
Crude fiber	4.66	4.97
Crude fat	4.37	4.80
Metabolisable energy, ME (MJ/kg)[2]	12.67	12.66

[1]Contained per kg diet: 10000 IU vitamin A; 2000 IU vitamin D3; 30 IU vitamin E; 3 mg vitamin K3; 2 mg vitamin B1; 6 mg vitamin B2; 20 mg vitamin B3; 13.5 mg vitamin B5; 3 mg vitamin B6; 0.06 mg vitamin B7; 0.8 mg vitamin B9; 0.05 mg vitamin B12; 10 mg vitamin C; 30 mg Mn; 110 mg Fe; 25 mg Cu; 100 mg Zn; 0.38 mg I; 0.36 mg Se; 0.3 mg Co; 60 mg antioxidant.

[2]ME calculated based of feed composition using regression equations (NRC, 1998).

The sample pH was performed in triplicate according to SR ISO 2917: 2007. The pH was determined using a portable pH Meter (HACK, Germany). Before the pH determination, the pH-meter was calibrated using buffer solutions of pH 7 and pH 4. Muscle samples (5 g) were minced and mixed with 5 ml distilled water (tissue-water mixture), then the electrode was inserted in the tissue-water mixture for pH measurement.

The colour was instrumentally measured using a Chroma Meter CR-400 (Minolta Co. Ltd, Tokyo, Japan) calibrated with a white ceramic tile on D65 illuminate. The colour values were expressed in CIE L*a*b* colour system (CIE, 1976) Lightness (L*), redness (a*) and yellowness (b*). The colour was measured concerning lightness (L*: 0 = black, 100 = white), and 2 colour coordinates a* equal to red (positive) or green (negative) and of b* equal to yellow (positive) or blue (negative). Colour was determined on the fat-free surface area of the muscle. The muscle colour measurement was performed in triplicate for each sample, using different instrument orientations.

Textural properties analysis was performed using a Perten TVT 6700 texturometer. Before analyse the all the meat samples were cut so that they were shaped like a cylinder with a diameter of 15 mm and a height of 25 mm. The meat samples were subjected to a double cycle compression, which was determined with a cylinder probe 20 mm diameter, stainless steel. The method allows the determination of firmness, springiness, resilience, cohesiveness, gumminess, and chewiness.

Statistical analysis

The data were submitted to variance analysis using the General Linear Model (GLM) of the SPSS program (SPPS, 2011). The results were expressed as mean values and standard error of the mean (SEM). Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSIONS

Currently, consumers are more knowledgeable regarding the nutritive value of the food and have become more interested in food quality. Regarding the meat quality, generally, the consumers are more interested in colour, and texture.

The present study was performed to obtain information about the effect of the addition of 8% (5:3 kg/kg) extruded linseed and walnut meal mix, on some quality parameters (pH, colour, and texture) of the muscles with the major economic importance (*Longissimus dorsi* and *Semitendinosus)* in pigs. In our previous studies we show the importance of these feeding ingredients on fatty acids profile and animal health (Gheorghe et al., 2018; Habeanu et al., 2019).

The pH, colour and texture parameters are some of the most important quality attributes of meat (Lee et al., 2012). These parameters depend on species and muscle type, feeding, slaughter method etc. (Migdał et al., 2007). The pH is the most direct way to acquire information on meat quality characteristics (Lefter et al., 2013; Tomivic et al., 2014).

In the current study, there was registered a pH decrease in the LD muscle (>2.3%; P = 0.001) as effect of dietary inclusion of extruded linseed and walnut mix (Table 2).

According to Tomivic et al. (2014), the ultimate meat pH is reached at 5.3-5.8 at various periods post-mortem. The pH results obtained in our study are in range and are consistent with the results obtained by other authors (Wiecek et al., 2008; Lee et al., 2012; Lefter et al., 2013; Tomovic et al., 2014; Furtado et al., 2019). Contrary to our results, regarding meat quality traits, Corino et al. (2002) and Riley et al. (2000), did not show any dietary effect on pH values.

Table 2. pH and instrumental colour parameter in pigs muscle

Muscle	Parameter	M[1]	E[2]	SEM[3]	*P-value*
Longissimus dorsi	pH	5.57[a]	5.44[b]	0.03	*0.001*
	L*	52.60[a]	50.38[b]	0.52	*0.004*
	a*	1.21[b]	3.72[a]	0.57	*0.000*
	b*	9.75	8.99	0.24	*0.110*
	Chroma C*	9.82	9.73	0.18	*0.829*
Semitendinosus	pH	5.59	5.58	0.01	*0.101*
	L*	49.31[a]	45.71[b]	0.82	*0.001*
	a*	3.24[a]	2.50[b]	0.18	*0.016*
	b*	9.74	9.70	0.23	*0.942*
	Chroma C*	10.27	10.02	0.22	*0.637*

[1]M: control group;
[2]E: experimental group;
[3]SEM: standard error of the mean;
L*(Lightness), a* (Redness), b* (Yellowness);
[a, b]Row means with different superscripts differ significantly (P<0.05).

The LD and ST muscle, colour from pigs fed ELW diet had similar Chroma values with the control group and (in ST muscle was noticed a little higher value compared with LD muscle). The LD muscle of pigs fed ELW diet resulted in significantly lower L* (<4.20%; P=0.001) and a higher a* (3.07-fold; P = 0.001) values, indicating a shift to darker and redder meat compared to control diet. Similar results of LD colour were obtained by Okrouhlá et al. (2013).

The ST muscle, of pigs fed ELW meal, resulted in a lower L* (<7.30%; P<0.001) and a* (<22.84%; P = 0.016) values, suggesting a shift into darker and less red meat, compared to control group. A darker appearance (L*) of the meat was also obtained by Riley et al. (2000) and Okrouhlá et al. (2013), who fed pigs with linseed. Contrary to our results, previous studies have not reported any effect on meat colour when linseed was included in pig diets (Bee et al., 2008; Corino et al., 2008; Juáreza, 2011).

The most important parameters that they have in view when they buy meat are tenderness, juiciness, gumminess etc. (Migdał et al., 2007; Warner et al., 2010). There are studies (Bindon & Jones, 2001; Maltin et al., 2003) which pointed that most consumers complained about the meat hardness.

From our knowledge, data regarding the effect of the addition of extruded linseed and walnut meal in pigs diet in the growing phase, on the textural parameters, are limited. In the present study, as presented in Table 3 no significant differences were recorded between groups. However, we observed that the pigs fed ELW diet, produced meat with lower hardness for LD (-7.73%; P>0.05) and ST (-23.18%; P>0.05) compared to the control group.

Table 3. Textural parameters in pigs muscle

Muscle	Parameter	M[1]	E[2]	SEM[3]	*P-value*[*]
Longissimus dorsi	Hardness (g)	1431.0	1320.5	290.2	*0.890*
	Springiness (adm)	1.0	1.0	0.001	*0.728*
	Resilience (adm)	3.0	3.0	0.32	*0.970*
	Cohesiveness (adm)	0.4	0.4	0.03	*0.486*
	Gumminess (g)	563.3	614.1	154.7	*0.905*
	Chewiness (g)	563.9	614.1	154.7	*0.906*
Semitendinosus	Hardness (g)	1754.0	1347.5	238.5	*0.508*
	Springiness (adm)	1.0	1.0	0.001	*0.963*
	Resilience (adm)	3.6	2.8	0.46	*0.546*
	Cohesiveness (adm)	0.4	0.4	0.04	*0.768*
	Gumminess (g)	728.9	541.4	147.9	*0.634*
	Chewiness (g)	729.4	541.9	147.9	*0.634*

[1]M: control group;
[2]E: experimental group;
[3]SEM: standard error of the mean;
*Means within rows do not differ significantly (P>0.05).

Lower values were also obtained for gumminess (<25.73%; P>0.05), and chewiness (<25.71%; P>0.05) in the ST muscle of pigs fed ELW diet compared to control group. Regarding the LD muscle, this recorded higher values for gumminess (>9.01%; P>0.05) and chewiness (>8.90%; P>0.05) compared to the control group.
Similar textural parameters of meat were also obtained by Wiecek et al. (2008) from pigs fed with linseed oil and slaughtered at 23 kg BW. However, previous studies feeding pigs with linseed also reported insignificant differences in physical meat quality parameters (Corino et al., 2008; Nurnberg et al., 2011).

CONCLUSIONS

The addition of 8% (5:3 kg/kg) extruded linseed and walnut meal mixture in growing pigs diet did not influence negatively the physical characteristics of the qualitative parameters of *Longissimus dorsi* and *Semitendinosus* muscle. Also, following the results mentioned above, we can recommend the inclusion of the mixture of extruded linseed and walnut in the pigs diet.

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REFERENCES

Andres, S., Murray, I., Navajas, E. A., Fisher, A. V., Lambe, N. R., Bunger, L. (2007). Prediction of sensory characteristics of lamb meat samples by near infrared reflectance spectroscopy. *Meat Science*, 76(3), 509–516.

Bee, G., Jacot, S., Guex, G., Biolley, C. (2008). Effects of two supplementation levels of linseed combined with CLA or tallow on meat quality traits and fatty acid profile of adipose and different muscle tissues in slaughter pigs. *Animal*, 2 (5), 800–811.

Bindon, B. M., Jones, N. M. (2001). Cattle supply, production systems and markets for Australian beef. *Australian Journal of Experimental Agriculture*, 41, 861–877.

Brunschwig, P. (2003). Le tourteau de noix. Institut de l'Elevage. http://www.inst-elevage.asso.fr/spip.php.

CIE. (1976). *Commission Internationale de l'Eclairage*, 18th Session, London, UK. September 1975. CIE publication 36.

Czech, A., Ognik, K., Laszewska, M., Sembratowicz, I. (2017). The effect of raw and extruded linseed on the chemical composition, lipid profile and redox status of meat of turkey hens. *Animal Science Papers and Reports,* 35(1), 57–69.

Corino, C., Magni, S., Pagliarini, E., Rossi, R., Pastorelli, G., Chiesa, L. M. (2002). Effects of dietary fats on meat quality and sensory characteristics of heavy pig loins. *Meat Science*, 60, 1–8.

Corino, C., Musella, M., Mourot, J. (2008). Influence of extruded linseed on growth, carcass composition, and meat quality of slaughtered pigs at one hundred ten and one hundred sixty kilograms of live weight. *Journal of Animal Science*, 86(8), 1850–1860.

Delgado-Licon, E., Martinez-Ayala, A.L., Rocha-Guzman, N.E., Gallegos-Infanate, J.A., Atienzo-Lazos, M., Drzewicki, J., Martínez-Sánchez, C.E., & Gorinstein, S. (2009). Influence of extrusion on the bioactive compounds and the antioxidant capacity of the bean/corn mixtures. *International Journal of Food Sciences and Nutrition*, 60(6), 522–532.

FAO (2012). FAOSTAT. www.fao.org.

Furtado, E. Bridi, J. G., Barbin, A. M., Barata, D. F., Peres, C. C. P., Barbon, L. M., Andreo, A. P. A, Giangareli, B., Terto, D. K., Batista, J. P. (2019). Prediction of pH and color in pork meat using VIS-NIR Near-infrared Spectroscopy (NIRS). *Food Science and Technology Campinas*, 39(1), 88–92.

Food Report Romania. (2016). https://www.dutchromaniannetwork.nl/wp-content/uploads/2017/01/Food-Report-Romania-2016.pdf

Giannico, F., Colonna, M. A, Coluccia, A., Crocco, D., Vonghia, G., Cocca, C., Jambrenghi, A. C. (2009). Extruded linseed and linseed oil as alternative to soybean meal and soybean oil in diets for fattening lambs. *Italian Journal of Animal Science*, 8(2), 495–497.

Ghasemi, K., Ghasemi, Y., Ehteshamnia, A., Nabavi, S.M., Nabavi, S.F., Ebrahimzadeh, M.A., Pourmorad, F. (2011). Influence of environmental factors on antioxidant activity, phenol and flavonoids contents of walnut (*Juglans regia* L.) green husks. *Journal of Medicinal Plants Research*, 5(7), 1128–1133

Gheorghe, A., Hăbeanu, M., Lefter, N. L., Grigore, D. M. (2018). Effect of dietary extruded linseed and walnut meal mixture (8:1) on performance and plasma protein profile in weaned piglets. *Bulletin UASVM Animal Science and Biotechnologies*, 75(2), 121–126.

Hăbeanu, M., Lefter, N., Gheorghe, A., Tabuc, C., Dumitru, M., Ciurescu, G., Palade, M. (2017). Effects of dietary peas mixed with linseed (3:1) on the growth performance, enteritis and certain serum parameter in weaned piglets. *Food and Feed Research*, 44 (2), 173–180.

Habeanu, M., Gheorghe, A., Ropota, M., Toma, S.M., Marin, M. (2018). Changes in fatty acids composition of Longissimus dorsi muscle, brain, liver and heartas effect to hempseed addition in pigs feeding. *AgroLife Scientific Journal*, 7(2), 61–67.

Hăbeanu, M., Lefter, N. L., Gheorghe, A., Untea, A., Ropotă, M., Grigore, D. M., Vărzaru, I., Toma, S. M. (2019). Evaluation of performance, nitrogen metabolism and tissue composition in barrows fed an n-3 PUFA-rich diet. *Animals*, 9, 234.

Heuzé, V., Tran, G., Hassoun, P., Lebas, F. (2017). Walnut (*Juglans regia*). Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. https://www.feedipedia.org/node/38.

Heuzé, V., Tran, G., Nozière, P., Lessire, M., Lebas, F., (2018). Linseed meal. Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. https://www.feedipedia.org/node/735.

Juáreza, M., Dugana, M. E. R., Aldaia, N., Aalhusa, J. L., Patienceb, J. F., Zijlstrac, R. T., Beaulieu, A. D. (2011). Increasing omega-3 levels through dietary co-extruded flaxseed supplementation negatively affects pork palatability. *Food Chemistry*, 126(4), 1716–1723.

Karlovic, S., Ježek, D., Blažic, M., Tripalo, B., Brncic, M., Bosiljkov T., Šimunek, M. (2009). Influence of refrigeration and ageing time on textural characteristics of fresh meat. *Croatian Journal of Food Science and Technology*, 1(2), 1–6.

Law 305/2006. http://www.monitoruljuridic.ro/act/lege-nr-305-din-12-iulie-2006-privind-ratificarea-convetiei-europene-pentru-protectia-animalelor-vertebrate-utilizate-in-experimente-si-alte-scopuri-stiintifice-adoptata-la-strasbourg-la-18-martie-1986-74186.html

Law 43/2014 http://www.monitoruljuridic.ro/act/lege-nr-43-din-11-aprilie-2014-privind-protec-ia-animalelor-utilizate-n-scopuri-tiin-ifice-emitent-parlamentul-157944.html

Lee, S. H., Choe, J. H., Choi, Y. M., Jung, K. C., Rhee, M. S., Hong, K. C., Lee, S. K., Ryu, Y. C., Kim, B. C. (2012). The influence of pork quality traits and muscle fiber characteristics on the eating quality of pork from various breeds. *Meat Science*, 90, 284–291.

Lefter, N. A., Drăgotoiu, D., Gheorghe, A., Chedea, S. V., Ciurescu, G., Hăbeanu, M. (2013). Effect of the dietary by-product grape seed cake on performances and carcass quality of TOPIGS pigs. *Bulletin UASVM Animal Science and Biotechnologies*, 70(2), 214–222.

Leterme, P., Eastwood, L., Patience, J. (2015). Flaxseed and Flaxseed Meal in Swine Nutrition. Proceedings of the Western Nutrition Conference, Saskatoon, SK, Sept. 25-27, 241–252.

Maltin, C., Balcerzak, D., Tilley, R., Delday, M. (2003). Determinants of meat quality: tenderness. Proceedings of the Nutrition Society, 62, 337–347.

Migdał, W., Wojtysiak, D., Palka, K., Natonek-Wiśniewska, M., Duda, I., Nowocień, A. (2007). Chemical composition and texture parameters of some selected muscles of the Polish Landrace Fatteners slaughtered at different age. *Żywność Nauka Technologia, Jakość,* 55, 277–284.

McDonald, P., Edwards, R. A., Greenhalgh, J. F. D. (2002). *Animal Nutrition*. 6th Edition. London and New York, Longman Publishing House, 543.

Nurnberg, K., Nurnberg, G., Dannenberger, D., Hagemann, L., Paulke, T. (2011). Effect of extruded linseed on growth and lipids of muscle and back fat in pigs. *Fleischwirtschaft*, 91, 88–92.

National Institute of Statistics (2018). Disponibilităţile De consum ale populaţiei. Available at: http://www.insse.ro/cms/sites/default/files/field/publicatii/disponibilitatile_de_consum_ale_populatiei_anul_2018.pdf.

Okrouhlá, M., Stupka, R., Čítek, J., Šprysl, M., & Brzobohatý, L. (2013). Effect of dietary linseed supplementation on the performance, meat quality, and fatty acid profile of pigs. *Czech Journal of Animal Science*, 58 (6), 279–288.

Pogurschi E, Melania Munteanu, Nicoale Carmen Georgeta, Marin Monica, Corina Aurelia Zugravu - (2018). Rural-urban meat consumption in Romania.*Scientific Papers Series D, Animal Science*, 61 (2), 111–115.

Riley, P. A., Enser, M., Nute, G. R, Wood, J. D. (2000). Effects of dietary linseed on nutritional value and other quality aspects of pig muscle and adipose tissue. *Animal Science Journal*, 71, 483–500.

Rosenvold, K., Andersen, H. J. (2003). Factors of significance for pork quality - a review. *Meat Science*, 64(3), 219–237.

SPSS, (2011). Statistics version 20.0. IBM SPSS Inc, USA.

SR ISO 2917:2007, Carne şi produse din carne. Măsurarea ph-ului. Metodă de referinţă. ARSO.

Tomovic, V., Zlender, B., Jokanović, M., Tomovic, M., Sojic, B., Skaljac, S., Tasic, T., Ikonic, P., Soso, M., Hromis, N. (2014). Technological quality and composition of the M. *Semimembranosus* and M. *Longissimus dorsi* from Large White and Landrace Pigs. *Agricultural and Food Science*, 23(1), 9–18.

Warner, R. D., Greenwood, P. L., Pethick, D. W., Ferguson, D.M. (2010). Genetic and environmental effects on meat quality. *Meat Science*, 86, 171–183.

Wiecek, J., Skomiał, J., Rekiel, A., Florowski, T., Dasiewicz, K., Woźniak, A. (2008). Compensatory growth of pigs: quantitative and qualitative parameters of pork carcass of fatteners administered a mixture with linseed oil in the re-alimentation period. *Polish Journal of Food and Nutrition Sciences*, 58(4), 451–455.

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PRELIMINARY RESEARCH ON GROWTH RESPONSE AND HEALTH STATUS OF PIGLETS FED MILLET GRAIN AS A PARTIAL REPLACEMENT FOR TRITICALE

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Abstract

A 21 days trial was conducted to evaluate the performance and plasma biochemical markers on 40 pigs (8.14 ± 1.08 kg of average body weight and 30 ± 3 days of age) when 25% of triticale was replaced with millet in weaned diets. The group C (n = 20) received a conventional diet based on corn-triticale-soybean meal and in the M group (n = 20), 25% millet cv. 'Marius' replaced triticale (2 replicates per group). At 7 days post-weaning, the performances and plasma profiles (lipid, protein, mineral, enzyme) were evaluated. The blood samples collected from the piglets jugular vein (n = 10) were determined by a chemistry analyser Spotchem EZ SP-4430, Arkray, Japan. The performances of piglets fed either M or C diet were comparable (P>0.05). No effect (P>0.05) of dietary treatment on plasma metabolites was observed. However, the inorganic phosphorus (IP) decreased (-15%; P<0.04) in the M vs. C diet. The present study revealed that the replacement of 25% millet to the piglet's diet, even in the most critical period of their lives, maintains the performances and blood plasma parameters within the normal reference ranges, beneficial to the health status.

Key words*: health status, millet grain, performances, weaned piglet.*

INTRODUCTION

The interest in converting cereals, the most important energetically sources for feeding pigs, into ethanol production is increasing, in an exponential manner (Popp et al., 2016). To combat this, a raise in demand for alternative nutritional sources, containing beneficial nutrients, that can provide opportunities for diversifying the feedstuff matrix, has been in demand (Habiyaremye et al., 2017). The condition is not to affect the health, productivity and the development of animals and their products.

Millet grains are now receiving specific attention in terms of nutritional and health benefits (Changmei and Dorothy, 2014; Singh and Chauhan, 2019). Agrotechnical characteristics and the resistance to storage pests during long periods of time (Habeanu et al., 2019; Yenagi et al., 2013; Dipnarayan et al., 2016), enhance its value (Davis et al., 2003; Garcia and Dale, 2006; Saleh et al., 2013; Devi et al., 2014; Goron and Raizada, 2015). However, in Europe, millet varieties are cultivated in small amounts and usually used as feed for pets and less for piglets (Habiyaremye et al., 2017). Thus, millet are underutilized in many developed countries (Devi et al., 2014), including Romania. According to Singh and Chauhan (2019), and Habiyaremye et al., (2017), millet grain appear to have great nutritional potential as feed for piglets (Hăbeanu et al., 1019), particularly in the first week post-weaning, due to its richness in many vital nutrients (e.g. minerals, micronutrients, limiting amino acids, fiber, polyphenols) which offer several health benefits (Jones and Engleson, 2010). Moreover, millet, as they do not contain gluten, is easy to digest and hence is advisable for nourishment of humans and young animals (Habiyaremye et al., 2017).

In modern pig production, weaning is an abrupt process that induces distress for piglets due to

psychological, environmental or nutritional factors (Colson et al., 2012; Hăbeanu et al., 2015). Moreover, the literature data suggest that when the pigs' diet based of sow's milk is abruptly changed to a solid diet, especially in the first week after weaning, piglets suffer a nutritional stress (van Beers-Schreurs et al., 1998). Unfamiliarity with post-weaning feeding systems and diets contributed also, to inadequate protein and micronutrients intake, nutrient malabsorption and diarrhoea (Mavromichalis, 2014), with negative effects on productivity (Campbell, 2013). Therefore, beyond diet formulation for properly feeding the weaning piglets, it's also important to know how this category of animals responds to the composition of the diet. The evaluation of blood parameters provides important information about the function of certain tissues and organs (Luo et al., 2016) and, also efectiveness of dietary nutrients.

However, little is known about the interrelationships among dietary millet and concentration of certain plasma parameters as important indicators of health status.

In this context, the aim of study was to evaluate the effects of 25% dietary millet grain on performance and plasma metabolites during the first seven days post-weaning.

MATERIALS AND METHODS

The trial approved by ethics committee (No. 1493/12.03.2018) was conducted at the experimental farm of the National Research-Development Institute for Biology and Animal Nutrition (INCDBNA Balotesti). The animals used in the present study were treated in accordance with the EU Directive 2010/63/EU (OJEU, 2010).

Animals and diets

The experimental trial was conducted during 21 days and the study reported herein, only included information on first 7 days post-weaning on 40 weaning piglets Topigs, 20 ♀ and 20 ♂ with an average initial body weight (BW) of 8.14 ± 1.08 kg. The animals were assigned to two groups (20 pigs/group, 2 replicates/group): i). the control group (C), fed with a diet based on corn-triticale-soybean meal; ii). the experimental group (M), fed a compound feed similar to that of group C, where 25% millet replaces triticale. Both diets were isocaloric and isoenergetic with similar content in limiting amino acids (lysine, methionine + cysteine) and contained 0.01% phytase. The feed was given *ad libitum* in pelletized form. The intake was recorded daily. In order to determine the performances (body weight, BW; feed intake, FI; average daily gain, ADG) the piglets were weight after 7 days of experimental trial.

Chemical and biochemical analyses

The chemical analyses of the feed ingredients and feed compound, were measured using the SR EN ISO 17025:2005 standards, in the Laboratory of Chemistry and Nutrition Physiology of INCDBNA. The crude protein of the diet was determined by a semiautomatic classical Kjeldahl method using a Kjeltek auto 1030 - Tecator (SR 13325). The fat was extracted using an improved version of the classical method by continuous extraction in solvent, followed by fat measurement with Soxhlet after solvent removal (SR ISO 6492). The crude fibre was determined with a classical semiautomatic Fibertec-Tecator method (STAS 959715-77) and the ash by calcinations' at 550^{o} until constant mass (SR ISO 5984). The nitrogen free extractives (NFE) were calculated using the formula: NFE = DM - (CP + EE + GF + Ash), where: DM - dry matter; CP - crude protein; EE - ether extract; GF - gross fiber. The metabolisable energy (ME) was calculated with regression equations developed by the „Oskar Kellner" Institute of animal nutrition: ME = 5.01 DP + 8.93 EE + 3.44 GF + 4.08 DNFE, where: DP - digestible protein; EE - ether extract; GF - gross fiber; DNFE - digestible nitrogen free extractives.

After 7 days, blood samples from 10 pigs/group were collected from jugular vein. The plasma was obtained after the blood samples were centrifuged at 3000 × g for 15 min. A chemistry analyser Spotchem EZ SP-4430, (Arkray, Japan) was used to determine the plasma metabolites (triglycerides, TG; total cholesterol, T-Chol; high-density lipo-protein cholesterol, HDL-C; total protein, T-Pro; albumin, Alb; uric acid, UA; creatinine, Cre; total bilirubin, T-Bil; urea nitrogen, BUN; calcium, Ca; magnesium; Mg, inorganic

phosphorus, IP; aspartate aminotransferase, AST; alanine aminotransferase, ALT; lactate dehydrogenase, LDH; creatine kinase, CK).

Statistical calculations

Data were analysed using the SPSS V.20 (2011) software, General Linear Model procedure. The results were given as average value ± mean standard error. The effect was considered significant at $P<0.05$ and as trend at $0.1<P>0.05$.

RESULTS AND DISCUSSIONS

Chemical composition

Results for chemical composition analyses of millet 'Marius' and triticale, are presented in Table 1.

Nutritionally, millet grain compares closely with other major cereal grains (wheat, rice, maize), including triticale (Adeola et al., 1994; Amadou et al., 2013; FAOSTAT, 2014). However, millet had higher level in metabolisable energy, crude fat and minerals content compared to triticale. The crude fat content of the millet observed in this study is lower than the report of Kaur et al. (2014), and Saleh et al. (2013) for millet or some others millet varieties. The protein content averaged 9.86% for millet and 12.13% for triticale (23.02% less protein than the triticale).

The protein values for millet reported in this study were found to be by 1.2-1.4% lower than that reported in other studies (Kalinova and Moudry, 2006; Berglund, 2007; Saleh et al., 2013; FAOSTAT, 2014).

Contrary to our study, Dipnarayan et al. (2016) reported higher protein content (12.5%) of millet.

Table 1. The chemical composition of the main ingredients used in the experimental diets

Nutrients	Cereal grain	
	Triticale	Millet cv. 'Marius'
Dry matter (%)	88.90	87.19
Metabolisable energy (kcal/kg)	3004	3052
Crude protein (%)	12.13	9.86
Digestible protein (%)	9.46	7.40
Crude fat (%)	2.01	2.99
Crude fiber (%)	2.17	2.50
Minerals (%)	2.17	3.38
Calcium (%)	0.04	0.03
Total phosphorus (%)	0.94	0.28
Digestible phosphorus (%)	0.18	0.15
Lysine: Energy Ratio (g/1000 kcal)	1.36	0.75

Varietal differences of crude protein content in millet grain were described also in other reports and might be caused by genetic differences or variation in growing conditions (e.g. water, soils nutrients), including conditions during grain formation (Ravindran, 1991; Kalinova and Moudry, 2006).

Millet was lower in digestible protein (-18.71%) than triticale. The digestibility of protein in our study was nearly 5% less than the values (average, 71.3%) reported by Ravindran (1992) who investigated *in vitro* protein digestibility of six varieties of proso millet. Factors such as the presence of tannins, influence the protein digestibility of proso millet (Ravindran, 1992).

The fiber content of the millet used in our study was lower with 15.21% than triticale. Contrary to our results, Ravindran (1991), who tested six varieties of proso millet, observed that the fiber content ranged between 3.2-4.2%.

Regarding the mineral content, we noticed that millet contained a higher mineral amount (+55.8%) compared to triticale. Similar results regarding the mineral content between proso millet and other common cereal grains were also obtained by Dipnarayan et al. (2016) and Habiyaremye et al. (2017). However we found that macrominerals, such as Ca and total P, were lower in millet cv. Marius compared to triticale. Ravindran (1991) obtained similar

values for Ca and P when analysed grain samples of six varieties of common millet.
According to Adeola and Orban (1995), variations in chemical composition for cereals are expected, and are in close relationships with the genotype and environment (soil moisture content, level of nitrogen in the soil and the time of nitrogen fertilizer application).

Animal performance

All the animals used in the experiment remained healthy and consumed their diets. There was no mortality during the study.
Table 2 shows the performance of the 7 day post-weaning piglets. Initial average BW of the experimental animals was 8.12 ± 1.08 kg.

Table 2. Effects of using millet grain on productive performance of weaning piglets

Items	C	M	SEM	*P-value**
No. of pigs, animals/group	20	20	-	-
Weight at weaning, kg	8.14	8.15	0.201	*0.984*
Weight after 7 days, kg	9.69	9.55	0.280	*0.817*
ADG, g/day	0.222	0.200	0.180	*0.609*
ADFI, kg/day	0.254	0.242		
g gain: g feed	0.87	0.83		

Replacement of triticale with millet grain in the piglets diet had no significant effect on the average BW or ADG compared to control diet, after 7 days experimental period; this revealed that the inclusion of millet grains, did not affect the palatability of the piglets diets. Furthermore, the digestive processes (e.g. absence of diarrhoea or other disorders) were not affected by dietary treatments (data not shown).
The majority of the previous studies have been conducted to investigate the effect of millet grain in poultry nutrition (Ferket, 2000; Oso et al., 2014) while the reports on pigs are fewer. Yilkal et al. (2018) by increasing dietary level of finger millet grain up to 75% in layer ration observed no negative impact on production performance. Body weight gains did not differ between the broilers fed the control diet and the diets containing 14 or 28% whole millet in a study reported by Cisse et al. (2016). Similar results regarding pigs performance were also obtained by Adeola et al. (1996), how used 25% pearl millet as replacement of corn for 28 days in nursery (10 kg), respectively growing (20 kg) pigs, and Lawrence et al. (1995) when 0, 25, 50, 75, or 100% of corn was replaced with pearl millet in young pigs diets.

Plasma biochemical parameters

The results of plasma biochemical parameters are summarized in Table 3. At 7-days after weaning, the concentration of TG, T-Chol and HDL-C of pigs from the M group, were lower (P>0.05) than the C group. Moreover, the values of these important markers of lipid metabolism were within the normal range. The lack of significant differences among control and experimental groups for TG value were also reported by Li et al. (2019), feeding undernourished pigs at age of 9 weeks with pure maize diet and millet-based supplementary food for 3 weeks. However, some studies (Nishizawa and Fudamoto, 1990; Nishizawa et al., 1995; Shimanuki et al., 2006) conducted on mice and rats fed proso millet protein concentrate diets, during 21 days experimental period, reported a beneficial effect of millet protein on lipid metabolism. We speculated that in our study no physiological improvement was observed in the piglets on lipid parameters due to the short-term feed intervention.
T-Pro, Alb and UA are major plasma protein fractions that can be used as indicators of protein synthesis (Hellwing et al., 2007). Cre and BUN are important endogenous marker, most commonly used to measure kidney function, while T-Bil is an indicative of liver function (Radostits et al., 2000). Compared with the C diet, the concentrations of T-Pro, Alb, UA, Cre, T-Bil and BUN, in M diet remained at a similar level (P>0.05). Contrary to our study, improved of T-Pro and BUN levels were reported by Li et al. (2019) on malnourished pigs fed with corn and proso millet diet during 3 weeks.
In the present study, significant changes in serum AST, ALT, LDH and CK activities, as markers of liver function (Nishizawa et al.,

2002) and cardiovascular system (Radostits et al., 2000), were not observed between the dietary groups of C and M.

Table 3. Effects of using millet grain on plasma profile of weaning pigs

Plasma profile	Parameter	Limits	C	M	SEM	*P-value*
Lipid	TG, mg.dL^{-1}	33-50[1]	49.50	42.13	0.55	*0.298*
	T-Chol, mg.dL^{-1}	67-367[2]	78.75	68.75	3.28	*0.159*
	HDL-C, mg.dL^{-1}	-	32.50	27.50	2.15	*0.294*
Protein	T-Pro, g.dL^{-1}	5.8-8.3[1]	4.85	4.79	0.14	*0.840*
	Alb, g.dL^{-1}	2.3-4.0[1]	2.88	2.69	0.13	*0.523*
	UA, mg.dL^{-1}	-	1.00	1.00	-	-
	Cre, mg.dL^{-1}	0.8-2.3[1]	1.28	1.26	0.04	*0.884*
	T-Bil. mg.dL^{-1}	0-0.5[1]	0.30	0.34	0.04	*0.688*
	BUN, mg.dL^{-1}	8.2-25[1]	13.75	12.06	0.89	*0.397*
Mineral	Ca, mg.dL^{-1}	6.8-14.8[2]	14.05	12.46	0.55	*0.190*
	Mg, mg.dL^{-1}	2-3.5[1]	2.30	2.18	0.07	*0.422*
	IP, mg.dL^{-1}	5.5-9.3[1]	6.58[a]	5.70[b]	0.21	*0.042**
Enzyme	AST, U/L	18-84[2]	44.50	50.81	8.65	*0.748*
	ALT, U/L	31-75[1]	35.25	35.50	3.02	*0.969*
	LDH UI/L	380-630[3]	634.50	502.50	84.01	*0.095*^{T}
	CK UI/L	146-870[2]	440.75	346.13	218.61	*0.849*

Triglycerides, TG; total cholesterol, T-Chol; high-density lipoprotein cholesterol, HDL-C; total protein, T-Pro; albumin, Alb; uric acid, UA; creatinine, Cre; total bilirubin, T-Bil; urea nitrogen, BUN; calcium, Ca; magnesium, Mg; inorganic phosphorus, IP; aspartate aminotransferase, AST; alanine aminotransferase, ALT; lactate dehydrogenase, LDH; creatine kinase, CK. [1]Merck Veterinary Manual 2010; [2]Perri et al., 2017; [3]Radostits et al., 2000. Means within rows do not differ significantly (P>0.05). T = Tendence to be influenced by treatment.

However, the M group as compared to C group, tended to have lower plasma LDH activities (-26%, P = 0.095).

Significantly supressed elevation of serum LDH activities was also noticed by Nishizawa et al. (2002) by feeding the diet containing 20% protein of proso millet for 14 days as compared with those of rats fed a 20% casein diet. Thus, the author concluded that the intake of millet was considered to be a preventive food for liver injury induced by D-galactosamine.

The plasma Ca and Mg concentrations were not significantly affected by the millet diet. Regarding the plasma IP we noticed that pigs fed millet diet registered lower values (-15.43%, P = 0.042) compared to control diet. The values obtained were in physiological limits. Thus, reduction the IP plasma content in our study was surprising since previous reports (Lei et al., 1993; Murry et al., 1997) have demonstrated an increase in serum IP concentration when millet and different phytase levels were added to the pigs diets, and in our study it was expected that the plasma IP level would either remain constant or increase. However, in millet, information is limited on the different types of compound that inhibited mineral absorption (Krishnan and Meera, 2018). The above workers also reported that the phytic acid, polyphenols and fibres present in the millet varieties is a nutritional concern, due to the possible interference with mineral absorption at the intestinal level (Ravindran, 1991).

CONCLUSIONS

The data of the present study shows that 25% of millet 'Marius' could be added to the piglets diet without causing significant changes in the performances and investigated blood parameters, except for the decreasing the plasma inorganic phosphorus levels. However, it might be an effect of millet compound, but further investigations are needed to confirm this.

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REFERENCES

Adeola, O. Rogler, J. C., Sullivan, T. W. (1994). Pearl millet in diets of White Pekin ducks. *Poultry Science,* 73(3), 425-435.

Adeola, O., Orban J. I. (1995). Chemical composition and nutrient digestibility of pearl millet (*Pennisetum glaucum*) fed to growing pigs. *Journal of Cereal Science,* 22, 177-184.

Adeola, O. King, D., Lawrence, B.V. (1996). Evaluation of pearl millet for swine and ducks. J. Janick (ed.), Progress in new crops. ASHS Press, Alexandria, VA. 177-182, from https://hort.purdue.edu/newcrop/proceedings1996/V3-177.html.

Amadou, I. Gounga, M. E, Le, G. W. (2013). Millets: nutritional composition, some health benefits and processing-A review. *Emirates Journal of Food and Agriculture,* 25, 501–508.

Berglund, D. R. (2007). Proso millet in North Dakota. http://mccc.msu.edu/wpcontent/uploads/2016/11/ND_2007_Proso-Millet-in-North-Dakota.pdf.

Campbell, J. M., Crenshaw, J. D., Polo, J. (2013). The biological stress of early weaned piglets. *Journal Animal Science Biotechnology,* 13(4), 19–22.

Changmei, S., Dorothy, J. (2014). Millet-the frugal grain. *International Journal of Scientific Research and Reviews,* 3(4), 75-90.

Colson, V., Martin, E., Orgeur, P., Prunier, A. (2015). Influence of housing and social changes on growth, behaviour and cortisol in piglets at weaning. *Physiology & Behavior,* 107(1), 59-64.

Davis, A. J., Dale, N. M., Ferreira, F. J. (2003). Pearl millet as an alternative feed ingredient in broiler diets. *The Journal of Applied Poultry Research,* 12(2), 137-144.

Devi, P. B., Rajendran, V., Sathyaseelan, S., Nagappa, G. M., Venkatesan, B. P. (2014). Health benefits of finger millet (*Eleusine coracana* L.) polyphenols and dietary fiber: a review. *Journal of Food Science and Technology,* 51(6), 1021–1040.

Directive (EU) No. 63 (2010). Official Journal of the European Union, L 276, 33-79.

Dipnarayan, S. M. V., Channabyre, G., Lalit, A., Manjusha, V., Kailash, C. B. (2016). Genetic and genomic resources of small millets. *Critical Reviews in Plant Sciences,* 35(1), 56-79.

FAOSTAT (2014). http://faostat3.fao.org/browse/Q/QC/E.

Ferket, P. (2000). Feeding whole grain to poultry improves gut health. *Feedstuff,* 72, 12–14.

Garcia, A. R., Dale, N. M. (2006). Feeding of unground pearl millet to laying hens. *Journal of Applied Poultry Research,* 15, 574-578.

Goron, T. L., Raizada, M. N. (2015). Genetic diversity and genomic resources available for the small millet crops to acceleratea New Green Revolution. *Frontiers in Plant Science,* 6(157), 1-28.

Habeanu, M., Lefter, N. A., Gheorghe, A., Tabuc, C., Untea, A., Surdu, I., Ciurescu, G., Balan, C.G., Dragomir, C. (2015). Changes in certain serum and faeces parameters in weaned piglets as a response to nutritional stress. *South African Society for Animal Science,* 45(2), 164-172.

Hăbeanu, M., Lefter, N. A., Gheorghe, A., Untea, A., Ropotă, M., Grigore, D. M., Varzaru, I., Toma, S. M. (2019). Evaluation of performance, nitrogen metabolism and tissue composition in barrows fed an n-3 PUFA-rich diet. *Animals,* 13, 9(5). pii: E234, 1-25.

Habiyaremye, C., Matanguihan, J. B., D'Alpoim Guedes, J., Ganjyal, G. M., Whiteman, M. R., Kidwell, K. K., Murphy, K. M. (2017). Proso millet (*Panicum miliaceum L.*) and its potential for cultivation in the Pacific Northwest, U.S.: A Review. *Frontiers in Plant Science,* 7(1961), 1-17.

Hellwing, A., Louise, F., Tauson, A.H., Skrede, A. (2007). Blood parameters in growing pigs fed increasing levels of bacterial protein meal. Brief communication. *Acta Veterinaria Scandinavica,* 49(33), 1-4.

Yilkal, T., Tegene, N., Negassi, A., Yadav, K. R. (2018). Effect of dietary replacement of maize with finger millet (*Eleusine coracana*) grain on production performance and egg quality of White Leghorn hens. *International Journal of Poultry Science,* 17(1), 40-50.

Jones, J. M., Engleson, J. (2010). Whole grains: benefits and challenges. *Annual Review of Food Science and Technology,* 1, 19–40.

Kalinova, J., Moudry, J. (2006). Content and quality of protein in proso millet (*Panicum miliaceum L.*) varieties. *Plant Foods for Human Nutrition,* 61(1), 45-49.

Kaur, K. D., Jha, A., Sabikhi, L., Singh, A. K. (2014). Significance of coarse cereals in health and nutrition: a review. *Journal of Food Science Technology,* 51(8), 1429-1441.

Krishnan, R., Meera, M. S. (2018). Pearl millet minerals: effect of processing on bioaccessibility. *Journal of Food Science &Technology,* 55(9), 3362–3372.

Lawrence, B. V., Adeola, O., Rogler, J. C. (1995). Nutrient digestibility and growth performance of pigs fed pearl millet as a replacement for corn. *Journal of Animal Science,* 73, 2026-2032.

Lei, X. G., Ku, P. K., Miller, E. R., Yokoyama, M. T., Ullrey, D. E. (1993). Supplementing corn-soybean meal diets with microbial phytase maximizes phytate phosphorus utilization by weanling pigs. *Journal of Animal Science,* 71(12), 3368-3375.

Li, X., Hui, Y. Ren, J., Song, Y., Liu, S., Che, L., Peng, X. Dai, X. (2019). Millet-based supplement restored microbiota diversity of acute undernourished pigs. *bioRxiv,* The Preprint Server for Biology, 1-26.

Luo, L., Wei, Z., Qi, G., Wenli, L., Jing, Z., Weina, X., Jianxiong, X. (2016). Weaning induced hepatic oxidative stress, apoptosis, and aminotransferases through mapk signaling pathways in piglets. *Oxidative Medicine & Cellular Longevity,* ID 4768541, 1-10.

Marin, M., Hodoșan, C., Diniță, G., Nicolae, C.G. (2017). The influence of the chemical composition of maize, barley and peas hybrids on the digestibility of compound feed for pigs. *Agrolife Scientific Journal*, 6(2), 127-132.

Mavromichalis, I. (2014). Introduction to piglet nutrition: Back to the basics! https://www.pigprogress.net/Special-Focus/Piglet-Feeding/Introduction-to-piglet-nutrition-Back-to-the-basics.

Merck & Co. (2010). The Merck Veterinary Manual, 10th ed., Merck Co., Inc., Kenilworth, NJ, USA.

Murry, A. C., Lewis, R. D., Amos, H. E. (1997). The effect of microbial phytase in a pearl millet-soybean meal diet on apparent digestibility and retention of nutrients, serum mineral concentration, and bone mineral density of nursery pigs. *Journal of Animal Science,* 75, 1284–1291.

Nishizawa, N. Oikawa, M., Hareyama, S. (1990). Effect of dietary protein from proso millet on the plasma cholesterol metabolism in rats. *Agricultural and Biological Chemistry,* 54, 229–230.

Nishizawa, N., Fudamoto, Y. (1995). The elevation of plasma concentration of high-density lipoprotein cholesterol in mice fed with protein from proso millet. *Bioscience, Biotechnology, and Biochemistry,* 59(2), 333–335.

Nishizawa, N. Sato, D. Ito, Y. Nagasawa, T. Hatakeyama, Y. Choi, M.R. Choi, Y.Y., Wei, Y. M. (2002). Effects of dietary protein of proso millet on liver injury induced by D-galactosamine in rats. *Bioscience, Biotechnology, and Biochemistry,* 66(1), 92–96.

Oso, A. O. Williams, G. A., Jegede, A. V., Sobayo, R. A., Idowu, O. M. O., Fafiolu, A. O., Sogunle, O. M., Akinola, O. S., Adeleye, O. O., Olorunsola, I. A. R., Ogunade, I. M., Osho, S. O., Obadire, F.O., Bamgbose, A. M. (2014). Effect of combination of whole millet feeding and mannan oligossaccharides supplementation on growth performance, serum biochemistry and relative organ weights of growing guinea fowl (*Numidia meleagris*). *Livestock Science,* 159, 46–52.

Perri, A. M., O'Sullivan, T. L., Harding, J. C. S., Wood, R. D., Friendship, R. M. (2017). Hematology and biochemistry reference intervals for Ontario commercial nursing pigs close to the time of weaning. *Canadian Veterinary Journal*, 58(4), 371–376.

Popp, J., Harangi-Rákos, M., Gabnai, Z., Balogh, P., Antal, G., Bai, A. (2016). Review. Biofuels and their co-products as livestock feed: global economic and environmental implications. *Molecules,* 21(285), 1-26.

Radostits, O., Gay, C., Hinchcliff, K., Constable, P. (2000). A textbook of the diseases of cattle, horses, sheep, pigs and goats. Veterinary medicine, 9th Ed. Saunders, London, 1819–1822.

Ravindran, G. (1991). Studies on millets: Proximate composition, mineral composition and phytate and oxalate contents. *Food Chemistry,* 39(1), 99–107.

Ravindran, G. (1992). Seed protein of millets: amino acid composition, proteinase inhibitors and *in-vitro* protein digestibility. *Food Chemistry,* 44(1), 13-17.

Saleh, A. S. M., Qing, Z., Jing, C., Qun, S. (2013). Millet grains: nutritional quality, processing, and potential health benefits. *Comprehensive Reviews in Food Science and Food Safety,* 12, 280-295.

Shimanuki, S., Nagasawa, T., Nishizawa, N. (2006). Plasma HDL subfraction levels increase in rats fed proso-millet protein concentrate. *Medical Science Monitor,* 12(7), BR 221-6. E pub 2006 Jun 28.

Singh, S., Chauhan, E. S. (2019). Role of underutilized millets and their nutraceuticals importance in the new era. A review. *International Journal of Scientific Research & Reviews,* 8(2), 2844-2857.

SPSS (2011). Statistics v. 20.0., IBM, SPSS, Inc., USA.

van Beers-Schreurs, H. M., Nabuurs, M. J., Vellenga, L. Kalsbeek-van der Valk, H. J. Wensing, T. Breukink, H. J. (1998). Weaning and the weanling diet influence the villous height and crypt depth in the small intestine of pigs and alter the concentrations of short-chain fatty acids in the large intestine and blood. *Journal of Nutrition,* 128, 947–953.

Yenagi, N., Joshi, R., Byadgi, S., Josna, B. (2013). A hand book for school children: Importance of millets in daily diets for food and nutrition security. University of Agricultural Sciences, Dharwad, India. 1-24.

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EFFECTS OF USING PROBIOTICS AND PREBIOTICS ON CALVES HEALTH STATUS: AN REVIEW

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Abstract

The aim of the current review was to evaluate the efficacy of using microorganisms with probiotic effect and prebiotic substrate on calves' health status. Probiotics and prebiotics are currently subject to expansive research as alternative to antibiotics use in animal husbandry. This study reports the symbiotic effects on calves at weaning period (calf performance) and health applications (potential benefits in calf's health and safety use). Since preweaning period, diary calves are vulnerable due to innate immune system, not fully capable of protection the organism against pathogenic actions. Thus, in order to minimise the stress during weaning period and to encourage efficient valorisation of nutrients probiotics and prebiotics are used as natural feed supplements. The article describes interrelations of probiotics and physiological responses to weaning, given that such aspects are very important due to the detrimental balance provided from dietary and physiological mechanisms concerned weaning calves. The importance of exploiting this valuable resource rises with the demand of safer and more affordable alternatives for antibiotic growth promoters, reducing economic loss and promoting homeostasis, thus increasing farm profit.

Key words*: antibiotics, calves, health, probiotic, weaning.*

INTRODUCTION

In order to archive early stage development and to avoid potential pathogenic threats, a common practice was to administrate antibiotic formulas as growth promoters in dietary feed (Dennis et al., 2019).

Antibiotics have been by far the most cost-effective measure to maintain feed efficiency and health status in intensive livestock production systems, including ruminants. Since early 2006, antibiotics administration as feed additives in animal husbandry is prohibited in the European Union. Hence, antibiotics can only be administrated to animals as a treatment or in order to prevent disease and it needs a veterinarian prescription. Given the fact that antibiotics have been reported in milk even after 2006 (Pogurschi et al., 2015), it is necessary to encourage the use of prebiotics and probiotics in dairy cows and calves diet. Therefore, replacing the use of antibiotics as health and growth promoters in animal husbandry represents a real challenge, given the needed balance between costs and effectiveness.

Diarrhoea caused by enteric pathogens in un-weaned calves represents one of the most prevalent causes of morbidity and mortality, with reported incidences of up to 75% and 57%, respectively (Muktar et al., 2015, Cho and Yoon, 2014). Important measures can be taken in order to avoid diarrhoeic diseases in young ruminants, considering their significant effects on early development and later adult productivity.

Probiotics, which are live and viable microorganisms, administrated in sufficient amounts and on a specific time scale, could have beneficial effects on the animal's organism (WHO and FAO, 2002; FAO, 2009; ISAPP, 2013). Probiotics use are supported by the EU legislation (Regulation No. 767/2009), as natural alternatives to antibiotic additives in animal feed. As pharmaceutical application, biotherapeutic drugs formulas regarding

probiotics (one or more strains of the same genus or species) are specifically defined strains, tested clinically and proficient in gastrointestinal diseases (Sreeja and Prajapati, 2013). Often, biotherapeutic drug formulas may include microorganism with potential probiotic effect and other substances such as: oils, vitamins, rehydration salts and prebiotics (Markowiak et al., 2017).

Along with probiotics, prebiotics are accepted as likely substitutes for antibiotic use in animals (Gibson et al., 2017). Gibson et al. (1995) were the first to observe that prebiotics are a group of substances that interact indirectly with the hosts metabolism, in order to induce proliferation in a selective approach among host microbiome, serving as nutritive substrate for non-pathogenic bacteria.

Symbiotics are the synergistic combination of probiotics and prebiotics, which can offer to different extents beneficial effects to cattle (Radzikowski, 2017). In calves, symbiotic consumption has several proven health benefits, including an increase of intestinal beneficial bacteria, improved immune modulatory responses and reduced incidence of enteritis (Shimizu et al., 2018).

In addition, calf's intestinal microbiome has a major impact on health status, thus protecting the organism from environmental stressors. Adding physiological functions which include storage, conversion and transportation of nutrients. In neonatal calves, the gastrointestinal system (GIT) is unique, experiencing important changes between birth and the first year of life (Yeoman et al., 2018). The digestive system of new born calves in sterile, GIT colonisation starts instantly after birth. During the suckling period, calf's intestinal microbiome is represented mostly of *Lactobacillus* spp., *Lactococcus* spp., *Citrobacter* spp. and *Leuconostoc* spp. Diversity and complexity rises as a result of dietary, physiological and environmental challenges (Diao et al., 2019). At the age of three months, in the calf microbiome, beside existent bacteria an abundance of other genera are being found, including: *Bifidobacterium* spp., *Bacteriodes* spp., *Firmicutes* spp. and *Faecalibacterium* spp. (Oikonomou et al., 2013). From this age, a dynamic microbial complex expands, and it is encouraged by innate immune system to become similar as ones functioning in adults.

Enteric diarrhoea is one of the most prevalent causes of morbidity and mortality among diary calves. For this reason, it is important to prevent pathogenic actions and to encourage healthy approaches (Manyi-Loh et al., 2016).

In this context, the paper presents an evaluation of symbiotic manipulation of gut microbiome in order to optimise calf's performance and health status.

Potential benefits in using prebiotics and probiotics in calves

Current applications of probiotic and symbiotic on young pre ruminant animals, generally targets the gastrointestinal system, by stabilizing and enhancing lower intestine microbiome, thus minimizing the rate of gastrointestinal disorders, by supporting health and optimal nutrition. Probiotic, prebiotic and the combination between them, symbiotic claims a potential positive effect on calf health (Dar et al., 2017) and calf performance (Ysmail, 2019). Gut microbiota has a metabolic active role, having also protective and trophic functions, being positively modulated by beneficial microorganisms, in combination with or without carbohydrate substrates (Rowland et al., 2018). Previous studies have shown the beneficial health aspects and potential, throughout the manipulation of intestinal barrier microbiota, facilitating beneficial bacteria proliferation, while reducing the incidence of neonatal calf diarrhoea (Jyotimala et al., 2019), while also improving weight gains (Radzikowski, 2017). The combination of mannan oligosaccharide and *Streptococcus faecium* was investigated by Morrison et al. (2010), as addition to milk replacer on calf performance and health. Results shown a significant improvement of fed concentrate intake and calf faecal consistency, thus improving calf performance.

Lactic acid bacteria such as *Lactobacillus* spp. and *Bacillus* spp. are the most widespread probiotic supplements used and often regarded as feasible in common feeding techniques, improving average daily gains (Ballou et al., 2019; Cantor et al., 2019) and decreasing the incidence of diarrhoea (Jiang et al., 2020). Dar et al., 2017, evaluated the impact of symbiotic

combination between *Lactobacillus* spp. and mannan oligosaccharide on serum biochemical profile of calves. Results shown that symbiotic supplementation had reduced the serum triglycerides and cholesterol, unaffecting serum glucose, bilirubin, creatinine and urea.
On the other hand, symbiotic administration of *Enterococcus faecium* and lactulose could improve calf performance (Fleige et al., 2007), by modulating the intestinal barrier physiology, thus decreasing the height of ileal villus, the crypts depth in the cecum and the area of lymph follicles from Peyer's patches.

Inclusion and general use of prebiotics and probiotics in pre weaned calves

Given the economic implications of dairy calves rearing, in order to improve calf's growth, health and future adult development, farmers give an increasing interest to calf weaning techniques. In addition, intensive dairy farming demands an accelerated weaning process (Jami et al., 2013), from pre ruminant to ruminant with minimum time and less economic resources.
Furthermore, available research results to improve calf's nutrition and development had become more available with the progress of biochemistry and biotechnologies.
The next generation of growth and health promoters are probiotic, prebiotic and symbiotic, in order to provide solutions to the antibiotic's restrictions put in place by EU Directives in all Member States since year 2006. Recent research demonstrates the multitude of biotechnological approaches applied in dairy calf's diet supplementation (Table 1.).
Colostrum administration together with the milk diet (milk quantity, quality and number of meals per day) are dependent factors that support the future development and stabilisation of calves' gastrointestinal microbiome and adaptive immune system (Neamt et al., 2019; Marin et al., 2020). For instance, probiotic, prebiotic and symbiotic administration could be included in both liquid and solid diets. During the first weeks of life, milk replacer -based diets could be improved with probiotic or symbiotic supplementation in diary calf farms (Mehdi Bahari, 2017).

Table 1. Example of biotechnological growth promoting formulations used in calf nutrition

Name of formula	Microorganism +/- Prebiotic substrates	Producer, recommendation of administration
Symbiotics		
Biomin©IMBO	*Enterococcus faecium* + FOS	Biomin IMBO, mixed directly in feed
Prebiotics		
BionatStart	MOS, β-glucans	1 x 25 ml/calf/ day
MetSac MOS	MOS, β-glucans	-
PROFEED®	scFOS	10 g/calf/day
Probiotics		
Cylactin	*Enterococcus faecium*	-
Lactiferm	*Enterococcus faecium*	5×10^8 - 2×10^{10} CFU/kg feed
Oralin	*Enterococcus faecium*	1×10^9 CFU/kg feed
BioEnterom	*Enterococcus faecium*	1×10^8 UFC/ml/calf/day
Cernivet LBC ME10	*Enterococcus faecium*	-
BioPlus2B®	*Bacillus subtilis*, *Bacillus liqueniformis*	0.45 kg/1 ton of solid feed
Probios Max®	Lactic acid bacteria + vitaminic complex	Unique dose- 5 mg at calf birth
Probiosacc C-I	*Saccharomyces cerevisiae*	-
Yea Sacc	*Lactobacillus rhamnosus, Enterococcus faecium*	-

FOS - fructooligo saccharide; MOS-mannanoligosaccharide

In addition, Ulger (2019) found that by supplementing calf diet, during the first 56 days of life (suckling period) with probiotic (*Lactobacillus acidophilus, Lactobacillus casei, Enterococcus faecium* and *Bifidobacterium bifidum*) improves calf performance and decreases blood triglyceride and iron levels. Among administration mode, diary calves' breeders often are substituting whole milk diets with artificial milk replacers. Efficacy of biotechnological application such as probiotic and symbiotics is directly affected by certain factors and circumstances. However, only an appropriate and correct dosage of administration could manifest maximum positive attributes of the probiotic strain. Other relevant factor is represented by the physical form of administration: liquid, powder, suspensions, capsules, gel, paste or pellets. In addition, dosage requirements are depending in the same time of physical form and biological potential of probiotic and symbiotic formula. Furthermore, circumstances regarding individuality and environmental conditions (e.g. diseases exposure) are criteria can be controlled at farm level throughout the

implementation of biosecurity and veterinary measures.

Effects of prebiotics and probiotics on calf's growth and feed conversion

Future productivity and performance of dairy farms request adequate calf nutrition and care. In addition to banning antibiotic use at farm level, probiotic and prebiotic approaches represent a potential key to establish a new generation of growth promoters. Early stage development and weaning are important due to the fact that they are facilitating changes in metabolism absorption and growth (Govil et al., 2017). Furthermore, ruminal development is conditioned by solid feed administration and intake, within significant effects on the ruminal fermentation processes. The effects of probiotic are generally dependent on the specific microorganisms producing antimicrobial substances, and also on the digestive enzyme stimulation capacity. Prebiotic use showed an improvement of growth performances by improving average daily gain, feed intake and digestibility. In calf feeding, carbohydrate represent the main nutrient in producing volatile fatty acids, with potential to increase nutrient digestibility (Nagpal et al., 2015) and absorption, thus increasing feed efficiency. Ghosh and Mehla (2012) observed an increase in performance weight gain, an improvement of feed intake and feed conversion efficiency by administrating 4 g/day/calf of a mannan oligosaccharide as supplement to calf feed. Similar results were shown by Grand et al., 2013, throughout direct administration of different quantities (3 g or 6 g) of short chain fructo-oligosaccharides, enhancing the growth performance of weaned calves and decreasing feed conversion ratio, while improving carcass weight.

Prebiotic supplementation such as inulin and lactulose, in young pre-ruminant regulates the mRNA gene expression involved in intestine inflammation (Masanetz et al., 2011) and it is believed to facilitate nutritive absorption. Along with health benefits, current prebiotic mechanisms could increase macro elements absorption, for instance Cu, Ca, Mg, Fe and Zn, also Na+ and colon water absorption (Singh et al., 2017). Other researchers have observed an improved capacity of prebiotics, like fructans in colon calcium transporters (Haq and Khan, 2018) increasing bone calcium formation and decreasing calcium losses (Garcia-Vieyra et al., 2014).

Probiotic lactic acid bacteria had influenced productive performance by stimulating feed intake and increasing average daily weight gains, also improving health, thus reducing the incidence of diarrhoea among young ruminants (Wallace et al., 1993; Mehdi Bahari, 2017).

Marcondes et al. (2016) research has shown that symbiotic containing mannan-oligossacharides [(MOS; prebiotic), *Lactobacillus acidophilus*, *Enterococcus faecium*, *Bacillus subtilis*, *Saccharomyces cerevisiae* and cellulase, xylanase and hemicellulose (fibro lytic enzymes)] supplementation had no effect on calves performance, instead was found that the symbiotic Bioformula Leite could represent an bio-control option in calf diarrhoea during the pre-weaning period.

Effects of prebiotics and probiotics on calf's health

The capacity of symbiotics on maintaining an eco-balance in the gastro-intestinal microbiome has significantly impacted calf's overall status health status (Malmuthuge et al., 2015; Pandey et al., 2015). Frequently, calf diarrhoea, scours and weight losses are results of stressors generated by husbandry practice (such as ear tagging, vaccination programs, weaning, heat or cold stress, regrouping), pathogens (*Clostridium* spp., *Escherichia* spp., *Enterococcus* spp. and *Shigella* spp.) and bacterial toxins (Taijima et al., 2001). Calf gastro-intestinal microbiome has proven to be able of modulation (Uyeno et al., 2015), as an adaptative response (Cammack et al., 2018) to environmental challenges. Opportunistic pathogens like *E. coli* are the most prevalent causes on neonatal diary calf's diarrhoea (Cho et al., 2012). In according with Muktar et al. (2015), around 80% of neonatal calf diarrhoea positively tested for at least one enteric bacterial infection, denoting that still pathogen infections are main causes. It has been accepted that the first 24 h after birth are crucial to young ruminants, within a need for adequate amount of colostrum administration (Meganck et al., 2014) starting from the first hour after

calving, in order to acquire adaptive immune system (Godden et al., 2019). Calf suckling period is often associated with morbidity and mortality (Meganck et al., 2014) and represents the major economic loss factor. Mokhber-Dezfouli et al. (2007) tested a dietary probiotic mixture (*Lactobacillus, Bifidobacterium bifidum, Enterococcus faecium, Streptococcus thermophilus, Aspergillus oryzae* and *Candida pinotopesti*) added in concentrates (25 g/ head/day) and evaluated performance and health status. At the end of the experiment (90 days) body weight gain of the experimental group increased by 7 kg over the control group, and had shown lower diarrhoea incidence.

Satik and Gunal (2017), studied a natural probiotic (kefir), with the administration having a positive effect on lactic acid bacteria populations, related to pathogenic competitive exclusion, proving thus beneficial during the first eight weeks of life.

Li et al. (2012) and Loor et al. (2016) found throughout the sequencing method that calf microbiome development is age-dependent and at the age of two weeks, calf microbiome colonisation is preponderant colonised by *Pervotella* spp., compared to

six weeks of age, when the vast majority of populations are *Bacterioidetes* spp.

Safety measurements in using prebiotics and probiotics

As the legislation enforces, in order to characterize the microorganism's probiotic action, the following indicators should be used: stress tolerance, adhesion ability, own pathogenic activity, antipathogenic activity, safety assessment and clinical trials (FAO, 2002). New approaches of the pharmaceutical industry regarding antibiotic replacement include therapies such as probiotics and symbiotics, and they are displaying them as less-aggressive methods. Pharmaceutical products impose advanced production technologies and have requirement such as isolation of bacteriologic strains with probiotic potential, description based on ecosystems, geographical region particularisation, selection and characterisation, reproduction at industrial scale. In most cases, non-selected probiotics administration to livestock had low or/and no effects on performance and/or health condition (Fuller, 1989). This might be a consequence of using probiotics which were isolated from other regions or even from other animal species.

In addition, in most publications, authors tend to discuss the efficacy of using probiotics rather than safety of usage. Therefore, in future research it would be advised to evaluate the safety of adding symbiotics in livestock nutrition. Shanahan (2012) pointed the general safety measurements of the prebiotic and symbiotic pharmaceutical formulation, as follows:

- Generalisation of safety assessments on probiotic or symbiotic formulation cannot be applied on similar probiotic or symbiotic products;
- Adverse effects and severity of effects could be specific to susceptibility by individuality;
- Raising awareness among public consumers and farmers, given the wide range of effects and influencing factors in prebiotic administration.

Microorganisms with probiotic attributes are considered generally safe, although often risk administration assessment did not have been made. Regularly, risk as gastro-intestinal or systemic infection on host, detrimental metabolic or toxic effects also immune system hyperstimulation are not taken into consideration, frequently with serious consequences.

Effects of prebiotics and probiotics on calf's immune responses

Immunomodulatory actions based on establishment and enhancement of innate and adapted immune system, is involved in the natural immune response mechanism and is directly influencing the animal health (Wu et al., 2019).

Intensive dairy farming, focused on productivity and product quality, requires calf artificial rearing, as a consequence, acquired microbiota and immune system of calves is often reduced and vulnerable to bacterial pathogen colonisation (Marcondes et al., 2016). In comparison with extensive production systems, the intensive calf management requires alternative solutions to support innate immune system and to improve intestinal microbiome. Roodposhti and Dabiri (2012)

studied the effect of symbiotic consisting of probiotic bacteria and two types of fungi and prebiotic substances (*Saccharomyces cerevisiae* and cell wall polysaccharide) on daily growth of calves, traces of *E. coli* in calf faeces and IgG immunoglobulin content from calf blood. Research was conducted during 8 weeks, administrating to calves from the control group 1 g probiotics and 4 g probiotic, results displayed an increase on growth performance and improvement in antibody production by 0.120 mg/ml, while decreasing the number of pathogenic populations of *E. coli* by 5%.

Indart et al. (2012) studied the administration of multispecies and multi-strain probiotic (*L. helveticus, L. casei, L. fermentum, L. paracasei, L. parabuchneri, Lactobacillus gasseri* and *Lactobacillus panis, Sacchoromyces cerevisiae* and *Pichia kudravzevii*) on calf pre-weaning period, results indicating a significant increase on metabolic and microbicide activity in peripheral blood neutrophils.

Similar results were shown by Qadis et al. (2014) regarding probiotic (*Lactobacillus plantarum, Enterococcus faecium* and *Clostridium butyricum*) administration on pre-weaned calves, with positive outcomes displayed on peripheral blood leukocytes, inducing a stability among commensal microflora.

CONCLUSIONS

Probiotic, prebiotic and symbiotic use were shown to have positive effects when administrated to claves on both performance and health. In addition, enhancing the calf development may prove an important pathway to maximise production potential as adult. Besides productive benefits, health status can be modulated throughout the immune response, protecting calves via antipathogenic action. New-born calf microbiome manipulation throughout characterisation and identification of ideal interactions between symbiotics could represent an important key in order to improve overall production. Further research is needed, considering the lack of knowledge and low understanding of the complex mechanisms of interactions, regulation and overall profitability and feasibility.

REFERENCES

Ballou, M. A., Davis, E. M., Kals, B. B. (2019). Nutraceuticals: An Alternative Strategy for the Use of Antimicrobials. *Veterinary Clinics of North America: Food Animal Practice*, 35(2), 507-534.

Cantor, M. C., Stanton, A. L., Combs, D. K., Costa, J. H. C. (2019). Effect of milk feeding strategy and lactic acid probiotics on growth and behaviour of dairy calves fed using an automated feeding system. *Journal of Animal Science,* 97(3), 1052-1065.

Cho, Y., Yoon, K. J. (2014). An overview of calf diarrhea - infectious etiology, diagnosis, and intervention. *Journal of Veterinary Science,* 15(1), 1-17.

Dar, A., Singh, S., Palod, J., Ain, K., Kumar, N., Farooq, F. (2017). Effect of Probiotic, Prebiotic and Symbiotic on Haematological Parameters of Crossbred Calves. *International Journal of Livestock Research,* 7(4), 128-136.

Dennis, T. S., Suarez-Mena, F. X., Hu, W., Hill, T. M., Quigley, J. D., Schlotterbeck, R. L. (2019). Effects of milk replacer feeding rate and long-term antibiotic inclusion in milk replacer on performance and nutrient digestibility of Holstein dairy calves up to 4 months of age. *Journal of Dairy Science,* 102(3), 2094-2102.

Diao, Q.,Zhang, R., and Fu, T. (2019). Review of Strategies to Promote Rumen Development in Calves. *Animals (Basel)*, 9(8), 490.

FAO, Food and Agriculture Organization of the United Nations. (2009). Guidelines for the evaluation of probiotics in food. Retrived in 01.02.2020 from www.fao.org/3/i0680e/i0680e00.htm.

FAO. Guidelines for the evaluation of probiotics in food. (2002). Report of a Joint FAO/WHO Working Group on Drafting Gidelines for the evaluation of probiotics in food; 30.04–01.05.2002, London, Ontario, Canada;

Fleige, S., Preißinger, W., Meyer, H.H.D., Pfaffl, M.W. (2007) .Effect of lactulose on growth performance and intestinal morphology of pre-ruminant calves using a milk replacer containing *Enterococcus faecium*. *Animal,* 1(3), 367-373.

Garcia-Vieyra, M.I., del Real, A. and Lopez, M.G. (2014). Agave fructans: Their effect on mineral absorption and bone mineral content. *Journal of Medicinal Food*, 17, 1247-125.

Ghosh, S. and Mehla, R. K. (2012). Influence of dietary supplementation of prebiotics (mannanoligosaccharide) on the performance of crossbred calves. *Tropical Animal Health Production,* 44, 617-622.

Gibson, G. R. and Hutkins, R. (2017). The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Review Gastroenterology Hepatology*, 2017; 14(8), 491-502.

Gibson, G. R. and Roberfroid, M. B. (1995) Dietary Modulation of the Human Colonic Microbiota: Introducing the Concept of Prebiotics. *Journal of Nutrition*, 125, 1401-141.

Godden, S. M., Lombard, J. E., Woolums, A. R. (2019). Colostrum Management for Dairy Calves. *Veterinary Clinics: Food Animal Practice*, 35(3), 535-556.

Govil, D. S., Yadav, A. K., Patil, S., Nayak, R. P. S., Baghel, P. K., Yadav, C. D., Malapure D. T. (2017). Feeding management for early rumen development in calves. *Journal of Entomology and Zoology Studies*, 5(3), 1132-1139.

Grand, E., Respondek, F., Martineau, C., Detilleux, J. and Bertrand, G. (2013). Effects of short-chain fructooligosaccharides on growth performance of preruminant veal calves. *Journal of Dairy Science* 96(2), 1094-101.

Haq, Z., and Khan, A. A., (2018). Prebiotics: The gut ecology modifiers. *Journal of Entomology and Zoology Studies,* 6(3), 1816-1820.

Indart, M., Cerone, S., Esteban, E.N., Yaniz, G. D., Inza, A. G., Landi, H. (2012). Multispecies multi strain probiotic effects on calf's development and health. *Open Journal of Veterinary Medicine*, 2, 225-229.

Jami, E., Israel, A., Kotser, A., Mizrahi, I. (2013). Exploring the bovine rumen bacterial community from birth to adulthood. *ISME Journal*, 7, 1069-1079.

Jiang, X., M, G.M., Cui, Z.Q., Li, Y., Zhang, Y.G., (2020). Effects of fermented corn gluten meal on growth performance, plasma metabolites, rumen fermentation and bacterial community of Holstein calves during the pre-weaning period. *Livestock Science,* 231.

Jyotimala, S., Aayush, Y., Tripti, K., Prasanna, P. and Pankaj K. P. (2019). Probiotic supplementation to produce healthier calves: A short note. *The Pharma Innovation Journal*, 8(3), 94-495.

Li, R. W., Connor, E. E., Li, C., Baldwin, V. R., Sparks, M. E. (2012). Characterisation of the rumen microbiota of pre-ruminant calves using metagenomic tools. *Environmental Microbiology*, 14, 129-139.

Loor, J. J., Elolimy, A. A., McCann, J. C. (2016). Dietary impacts on rumen microbiota in beef and dairy production. *Animal Frontiers,* 6(3), 22–29.

Malmuthuge, N., Griebel, N., Guan, P. J. (2015). The Gut Microbiome and Its Potential Role in the Development and Function of New born Calf Gastrointestinal Tract. *Frontiers in Veterinary Science*, 2, 36.

Manyi-Loh, C. E., Mamphweli, S. N., Meyer, E. L., Makaka, G., Simon, M., & Okoh, A. I. (2016). An Overview of the Control of Bacterial Pathogens in Cattle Manure. *International journal of environmental research and public health*, 13(9), 843.

Marcondes, M. I., Pereira, T. R., Chagas, J. C. C., Filgueiras, E. A., Castro, M. M. D., Costa, G. P., Sguizzato, A. L. L., Sainz, R. D. (2016b). Performance and health of Holstein calves fed different levels of milk fortified with symbiotic complex containing pre- and probiotics. *Tropical Animal Health Production*, 48(8), 1555-1560.

Marin M., Pogurschi E, Marin I, Nicolae C.G. (2020) - Influence of Natural Zeolites Supplemented with Inorganic Selenium on the Productive Performance of Dairy Cows. *Pakistan journal of zoology*, 52(2), 775-783.

Markowiak, P. and Silzewska, K. (2017). Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. *Nutrients,* 9(9), 102.

Masanetz, S., Preißinger, W., Meyer, H. H. D. and Pfaffl, M. W. (2011). Effects of the prebiotics inulin and lactulose on intestinal immunology and hematology of preruminant calves. *Animal,* 5(7), 1099-1106.

Meganck, V., Hoflack, G., Opsomer, G., (2014). Advances in prevention and therapy of neonatal dairy calf diarrhoea: a systematical review with emphasis on colostrum management and fluid therapy. *Acta Vetrinaria Scandinavica*, 56, 75-83.

Mehdi Bahari. (2017). A Review on the Consumption of Probiotics in Feeding Young Ruminants. *Approaches Poultry Dairy & Veterinary Science,* 1(2).

Mokhber-Dezfouli M. R., Tajik P., Bolourchi M., Mahmoudzadeh H. (2007). *Pakistan Journal of Biological Sciences*, 10(18), 3136-3140.

Morrison S. J., Dawson S., Carson A. F. (2010). The effects of mannan oligosaccharide and *Streptococcus faecium* addition to milk replacer on calf health and performance. *Livestock Science*, 131(2), 292-296.

Muktar, Y., Mamo, G., Tesfaye, B., Belina, D., (2015). A review on major bacterial causes of calf diarrhea and its diagnostic method. *Journal Veteterinary Medicine Animal Health*, 7, 173-185.

Nagpal, R., Shrivastava, B., Kumar, N., Dhewa, T., Sahay, H. (2015). Microbial Feed Additives. *Rumen Microbiology: From Evolution to Revolution*, Springer, New Delhi, 161-175.

Neamt, R. I., Ilie, D.E., Saplacan, S., and Cziszter, L.T. (2019). Effects of some factors on calves' viability and growth in Simmental cattle. *Research Journal of Biotechnology*, 14(1).

Oikonomou, G., Vieira Teixeira, A. G., Foditsch, C., Bicalho, M. L., Machado, V., V., and Bicalho, R. C., (2013). Fecal Microbial Diversity in Pre-Weaned Dairy Calves as Described by Pyrosequencing of Metagenomic 16S rDNA. Associations of *Faecali bacterium* Species with Health and Growth. *PLOS one*, v8(4).

Pandey, K. R., Naik, S. R., Vakil, B. V. (2015). Probiotics, prebiotics and synbiotics- a review. *Journal of Food Science and Technology*, 52(12),7577-7587.

Pogurschi, E., Ciric, A., Zugravu, C., Patrascu, D. (2015). Indentification of antibiotic residues in raw milk samples coming from metropolitan area of Bucharest. *Agriculture and agricultural Science Procedia,* (6), 242-245.

Qadis, A. Q., Goya, S., Yatsu, M., Yoshida, Y., Ichijo, T., and Sato, S. (2014). Effects of a Bacteria-Based Probiotic on Subpopulations of Peripheral Leukocytes and Their Cytokine mRNA. *Expression in Calves. Journal of Veterinary Medical Science*, 76(2), 189-195.

Radzikowski D. (2017). Effect of probiotics, prebiotics and synbiotics on the productivity and health of dairy cows and calves. *Wireless sensor networks,* 78, 193-198.

Roodposhti, P. M., Dabiri, N. (2012). Effects of Probiotic and Prebiotic on Average Daily Gain, Fecal Shedding of Escherichia Coli, and Immune System Status in New-born Female Calves. *Asian-Australasian Journal of Animal Sciences,* 25(9), 1255-1261.

Rowland, I., Gibson, G., Heinken, A., Scott, K., Swann, J., Thiele, I., Tuohy, K. (2018). Gut microbiota functions: metabolism of nutrients and other food components. *European journal of nutrition*, 57(1), 1-24.

Satık, S. and Günal, M. (2017). Effects of Kefir as a Probiotic Source on the Performance and Health of Young Dairy Calves. *Turkish Journal of Agriculture - Food Science and Technology*, 5(2), 139-143.

Shanahan, F. (2012). A commentary on the safety of probiotics. *Gastroenterology Clinics of North America*, 41(4), 869.

Shimizu, K., Yamada,T., Ogura, H., Mohri, T., Kiguchi, T., Fujimi, S., Asahara, T., Yamada, T.,Ojima, M., Ikeda, M., Shimazu, T., (2018). Synbiotics modulate gut microbiota and reduce enteritis and ventilator-associated pneumonia in patients with sepsis: a randomized controlled trial. *Critical Care*, v.22.

Singh, A., Kerketta, S., Yogi, R., Kumar, A., Ojha, L. (2017). Prebiotics: The New Feed Supplement for Dairy Calf. *International Journal of Livestock Research*, 7(8), 1-17.

Sreeja, V. and Prajapati, J. (2013). Probiotic Formulations: Application and Status as Pharmaceuticals - A Review. *Probiotics and Antimicrobial Proteins,* 4(4), 217-290.

Tajima, K., Aminov, R.J., Nagamine, T., Matsui, H., Nakamura, M., Benno, Y. (2001) Diet-dependent shifts in the bacterial population of the rumen revealed with realtime PCR. *Applied Environmental Microbiology,* 67, 2766-2774.

Ulger Ismail. (2019). Effects of pre-weaning probiotic treatments on growth performance and biochemical blood parameters of Holstein calves. *Indian Journal of Animal Research*., (53), 644-647.

Uyeno, Y., Shigemori, S., Shimosato, T., (2015). Effect of probiotics/prebiotics on cattle health and productivity. *Microbes and Environments*, 30, 126-132.

Wallace, R. J., Newbold, C. J. (1993). Rumen fermentation and its manipulation: the development of yeast cultures as feed additives. *Biotechnology in the Feed Industry. Alltech Technical Publications*, Nicholasville, USA., 173-192.

Wu, D., Lewis, E. D., Pae, M., Meydani, S. N. (2019). Nutritional Modulation of Immune Function: Analysis of Evidence, Mechanisms, and Clinical Relevance. *Frontiers in immunology*, 9, 3160.

Yeoman, C. J., Ishaq, S. L., Bichi, E., Olivo, S. K., Lowe, L., Aldridge, B. M. (2018). Biogeographical Differences in the Influence of Maternal Microbial Sources on the Early Successional Development of the Bovine Neonatal Gastrointestinal tract. *Scientific Reports*, 8, 3197.

Ulger Ysmail. (2019). Effects of pre-weaning probiotic treatments on growth performance and biochemical blood parameters of Holstein calves.*Indian Journal of Animal Research*., 53, 644-647.

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COMPARATIVE STUDY ON METABOLIC BIOMARKERS IN LACTATING DAIRY COWS AND BUFFALOES

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Abstract

The knowledge of haematological and urine biomarkers is useful to diagnose various metabolic and pathological disorders, which have a negative impact on the overall performances of dairy species. The aim of this study was to investigate the haematological and urine parameters in lactating dairy cows comparative with lactating buffaloes. The study was carried out on sixty-eight Romanian Black and Spotted cattle and on fifty Romanian buffalo. The obtained values for hemoglobin concentration (HGB), hematocrit percentage (HCT), white blood cells count (WBC), lymphocytes percentage (LY), monocytes percentage (MO), and neutrophil percentage (NE), varied significantly ($p<0.01$) between cows and buffaloes. For other haematological indices, such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), statistical differences were recorded. Urine examination of cows and buffaloes showed that all the parameters studied were within the normal physiological limits, with minor fluctuations for protein level (30 mg/dl). The obtained results could serve as baseline information for comparison in conditions of nutrient deficiency, physiology and health status of dairy herd's.

Key words*: buffaloes, cows, haematological profile, urine profile*.

INTRODUCTION

Haematological studies are useful in the diagnosis and prognosis of many diseases in farm animal's (Olafedehan et al., 2010; Togun et al., 2007; Parvu et al., 2003) and it plays a vital role in the physiological, nutrition and pathological status of an organism (Doyle, 2006). According to Xie et al. (2013), significant variations in the blood metabolic profile depend on genetic (breed and genotype) and non-genetic factors (age, sex, physiological status). Seasons and diet, also, influence the blood parameters of farm animal's (Knaus, 2013; Varra et al., 2017; Garkal et al., 2016; Radkowska and Herbut, 2014). Changes in haematological parameters are often used to determine status of the body and to determine stresses due to environmental, nutritional and pathological factors (Afolabi et al., 2010). Urinalysis is a useful and inexpensive tool to detect metabolic diseases such as diabetes mellitus, liver diseases, glomerulonephritis and urinary tract infections (Finco, 1997). There exists difference between the cattle and buffaloes regarding utilization of feed. Buffaloes have better digestive ability than cattle to utilize poor quality roughage (Agarwal et al., 2008) and better degrade crude protein and protein free dry matter (Terramoccia et al., 2000). With this background, the aim of the present study was to investigate the haematological and urine parameters of apparently healthy Romanian Black and Spotted cows and Romanian buffalo and, also, to compare the metabolic profile between both groups. The determination of these parameters could then aid in exploring the differences in the metabolism of this and their organ function.

MATERIALS AND METHODS

Sixty-eight multiparous Romanian Black and Spotted dairy cows and fifty multiparous Romanian buffalo cows, clinically healthy, were screened for metabolic profile (haematological and urine indicators) during the autumn season (October, 2019). The study was carried out at the Experimental Farm of the Research and Development Institute for Bovine

Balotesti, and at the Experimental Farm of the Research and Development Station for Buffalo Sercaia, Romania. The cows and buffaloes were housed under tied stanchion barn conditions. Feeding system was differentiated, for cows, the diet/head/day consisted of 4 kg alfalfa hay, 20-25 kg corn silage and 3-4 kg concentrates, and, for buffaloes, the diet/head/day consisted of 4 kg sedge, 7 kg marsh hay and 28 kg corn silage. The lactating dairy cows and buffaloes received salt and water *ad libitum*. For the haematological examinations, blood samples (1-2 ml) were collected aseptically from the jugular vein of each animal, 2-4 hours after the morning feeding, in vacutainer tubes with disodium-ethylene diamine tetra acetic acid ($EDTA/Na_2$). After harvesting, the samples were chilled to +4 °C. Urine samples were collected in 50 ml sterilized vials from both groups studied as free catch (mid stream voided) during micturition. Haematological parameters (RBC, HGB, HTC, MCV, MCH, MCHC, WBC, LY, MO, NE) were determined using automated hematology analyzer Abacus Junior Vet 5. Urine examination (bilirubin, urobilinogen, ketones, ascorbic acid, glucose, protein, blood, pH, nitrites, leukocytes, specific gravity) were determined with the DocUReader urine analyzer, used for *in vitro* diagnostics. Means ± (Standard Deviations) and coefficients of variation (CV) of blood indicators were calculated. Data were analyzed to compare the mean values of cows and buffaloes by applying independent mean t-test and 95% confidence intervals at 0.05 significance level using a descriptive statistical tool (computer software Microsoft Excel). The experimental procedures were performed in accordance with the *Romanian Law no. 43/2014* and the *Council Directive 2010/63/EU* on the protection of animals used for scientific purposes.

RESULTS AND DISCUSSIONS

The Table 1 shows means values of blood parameters in lactating dairy cows and buffaloes studied, and the Table 2 shows haematology reference intervals in bovine.

Table 1. Haematological parameters in lactating dairy cows and buffaloes

Parameters	Group I Cows		Group II Buffaloes		P	Reference limits
	X ± SD	CV, %	X ± SD	CV, %		
RBC, $10^6/\mu L$	6.56±1.34	20.43	6.79±1.24	18.26	0.114	5-8
HGB, g/dL	9.48±1.38	14.56	12.29±2.27	18.47	0.000	9-11
HCT, %	28.50±3.21	11.26	37.69±7.10	18.83	0.000	32-38
MCV, fl	43.98±4.41	10.03	55.46±2.83	5.10	0.000	40-60
MCH, pg	14.9±1.65	11.08	18.14±1.37	7.55	0.000	11-17
MCHC, g/dL	33.93±2.03	5.98	32.71±2.40	7.33	0.012	30-36
WBC, $10^3/\mu L$	8.37±2.27	27.12	9.86±2.14	21.70	0.000	6.5-9.5
LY, %	54.10±10.14	18.74	45.93±7.41	16.13	0.000	45-61
MO, %	7.03±2.81	39.97	5.21±2.00	38.39	0.003	0-4
NE, %	38.74±8.97	23.15	48.84±7.86	16.09	0.000	15-41

RBC - red blood cells, HGB - hemoglobin, HCT - hematocrit, MCV - mean corpuscular volume, MCH - mean corpuscular hemoglobin, MCHC - mean corpuscular hemoglobin concentration, WBC - total white blood cells, LY - lymphocytes, MO - monocytes, NE - neutrophils

Table 2. Haematology reference interval in bovine (source Roland et al., 2014)

Parameters	Wood and Quiroz-Rocha, 2010	George et al., 2010	Kraft and Dürr, 2005
RBC, $10^6/\mu L$	4.9-7.5	5.1-7.6	5-10
HCT, %	21-30	22-33	28-38
HGB, g/dL	8.4-12.0	8.5-12.2	9-14
MCV, fl	36-50	38-50	46-65
MCH, pg	14-19	14-18	11-17
MCHC, g/dL	38-43	36-39	31-34

RBC - red blood cells, HCT - hematocrit, HGB - hemoglobin, MCV - mean corpuscular volume, MCH - mean corpuscular hemoglobin, MCHC - mean corpuscular hemoglobin concentration

The results revealed no significant variation ($P>0.05$) in RBC count for the group I and for the group II studied. A significant variation ($p<0.01$) for HGB, HCT, WBC, LY, MO, and

NE, between the group I and the group II, was observed. The MCV, MCH, MCHC indices were, also, found to be significantly ($P<0.01$ for MCV and MCH; $P<0.05$ for MCHC) between groups studied, this erythrocyte indices are usually used for morphological classification of anaemia. The recorded mean values for RBC (6.56±1.3410^6/µL), HGB (9.48±1.38 g/dL), MCV (43.98±4.41 fl), MCH (14.9±1.65 pg), MCHC (33.93±2.03 g/dL), WBC (8.37±2.27 10^3/µL), LY (54.10±10.14%), and NE (38.74±8.97%) parameters were situated in the normal physiological limits in lactating dairy cows. However, in the present study, a lower mean value for HCT (28.50±3.21%) and a high mean value for MO (7.03±2.81%) than normal physiological limits were recorded. According to Research Animal Resources (2009), the reference values for cow are situated between 8-15 g/dL for HGB, 40-60 fl for MCV, 11-17 pg for MCH, 30-36 mg/dL for MCHC, 4-12 10^3/µL for WBC, 40-70 % for LY, and 1-6 % for MO. Merck Manual (2012) related the following rage of values for cow: 5-10 10^6/mm^3 for RBC 10-15 g/dL for HGB, 39-55 fl for MCV, 13-17 pg for MCH, and 30-36 mg/dL for MCHC. The recorded mean values for RBC (6.79±1.24 10^6/µL), HCT (37.69±7.10 %), MCV (55.46±2.83 fl), and LY (45.93±7.41 %), parameters were situated in the normal physiological limits in lactating buffaloes with slight increase for HGB (12.29±2.27 g/dL), WBC (9.86±2.14 10^3/µL), MCH (18.14±1.37 pg), MO (5.21±2.00%) and high increase for NE (48.84±7.86%) mean values. Mahmood et al. (2013) related means values of 6.73±1.28 10^6/µL for RBC, 11.20±2.06 g/dL for HGB, 31.69±5.39% for HCT, 47.97±4.03 fl for MCV, 17.39±1.73 pg for MCH, and 36.02±0.97 for MCHC. Shahzadi et al. (2014) reported the following values in lactating buffaloes: 5.38±0.25 10^6/µL for RBC, 9.94±0.47 g/dL for HGB, 8.32±0.78 10^3/µL for WBC, 27.17±1.27 % for HCT, 51.75±2.08 fl for MCV, 19.85±0.63 pg for MCH, and 37.85±0.45 for MCHC. The obtained values for the coefficient of variation, for MCV and MCHC, were below that critical threshold of 10%, indicated a very homogeneous population, in cows. The coefficient of variation calculated for RBC, HGB, HCT, MCH, and LY was lower than 20%, expressing a homogeneous population. For the WBC and NE, the coefficient of variation was 27.12%, respectively 23.15%. However, for the MO, the coefficient of variation was 39.97% expressing a heterogeneous population. The coefficient of variation calculated for MCV, MCH, MCHC was lower than 10%, expressing a very homogeneous population, in buffaloes. For the RBC, HGB, HTC, WBC, LY, and NE the coefficient of variation was lower than 20%, expressing a homogeneous population. For the MO, the coefficient of variation was 38.39% expressing a heterogeneous population. Results of the urinalysis in lactating dairy cows and buffaloes are shown in Table 3.

Table 3. Urine parameters in lactating dairy cows and buffaloes (twenty heads/group)

Parameters	Group I Cow	Group II Buffalo	Reference limits	References values	
				Kim et al., 2010	Zanetti et al., 2008
Bilirubin, mg/dL	Negative	Negative	Negative	Negative	
Urobilinogen, mg/dL	Normally	Normally	Normally	Negative	
Ketones, mg/dL	Negative	Negative	Negative	Negative	
Glucose, mg/dL	Negative	Negative	Negative	Negative	
Protein, mg/dL	30	30	Negative	Negative	
Blood, Ery/µL	Negative	Negative	Negative	Negative	
pH	7-8	7-8	5-7	7.0-8.4	
Nitrite	Negative	Negative	Negative	-	
Leukocytes, Leu/µL	Negative	Negative	Negative	Negative	
Specific gravity	1.020-1.030	1.010-1.025	1.015-1.025	1.020-1.040	

Urine examination of healthy cows and buffaloes showed that all the parameters were within the normal physiological limits with minor fluctuations for urine proteins (30 mg/dL). The obtained results are in agreement with the obtained results by Kim et al., 2010 and Zanetti et al., 2008. Normally, no bilirubin is detectable in the urine. Values of 0.5-1 mg/dL bilirubin are indicated. Higher values of bilirubin are associated with early liver damage

or disease. Urobilinogen is normally present in the urine in small quantity. Concentrations of > 2 mg/dL are considered to be pathological. Raised levels may be due to cirrhosis, hepatitis, hepatic necrosis and pernicious anaemia. The presence of ketones in urine is called ketonuria. Normally, the urine is free of ketones. Animals in late pregnancy and early post parturition may develop ketosis (pregnancy toxaemia), a severe and sometimes fatal disorder. Ketonuria occurs in diabetes mellitus and starvation, vomiting and diarrhoea may also result in ketosis. Values of 50 mg/dL acetone are indicated. Glucose cannot be detected in the urine although small amounts are secreted by the healthy kidney. The presence of urine glucose is called glucosuria. Pathologic glucosuria is associated with milk fever in cattle. Values of 40 mg/dL glucose are indicated. Protein in urine is most frequently evaluated, which primarily assesses the albumin content. Normally, no protein is detectable in the urine (Radostis et al., 2008). The presence of protein in urine is called proteinuria. Pathological causes of proteinuria include renal disease, urinary tract infections and haematuria. Many diseases can contribute to proteinuria because the inflammatory response can cause glomerulonephritis (Darling et al., 2009). Proteinuria may result from glomerulonephropathy, tubular transport defects, inflammation or infection within the urinary tract. The increased protein level in the urine might be due to acute nephritis or inflammatory exudation resulting from pyelitis, urethritis, cystitis and urolithiasis (Naghy, 2009). Values of 15 mg/dL albumine are indicated. Blood (erythrocytes) in the urine can be associated with haematuria. Values of approximative 5 erythrocytes/µL are indicated.
Urine pH is a measurement of the kidneys ability to conserve hydrogen ions. It is influenced by diet, recent feeding, bacterial infection, metabolic alkalosis, and urinary retention. The urinary pH in normal cattle is usually on the alkaline side and may range from 7.4 to 8.4 (Mavangira et al., 2012). In bovine obstructive urolithiasis, urine is usually alkaline (Sharma et al., 2006). Nitrites are formed by the breakdown of urinary nitrates. The presence of nitrites suggests bacterial infection such as *Escherichia coli*, *Staphylococcus* and *Klebsiella*. Values of 0.05-0.1 mg/dL nitrite are indicated. Urine of healthy animal's do not contain any leucocytes. Leucocytes in the urine are associated with urinary tract infections. Values of 10-20 leuocytes/µL are indicated. Specific gravity is a valuable test for renal disease (Parrah et al., 2013). Urine with a specific gravity outside the range 1.020-1.040 suggests alteration by the renal tubules. High specific gravity (>1.045) is associated with nephrotic syndrome, dehydration, acute glomerulonephritis, heart failure, liver failure. Low specific gravity (<1.005) is associated with diabetes insipidus, nephrogenic diabetes insipidus, acute tubular necrosis, or pyelonephritis (Kraft and Dürr, 2005). Specific gravity in health varies with the level of hydration and fluid intake. The range of specific gravity of urine in normal cattle is between 1.025-1.045 with an average of 1.035 (Kannan and Lawrence, 2010), and in obstructive urolithiasis it ranges from 1.008 to 1.025 (Braun, 2006).

CONCLUSIONS

The obtained data revealed significant differences for most blood parameters ($p<0.01$) studied. Urine examination of lactating dairy cows and buffaloes showed that all the parameters studied were within the normal physiological limits, with minor fluctuations for protein level (30 mg/dl). These differences are likely due to different feeding habit, diet and metabolism. The obtained results could serve as baseline information for comparison in conditions of nutrient deficiency, physiology and health status of dairy herd's. Monitoring the health of farm animal's is useful to the assessment of animal body status health and welfare. The changes in blood and urine constituents can reflect the physiological condition, nutritional and pathological status of dairy herds.

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REFERENCES

Afolabi, K. D., Akinsoyinu, A. O., Olajide, R., Akinleye, S. B. (2010). Haematological parameters of the Nigerian local grower chickens fed varying dietary levels of palm kernel cake, *Proceedings of 35th Annual Conference of Nigerian Society for Animal Production*, p.247.

Agarwal, N., Kamra, D. N., Chatterjee, P. N., Kumar, R., Chaudhary, L. C. (2008). *In vitro* methanogenesis, microbial profile and fermentation of green forages with buffalo rumen liquor as influenced by 2-Bromoethanesulphonic acid, *Asian Australasian Journal of Animal Sciences,* 21, 818-823.

Braun, U. (2006). Ultrasound as a decision- making tool in abdominal surgery in cows, *Veterinary Clinics of North America Food Animal Practice*.

Darling, A. L., Millward, D. J., Torgerson, D. J., Hewitt, C. E., Lanham. S. A. (2009). Dietary protein and bone health: a systematic review and meta-analysis, *American Journal Clinical Nutrition*, 90, 1674-1692.

Directive 2010/63/EU *regarding handling and protection of animals used for scientific purposes*, OJEU L 276/33 IA.

Doyle, D. (2006). William Hewson (1739-74). The father of haematology, *British Journal of Haematology*, 133(4), 375-381.

Finco, D. R. (1997). Kidney function. In: *Clinical Biochemistry of Domestic Animals,* Edt. By Kaneko, J. J., Harvey, J. W. and Bruss, M. L. New York, USA: Academic Press Publishing House, pp 441-484.

Garkal, R. A., Kekan, P. M., Kharde, S. D., Munde, V. K., Narladkar, B. W., Vaidya, M. S. (2016). Effect of Different Housing Systems On Haematological Parameters of Buffaloes, *Buffalo Bulletin*, 35(2), 191-197.

George, J.W., Snipes, J., Lane, V.M. (2010). Comparison of bovine hematology reference intervals from 1957 to 2006, *Veterinary Clincal Pathology,* 39, 138-148.

Kannan, K.V.A., Lawrence, K.E. (2010). Obstructive urolithiasis in a Sannen goat in NewZealand, resulting in a ruptured bladder, *New Zealand Veterinary Journal*, 58(5), 269-271.

Kim, N. H., Jeong, J. S., Kwon, H. J., Lee, Y. M., Yoon, H. R., Lee, K. R., Hong, S.P. (2010). Simultaneous diagnostic method for phenylketonuria and galactosemia from dried blood spots using high-performance liquid chromatography pulsed amperometric detection, *Journal of Chromatography B*, 878(21), 1860–1864.

Knaus, W. (2013). Re-thinking dairy cow feeding in light of food security. *AgroLife Scientific Journal*, II (1), 36-40.

Kraft, W., Dürr, U. M. (2005). Klinische Labordiagnostik in der Tiermedizin (Clinical laboratory diagnostics in veterinary medicine), *6th edition Schattauer*, Stuttgart, Germany. pp.186- 203 and 483- 484.

Law no. 43/2014 regarding *handling and protection of animals used for scientific purposes*, OM no.326/Parth I.

Mahmood, A., Khan, M. A., Younus, M., Khan, M. A., Ahad, A., Ahmad, M., Iqbal, H. J., Fatima, Z. andAnees, M. (2013). Haematological and Biochemical Risk Factors of Parturient Haemoglobinuria in Buffaloes, *Journal of Animal and Plant Sciences*, 23, 364-368.

Mavangira, V., Cornish, J. M., Angelos, J.A. (2012). Effect of ammonium chloride supplementation on urine pH and urinary fractional excretion of electrolytes in goats, *Journal of American Veterinary Medical Association*, 11, 1299-1304.

Merck Manual (2012). Haematologic reference ranges, *Mareck Veterinary Manual*, (http://www.merckmanuals.com/).

Nagy, D. W. (2009). Urolithiasis in small ruminants Proceedings on *Animal feed Resources*, CVC in Kansas City.

Olafedehan, C. O., Obun, A. M., Yusuf, M. K., Adewumi, O. O., Oladefedehan, A. O., Awofolaji, A. O., Adeniji, A. A. (2010). Effects of residual cyanide in processed cassava peal meals on haematological and biochemical indices of growing rabbits, *Proceedings of 35th Annual Conference of Nigerian Society for Animal Production*, p.212.

Parrah, J. D., Moulvi, B. A., Gazi, M. A., Makhdoomi, D.M., Athar, H., Din, M.U., Dar S., Mir, A.Q. (2013). Importance of urinalysis in veterinary practice - A review, *Veteterinary World,* 6(9), 640-646.

Parvu, Gh., Costea, M., Pirvu, M., Nicolae, B. (2003). *Treaty by animal nutrition.* Bucharest, RO: Coral Sanivet Publishing House.

Radkowska, I., Herbut, E. (2014). Hematological and biochemical blood parameters in dairy cows depending on the management system, *Animal Science Papers and Reports*, 32(4), 317-325.

Radostitis, O. M., Blood, D. C., Gray, G. C., Hinchcliff, K.W. (2008). *A text book of the disease of cattle, sheep, pig, goat and horse*, London, UK: Veterinary Medicine, Bailliere Tindall Publishing House, p. 1877.

Research Animal Resource (2009). Reference values for laboratory animals: Normal, haematological values, RAR Websites, *RAR, University of Minnesota*, Retrieved from, http:// (www.ahc.umn.edu/rar/refvalues.html).

Roland, L., Drillich, M., Iwersen, M. (2014). Hematology as a diagnostic tool in bovine medicine, *Journal of Veterinary Diagnostic Investigation*, 26(5), 592-598.

Sharma, P. D., Singh, K., Singh, J., Kumar, A. (2006). Bacteriological, biochemical and histopathological studies in uroperitoneum in buffalo calves (Bubalus bubalis), *Indian Journal of Animal Sciences*, 76(2), 124-126.

Shahzadi, F., Iqbal, F., Aziz, T., Saleem, R., Abbas, M. N. (2014). Iron status and haematological profile of lactating and non-lactating buffaloes, *Journal of Global Innovations Agricultural Social Sciences*, 2(1), 28-30.

Terramoccia, S., Bartocci, S., Amici, A., Martillotti, F. (2000). Protein and protein-free dry matter rumen degradability in buffalo, cattle and sheep fed diets

with different forage to concentrate ratios, *Livestock Production Science,* 65, 185-195.

Togun, V. A., Oseni, B. S. A., Ogundipe, J. A., Arewa, T. R., Hammed, A. A., Ajonijebu, D. C., Mustapha, F. (2007). Effects of chronic lead administration on the haematological parameters of rabbits – a preliminary study, *Proceedings of the 41st Conferences of the Agricultural Society of Nigeria*, p. 341.

Varra, M., Kumar, T., Rao, T., Chakravarthi, M. (2017). Comparative Studies on Blood Biochemical Profile of Jersey Cross Bred Cows and Graded Murrah Buffaloes in Conditions of Green Fodder Scarcity and Climate Change, *International Journal of Livestock Research,* 7(12), 74-80.

Wood, D., Quiroz-Rocha, G.F. (2010). Normal hematology of cattle, In: *Schalm's veterinary hematology*, Wiley, Ames, IA, SUA: Weiss DJ, Wardrop KJ Publishing House, 6th ed., pp. 829-835.

Xie, L., Xu, F., Liu, S., Ji, Y., Zhou, Q., Wu, Q., Xie, P. (2013). Age and Sex Based Hematological and Biochemical Parameters for *Macaca fascicularis*, *PLoS ONE*, 8(6), doi:10.1371/journal.pone.0064892.

Zanetti, G., Paparella, S., Trinchieri, A., Prezioso, D., Rocco, F., Naber, K.G. (2008). Infections and urolithiasis: current clinical evidence in prophylaxis and antibiotic therapy, *Italiano di Urologia i Andrologia*, 80(1), 1-5.

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RESEARCH ON THE IMPACT OF THE ADDING THE APPLE VINEGAR IN RATION OF THE HEAVY LAMBS AND MONITORIZING THE RUMINAL pH AND AVERAGE DAILY GAIN

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Abstract

The study's objectives are based on the monitoring of ruminal pH and average daily gain (ADG) in Karakul of Botoşani lambs, following the addition of apple vinegar to ration. The study was conducted on a group of 90 weaned lambs. The ration consisted 30% chopped natural hay and 70% feed concentrated. The lambs were divided into 3 equal batches: control group (G1), experimental group (G2) at which ration was supplemented with 8 ml of apple vinegar/day/animal and experimental group (G3) was supplemented with 12 ml of apple vinegar/day/animal. As a result of study, was observed differences between the control group and the experimental groups. Thus at the batch G1 there was a daily average increase of 260 g, at the batch G2 290 g and at G3 310 g. Control group (G1) showed an average ruminal pH of 5,4 and experimental groups (G2 , G3) an average of 5,9. There are differences between the groups studied through variations of ruminal pH and ADG denoting that apple vinegar may have a potential influence.

Key words*: apple vinegar, lambs, pH.*

INTRODUCTION

Sheep have a physiological capacity for meat production, which is a source of protein to feed the people. Use of high-concentrate diets for feedlot lambs will boost their efficiency, as the ingredients that make up these diets typically have high concentrations of digestible constituents (INRA, 1988). The potential effect of the reduction in the availability of forage is catastrophic and, ultimately, livestock farmers rely on concentrates and grains as a major source of nutrients, which, in turn, reduces the profitability of sheep farming. In this sense, livestock farmers have started to use alternate feeds, by-products and certain additives in ruminant diets as a way of lowering feed costs (Yáñez-Ruiz, 2004). The vinegar has been used for domestic and cooking purposes for decades. In folk medicine, vinegars are known to be perfect for various forms of health problems. Vinegars are derived worldwide from various sources of carbohydrate, including cane, corn, rice, grapes, plums and other fruit juices (Barneveld, 1999). The famous and common vinegar of today's whole food world is apple vinegar (AV), which has been proclaimed to have several different health benefits. AV is produced from fermented apple juice, where the bacteria and the yeast convert the fruit sugars into ethanol cider and transform ethanol into acetic acid in a second fermentation phase (Barry and McNabb, 1999). Different theories have been suggested to clarify the possible health effects of AV (Archimède et al., 2010; Barneveld, 1999). Antiglycemic properties may be due to slower gastric emptying, reduced disaccharidase activity, enhanced insulin sensitivity, or increased glycogen production (Ogimoto and Imai, 1981). Acetic acid is the dominant organic acid in AV. Succinic, ascorbic, formic, citric and oxalic acids are other acids present in AV (Barneveld, 1999). The following processes have been proposed to explain the lowering effects of acetic acid on blood glucose levels, blood lipids, and BW: tardy gastric emptying, decreased disaccharidase activity, stimulation of

adenosine monophosphate-activated protein kinase (AMPK) pathway, increased insulin secretion, enhanced postprandial satiety, and decreased energy intake (Newbold et al., 2015). A broad variety of feed additives has been researched and implemented for the modulation of rumen fermentation in the production of sheep. In addition to supplying direct nutrients, such as rumen-protected lysine, supplementation of AV was a key nutritional approach to modifying rumen function and animal performance (Chai et al., 2015; Patra and Saxena, 2011). Use of high-concentrate diets, primarily grains, is increasingly frequent in ruminant feed to increase body weight. Nevertheless, this form of diet includes carbohydrates that are readily fermentable in rumen and raise the risk of disorders such as ruminal acidosis and hepatic abscesses (Abarghue et al., 2010; Dehority, 1984; Yáñez-Ruiz, 2004). This disease is primarily attributed to the excessive accumulation of organic acids in rumen, which causes a decrease in ruminal pH (< 5.8) and lesions in the gastrointestinal barrier (Marie-Magdeleine et al., 2014; Martinele et al., 2014). Ruminal acidity contributes to the death of the Gram-negative bacteria with the resulting release of endotoxins (lipopolysaccharides, LPS), affecting the productive performance of the animal (Haro et al., 2019; Kamra, 2005).

MATERIALS AND METHODS

Animals and experimental design

The experiment was carried out between April and June (2019) at a private farm from the village Albesti, county Botosani, Romania. A total of 90 weaned lambs, Karakul of Botoşani with average 25.5 kg body weight (bw) were evaluated. The animals were divided in three groups; each provided with food and water, which consisted in various levels of apple vinegar. In pre-experimental period, the animals were respectively numbered with an ear tag and then subject to the control of endo- and ectoparasites with doramectin at 0.3 mg kg^{-1} and immunized for clostridiosis (Covexin®9). The experimental period lasted 70 days, with 30 days of adaptation and 40 days of the experiment period.

Forage chemical analysis

The determination of TMR (total mixed ratio), was realized with the NIR Analyzer (Perten DA 7200). The diets were formulated supplying the nutritional requirements for sheep within the 20-30 kg weight range for daily gains of over 250 g according to the recommendations of NRC (2007). The TMR was made up of chopped natural hay (30%), corn grain (58.8%), soybean meal (8%), dicalcium phosphate (0.05%), limestone (1.9%), bicarbonate (0.75%) and premix mineral (0.5%). The remnants of food were collected daily and evaluated for chemical composition. Control group (G1) only benefited from TMR, experimental group (G2) the ration was supplemented with 8 ml of apple vinegar/day/animal and experimental group (G3) the ration was supplemented with 12 ml of apple vinegar/day/animal. In every tenth day of experimentation period all animals were weighed for average daily gain (ADG) determination and ruminal fluids (cca 20 ml) were obtained 2-4 h after the morning feeding, from three slaughter lambs randomly selected from each treatment. Each container with ruminal fluid was sealed, labelled and transported to the laboratory, where the pH was analyzed with the InoLab pH-meter. To determine body weight, we used a weighing "Platform scale". Lambs were weighed at the beginning and end of the experimental phase after a solid-feed starvation duration of 12 h (water available ad libitum). The average daily gain (ADG) was determined as the total weight gain (TWG) over the feedlot duration divided by the number of days of the experiment.

Statistical analysis

The results were analysis the ruminal pH and average daily gain means were compared with the Tukey test. Statistical calculations were performed with the IBM SPSS V.22 software.

RESULTS AND DISCUSSIONS

During the 40 days of experiment, four determinations were made (the ruminal pH and the body weight were analyzed four times), the observed results being recorded in Table 1 and Figure 1.

Table 1. Average daily gain determination

	First determination (tenth day)		Second determination (twentieth day)		Third determination (thirtieth day)		Fourth determination (fortieth day)		
ELEMENT	**Mean of AVG (g)**	**Standard deviation**	**Mean of AVG (g)**	**Standard deviation**	**Mean of AVG (g)**	**Standard deviation**	**Mean of AVG (g)**	**Standard deviation**	**p-value**
Group 1	*255*	*10.22*	*263*	*10.1*	*264*	*12.46*	*261*	*9.61*	*0.02*
Group 2	*281*	*7.93*	*299*	*9.45*	*286*	*8.5*	*294*	*8.45*	*0.013*
Group 3	*306*	*8.32*	*316*	*9.89*	*308*	*8.39*	*310*	*7.9*	*0.011*

As can be seen in Table 1, at the first analysis, performed on the tenth day of experiment, a mean of average daily gain of 255 g was recorded in the G1 group, 281 g in the G2 group and 306 g in the G3 group, these values were similar to those previously reported by Chai et al. (2015). Thus, significant differences are observed regarding the average daily gain, between the three groups taken in the study right from the first analysis of the results. The greater ADG in lambs from G2 and G3 group results in the first determination. At the second determination, a mean of average daily gain of 263 g in G1, 299 in G2 and 316 in G3 was observed. On the thirtieth day of the experiment, a mean of ADG of 264 g was recorded in the control group, 286 in G2 and 308 in G3. Significant differences between the three groups taken in the study are also observed in the last determination, made on the fortieth day of the experiment.

As an ultimate result of study, was observed differences between the control group and the experimental groups. Thus at the batch G1 there was a daily average increase of 260 g, at the batch G2 290 g and at G3 310 g. Values for these parameter were in the range previously reported by others for lambs which was fed with high-cereal concentrates and slaughtered at about 25-30 kg BW (Archimède et al., 2010; Detmann et al., 2012; Patra and Saxena, 2011). In the current study, the two groups had comparable initial BW; however, final BW and ADG was greater in the groups G2 and G3 compared with the control group, indicating that lambs fed on the AV were more efficient in converting nutrients to growth.

Ruminal pH is an important indicator of the status of the ruminal microbial ecosystem in ruminants (Guyader et al., 2014).

The pH of the rumen liquid sampled in this study varied from 5.2 to 6.24, these values being within below the normal physiological range of 6.5-6.7 as outlined by Van Soest (1994). In the control group, which did not benefit from AV, a lower pH is observed than in the other two groups. This low pH level (in G1) can lead to ruminal acidosis and as can be seen in Figure 1, AV acted as a buffer against lowering the pH, keeping it within normal limits.

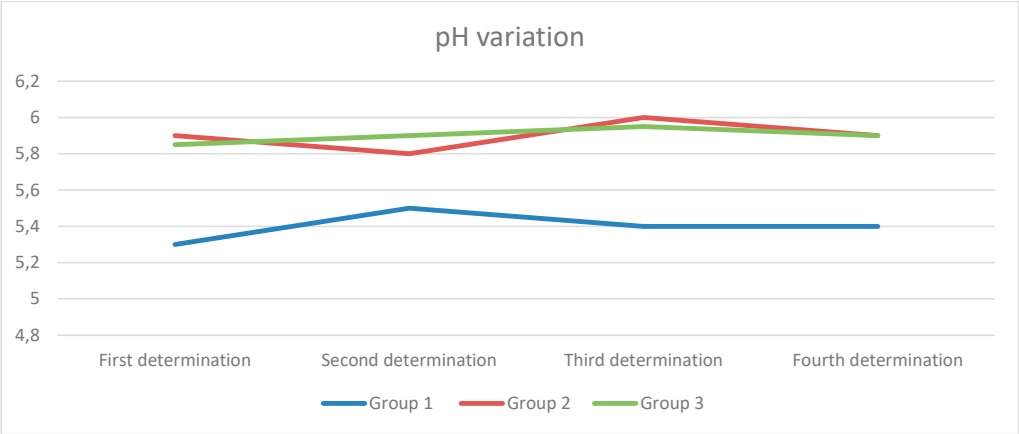

Figure 1. Ruminal pH variation in the three groups

Guyader et al. in 2014 indicated an optimum ruminal pH value for methanogen growth is between pH 6.0 and 7.5, with the maximum growth rate of this microorganism occurring at near-neutral pH, and a decrease in ruminal pH results in slower methanogen growth and reduced activity. In the cases of groups G2 and G3, which benefited from 8 ml of apple vinegar/day/animal, respectively 12 ml of apple vinegar/day/animal, it is observed that the ruminal pH remained constant between 5.8-6, values considered normal by other researchers (Göçmen et al., 2001; Heuzé et al., 2016).

CONCLUSIONS

In conclusion, when opting for the administration of a high proportion of concentrates, in order to fatten lambs, AV contributes significantly to improving the average daily gain by regulating the ruminal pH, maintaining ruminal health. Ruminal pH has a wide variety of effects on rumen physiology and fermentation, include methanogenesis. Ruminal pH is the result of interactions between the production of organic acids from microbial fermentation of feed, the bicarbonate flow into the rumen from saliva and the secretion through the ruminal epithelium, the absorption and passage of SCFA (short-chain fatty acid) and probably the absorption of ammonia. There were small differences between groups G2 and G3, so we can conclude that an amount of 8-12 ml of apple/day/animal vinegar is optimal for maintaining the ruminal environment under normal conditions. So we can see that apple vinegar has a significant potential to regulate ruminal pH and keep pH at normal values, preventing the occurrence of possible rumen disorders. The lambs in the groups that benefited from the AV supplement (G2 and G3) performed better than the animals in the control group, with ADG having clearly superior results.

REFERENCES

Abarghue, M. J., Rouzbehan, Y., Alipour, D. (2010). The influence of the grape pomace on the ruminal parameters of sheep. *Livestock Science*, 132, 73–79.

Archimède, H., González-García, E., Despois, P., Etienne, T., Alexandre, G. (2010). Substitution of corn and soybean with green banana fruits and Gliricidia sepium forage in sheep fed hay-based diets: effects on intake digestion and growth. *Journal of Animal Physiology and Animal Nutrition*, 94, 118–28.

Barneveld, S. L. (1999). Chemical and physical characteristics of grains related to variability in energy and amino acid availability in ruminants: a review. *Australian Journal of Agricultural Research*, 50, 650–660.

Barry, T. N., McNabb, W. C. (1999). The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. *British Journal of Nutrition*, 81, 263–272.

Chai, J., Diao, Q., Wang, H., Tu, Y., Tao, X., Zhang, N. (2015). Effects of weaning age on growth, nutrient digestibility and metabolism: And serum parameters in Hu lambs. *Anim. Nutr*., 1, 344–348.

Dehority, B. A. (1984). Evaluation of subsampling and fixation procedures used for counting rumen

protozoa. *Applied and Environmental Microbiology*, 48, 182–185.

Detmann, E., Souza, M. A., Valadares Filho, S. C. (2012). Métodos para Análise de Alimentos - INCT - Ciência Animal. Visconde do Rio Branco, Minas Gerais. 214 p.

Göçmen, B., Dehority, B. A., Talu, G. H., Rastgeldy, S. (2001). The rumen ciliate fauna of domestic sheep (Ovis ammon aires) from the Turkish Republic of Northern Cyprus. *The Journal of Eukaryotic Microbiology*, 48, 455–459.

Guyader, J., Eugene, M., Noziere, P., Morgavi, D. P., Doreau, M., Martin, C. (2014). Influence of rumen protozoa on methane emission in ruminants: a meta-analysis approach. *Animal*, 8, 1816–1825.

Haro, A. N., González, J., de Evan, T., de la Fuente, J., Carro, M. D. (2019). Effects of feeding rumen-protected sunflower seed and meal protein on feed intake, diet digestibility, ruminal and cecal fermentation, and growth performance of lambs, 415 [CrossRef].

Heuzé, V., Tran, G., Archimède, H. (2016). Banana leaves and pseudostems. Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. http:// www.feedipedia.org/node/686 Last updated on March 25, 2016, 11: 24.

INRA (1988). Alimentation des Bovins, Ovins et Caprins. INRA Paris.

Kamra, D. N. (2005). Rumen microbial ecosystem, *Current Science*, 89, 125–135.

Marie-Magdeleine, C., Udino, L., Philibert, L., Bocage, B. and Archimede, H. (2014). *In vitro* effects of *Musa paradisiaca* extracts on four developmental stages of *Haemonchus contortus*. *Research in Veterinary Science*, 96, 127–132.

Martinele, I., Santos, G. R. A., Matos, D. S., Batista, A. M. V., D'Agosto, M. (2008). População de protozoários de ovinos mestiços mantidos em pastagens naturais de caatinga. *Revista Brasileira de Saúde e Produção Animal*, 9, 280–292.

National Research Council (NRC) (2007). Nutrient requirements of small ruminants: sheep, goats, cervids and new world camelids, 6th edition. National Academy Press, Washington, District of Columbia.

Newbold, C. J., de la Fuente, G., Belanche, A., Ramos-Morales, E., McEwan, N. R. (2015). The role of ciliate protozoa in the rumen. *Frontiers in Microbiology*, 6, 1313. doi: 10.3389/fmicb.2015.01313

Ogimoto, K., Imai, S. (1981). Atlas of rumen microbiology. Tokyo: Japan Scientific Societies, 231.

Patra, A. K., Saxena, J. (2011). Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *Journal of the Science of Food and Agriculture*, 91, 24–37.

Van Soest, P. J. (1994). Nutritional Ecology of the Ruminant, 2nd ed. Ithaca, NY, USA: Cornell University Press Publishing House.

Yáñez-Ruiz, D. R., Moumen, A., Martín García, A. I., Molina Alcaide, E. (2004). Ruminal fermentation and degradation patterns, protozoa population, and urinary purine derivatives excretion in goats and wethers fed diets based on two-stage olive cake: effect of PEG supply. *Journal of Animal Science*, 82, 2023–2032.

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MEAT QUALITY OF BREAST FROM BROILERS FED A DIET SUPPLEMENTED WITH ORANGE AND RED GRAPEFRUIT DRIED PEEL

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Abstract

Utilization of agricultural wastes and residues resulted from food industry in animal nutrition is a matter of great concern nowadays. Dried citrus peel (DCP) and flax meal (FM) are potential sources of some valuable bioactive nutrients for animals and poultry. This experiment was conducted to evaluate the effects of dietary supplementation of FM used together with two different DCP: dried orange peel (DOP) and dried red grapefruit peel (DRGP) on few breast meat quality traits of broiler chickens. One hundred-twenty broiler chicks (1 day old) were randomly allocated to three groups for the starter phase (14 d) where they were fed with a standard diet. At day 14, they were individually weighed (average 440.77 g) and homogenous assigned to three dietary treatments comprised of: basal diet (C) with 4% FM, basal diet supplemented with 2% DOP and 4% FM (DOP) and basal diet supplemented with 2% DRGP and 4% FM (DRGP), for both grower and finisher phases. At the end of the experiment (d 42) 6 broilers chicks/group were slaughtered and samples of breast meat were collected, and assayed for chemical composition, texture profile analysis (TPA), color parameter and fatty acid composition. Results of the present study indicated that use of FM together with DOP and DRGP in broiler diets significantly ($P < 0.05$) improved the color and some texture parameters and didn't affected the chemical composition or fatty acids from raw chicken breast meat.

Key words*: food, fatty acids, citrus peel, texture parameters, meat color.*

INTRODUCTION

In recent years there has been an increasing social and ecological pressure for the efficient reuse of residues from the food and agricultural industry (Pfaltzgraff et al., 2013) due to the global intensification of food production leading to the creation of large quantities of co-products and food waste (Waldron, 2007). The use of secondary agro-industrial by-products in the animal feed industry reduces the environmental impact and improves profitability and capitalization of agricultural by-products, since feeding food residue to livestock is an efficient way to upgrade low quality materials into high quality foods (Elferink et al., 2008). They are in line with current legislation that strongly encourages the food industry to find new uses for the resulting by-products (Panouillé et al., 2007). Moreover, these unwanted materials represent a potential serious pollution problem, a loss of biomass and valuable nutrients (Laufenberg et al., 2003). In addition, industrial ecology and circular economy are considered leading principles for Eco-innovation, focusing on a *"zero waste"* society and economy, where waste can be used as raw materials (Mirabella et al., 2014). A number of agro-industrial by-products are generated from fresh *Citrus* fruits, after the main products of interest have been removed or extracted during processing or peeled for direct human consumption (Oluremi et al., 2007). Orange is a *Citrus* fruit consumed in large quantities worldwide in the peeled form and juice. During the production of orange juice, large quantities of residues and wastes (peel, pulp, seeds and whole orange fruits that do not meet the quality requirements) are generated (Rezzadori et al., 2012). The same process takes place in the case of grapefruit. These wastes are generally available in large quantities during the *Citrus* season and can therefore cause an environmental problem because they have no productive use. From a nutritional point of view, oranges are one of the

most abundant sources of vitamin C, carotenoids, flavonoids, essential oils, sugar, fiber and some minerals (Niu et al., 2008). Also, due to they're bioactive phytochemicals, grapefruit has health promoting properties (Chudnovskiy et al., 2014), flavonoids being considered the most important bioactive components present in grapefruit. Because of these abundant carotenoids, they have a positive effect on meat color and on some meat quality parameters when texture profile analysis (TPA) of the meat is tested. Regarding the flax meal it is already very well known that has an increased concentration of polyunsaturated fatty acids, especially α-linolenic acid (Vlaicu et al., 2017). When included in animal feeds, alone or in combination with other residues/wastes/meals it can improve the final product quality (Vlaicu et al., 2017b).

In this context, the present paper presents the potential usage of orange and red grapefruit peel in combination with flax meal in diet of broilers, regarding their effect on the raw breast meat color, texture profile and fatty acids composition.

MATERIALS AND METHODS

Experimental procedures were approved by the Ethical Committee of the National Research and Development Institute for Biology and Animal Nutrition, in accordance with the Romanian legislation (Law 206/2004, ordinance 28/31.08.2011, law 43/11.04.2014, Directive 2010/63/EU).

Materials. Fresh citrus fruits (oranges and grape fruits) were purchased from a local market in Bucharest, Romania. The juice was manually extracted with a press and the remained peels were chopped with a knife and spread on the floor at sun for drying. After drying, the peels were milled to the powder in a hammer mill with a 1-mm screen and stored in eremitic bags at room temperature until used.

Animals, diets and experimental design. A total of 120-day-old Ross 308 broiler chicks were purchased from a local hatchery. The broiler chicks were housed in an experimental hall with floor rearing, under controlled microclimate. They were reared on permanent litter of wood shaves (10-12 cm thick). The light regimen was according to the breeding guide, 23 h light/1 h dark. The day-old chicks were fed during the starter period (1-14 days) with a compound feed with corn, soybean meal and gluten, having 20.56% crude protein and 3140.03 kcal/kg metabolizable energy. At 14 days of age, birds were weighed individually and randomly divided into three equal treatments as follows: commercial control diet supplemented with 4% flax meal (C), a diet containing 4% flax meal and 2% dried orange peel (DOP), and a diet containing of 4% flax meal and 2% dried red grapefruit peel (DRGP). Diet was composed to meet the requirements suggested by the National Research Council (1994). The experimental feed and clean drinking water were available *ad libitum* throughout the experimental period.

Table 1. Ingredients of commercial diet for grower and finisher phases (15-42 days)

Ingredient, % as fed-basis	Grower	Finisher
	%	
Corn	35.50	39.34
Flax meal	4.00	4.00
Wheat	20.00	20.00
Soy meal	27.03	20.81
Gluten	4.00	6.00
Oil	4.20	4.70
Monocalcium phosphate	1.54	1.45
Calcium carbonate	1.40	1.33
Salt	0.36	0.33
Methionine	0.29	0.26
Lysine	0.41	0.48
Choline	0.05	0.05
Threonine	0.22	0.25
Premix for broilers	1.00	1.00
Total	**100.00**	**100.00**
Calculated analysis, %		
Metabolizable energy, kcal/kg	3126,76	3215,78
Crude protein, %	21.5	20
Crude fat, %	6.34	6,86
Lysine, %	1.29	1.19
Methionine, %	0.63	0.39
Methionine + cysteine, %	0.99	0.94

*DCP were added to basal diet at the expense of ground corn in the experimental diets.

**1 kg premix vitamin-mineral contains: = 1.350.000 IU/kg vit. A; 300.000 IU/kg vit. D3; 2700 IU/kg vit. E; 200 mg/kg Vit. K; 200 mg/kg Vit. B1; 480 mg/kg Vit. B2; 1485 mg/kg pantothenic acid; 2700 mg/kg nicotinic acid; 300 mg/kg Vit. B6; 4 mg/kg Vit. B7; 100 mg/kg Vit. B9; 1.8 mg/kg Vit. B12; 2500 mg/kg Vit. C; 7190 mg/kg manganese; 6000 mg/kg iron; 600 mg/kg copper; 6000 mg/kg zinc; 50 mg/kg cobalt; 114 mg/kg iodine; 18 mg/kg selenium; 50 g sodium monensin/kg.

By using a completely randomized design, the experiment was conducted on 40 birds per group. At the end of the trial, according to the experimental protocol, 6 broiler chicks/group

were slaughtered and samples of breast were collected in order to determine the proximate composition, color parameters, texture profile analysis (TPA) and the fatty acids composition in the raw breast chicken meat.

Proximate composition. The basic chemical composition analyses were determined on samples dried at 65^0C. Standardized methods complying with Regulation (CE) 152/2009 (Sampling and analytical methods for the official inspection of feeds) and ISO standards were used to determine the nutrient concentration. The dry matter (DM) was determined with the gravimetric method according to Regulation (CE) nr. 152/2009 and standard SR ISO 6496:2001; the crude protein (CP) was determined by the Kjeldahl method according to Regulation (CE) nr. 152/2009 and standard SR EN ISO 5983-2:2009; the crude fat (EE) was determined by extraction in organic solvents - the method complies with Regulation (CE) nr. 152/2009 and standard SR EN ISO 6492:2001; the meat ash was determined by calcinations at 5500 C (SR ISO 936, 2009).

Color determination. The breast raw meat color was measured by means of the CIE-Lab method, where L* (100 = white, 0 = black) represents the brightness, and a* (+ red, - green) and b* (+ yellow, - blue) are color parameters. The determinations were performed using the Konica Minolta Chroma Meter CR-400 device. Lightness (L*), redness (a*), and yellowness (b*) values were obtained using an average value from three repeated measurements taken at different locations on the surface of the meat.
Negative a* and b* values indicate the appearance of green and blue color of the meat.
The instrument was calibrated with a white calibration before the measurements. The total color difference ΔE^* was determined with the next formula:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where:

$$\Delta L^* = L^*_{\text{sample}} - L^*_{\text{control}}$$
$$\Delta a^* = a^*_{\text{sample}} - a^*_{\text{control}}$$
$$\Delta b^* = b^*_{\text{sample}} - b^*_{\text{control}}$$

L* = luminosity; a* = saturation index in (green/red) and b* = saturation index in (blue/yellow).

Warner-Bratzler shear test. This test measures the force (Newtons) necessary to shear a piece of meat. The analyses were carried out on raw chicken breast samples. The meat samples were cut from the breast so that the cross-section measures 20 mm and the muscle fibers are parallel to the length of the sample which was at least 40 mm. The cutting/shearing plane is perpendicular to the muscle fibers. Cutting/shearing test allows to determine the hardness (firmness) of the tissue. Parameter L that is measured is the maximum shear force (highest point on the curve) (N) which indicates the maximum shear strength of the sample. Load cell: 10 kg; Warner-Bratzler shear blade with a triangular slot cutting edge (triangular shear devices). Setting parameter: sample height: 50 mm, starting distance from sample: 5 mm, compression, 60 mm, initial speed: 1.5 mm/s; test speed: 1.5 mm/s; retract speed, 10 mm/s; trigger force, 40 g; date rat: 200 pps. The parameter recorded was the maximum shear force, that is the highest peak of the curve, which is the maximum resistance of the sample to shearing. Each sample was assessed 3 times.

Texture Profile Analysis (TPA). The TPA test simulates the biting action in the mouth. It consists of a 2-cycle compression test. Here the sample should have a smooth level surface with a diameter smaller than the flat faced cylindrical probe. This test gives the textural parameters of tenderness (hardness), adhesiveness, springiness, cohesiveness, chewiness and gumminess. It was determined by using a texture analyzer, Perten TVT 6700 texturometer, from Perten Instruments and a TexCalc 5 software. The TPA parameters of raw chicken breast meat was evaluated as described by Bourne (1978). Three cylinders of 10 mm height and 10.30 mm diameter were prepared from every sample. A double compression cycle test was performed up to 50% compression of the original portion height with a stainless-steel cylinder probe of 20 mm diameter. A time of 5 s was allowed to elapse between the two compression cycles. Force-

time deformation curves were obtained with a 10 kg load cell applied at a cross-head, speed of 2 mm/s. The data obtained from TPA curve were used for the calculation of textural parameters. Among the TPA parameters, hardness (N) is expressed as maximum force for the first compression. Adhesiveness (N × s), is expressed as negative force area for the first bite or the work necessary to pull the compressing plunger away from the sample. Springiness (m), ability of the sample to recover its original form after deforming force was removed and is calculated as the ratio of the time from the start of the second area up to the second sample reversal over the time between the start of the first area and the first sample reversal. Cohesiveness is a measure of the degree of difficulty in breaking down the samples internal structure. Gumminess and chewiness have been reported as products of hardness, cohesiveness. Chewiness is calculated as hardness × cohesiveness × springiness. Resilience reflects the re-deformation capacity of samples tissue after penetration.

Fatty acid composition. Fatty acids composition from meat samples was determined by gas chromatography. After lipid extraction from the samples, the fatty acids were transformed into methyl esters by transmethylation, and the components were separated in the capillary chromatograph column. The fatty acids (FA) were determined by gas chromatography by transforming the fatty acids from the sample in methyl esters, followed by component separation in capillary column, identification by comparison with standard chromatograms and quantitative determination of the fatty acids according to SR CEN ISO/TS 17764 -2: 2008, using Perkin Elmer Clarus 500 gas chromatograph, with capillary column injection system, high polarity stationary phase (BPX70: 60 m x 0.25 mm inner diameter and 0.25µm thick film).

Statistical analysis. The analytical data were compared by variance analysis (ANOVA) using Stat View for Windows (SAS, version 6.0). The difference between the means was considered significant at $P<0.05$. The results were expressed as mean ± standard deviation.

RESULTS AND DISCUSSIONS

In order to determine the quality of the raw chicken meat, the breast samples were analyzed to determine their proximate chemical composition (Table 2). From the data obtained it can be observed that the samples collected from group DRGP (4% FM and 2% DRGP), had the highest concentration of fat, being with 32.19% higher than group C and with 26.88% higher than DOP group. The same differences were also found in the analysis of DM, CP and Ash. Mourão et al. (2008) determined the chemical composition of some carcass traits by using 5% *Citrus* peels and he obtained similar values regarding the CP (22.90%) and DM (33.20%) in raw chicken meat. According to same authors, when the *Citrus* supplement was increased at 10% in the broilers diet, the results of proximate analyses had higher values.

Table 2. Proximate composition of raw breast meat

Item	C	DOP	DRGP
	g/100 g DM		
DM	25.79	26.43	27.40
OM	24.72	25.24	26.18
CP	22.56	22.87	23.29
EE	2.17	2.34	3.20
ASH	1.07	1.19	1.21

Where: DM = dry matter; OM = organic matter; CP = crude protein; EE - ether extractives; Ash.

Color parameters of the raw breast meat samples are shown in Table 3. Incorporation of DOP and DRGP in broiler diets had a significant impact on the color profile. Color parameter L* measured for chicken breast meat shows insignificant color variations in experimental groups compared to C group. The luminosity of the chicken breast meat from group DRGP, where the feed was supplemented with 2% grapefruit peel, decreased ($P<0.05$) significantly compared to that of group C. A significant increase ($P>0.05$) of the luminosity compared to the value of group C occurred in DOP where the chicken feed was supplemented with orange peels. The increase of the parameter a* in the DOP group indicates the intensification of the red color of the breast meat. Also, a significant decrease in yellow (b*) shows the chicken breast meat from DRGP group, followed by DOP group.

Table 3. Color parameters of the raw breast chicken meat samples in relation to the DCP added in broiler diet

Group	L*	a*	b*	ΔE*
C	54.34 ± 4.09[a]	2.22 ± 2.37[a]	13.44 ± 2.49[a]	0.00
DOP	54.01 ± 1.19[a]	2.61 ± 1.88[a]	12,64 ± 1.63[a]	0.95
DRGP	51.30 ± 3.50[b]	1.21 ± 0.81[b]	10.78 ± 1.67[b]	4.16

Means within a row with no common superscript differ (P < 0.05).

The chicken breast has a less intense yellowish hue when supplementing the chicken with grapefruit peel, which intensifies when supplementing the feed with orange peel (DRGP). Overall, the data showed a reduction of breast meat yellowness in animals consuming DRGP, showing that the usually undesirable yellow tones in the meat were less developed. This observation could be due to low levels of yellow bioactive molecules in grapefruit peel, which could reduce intestinal absorption of pigments. Castañeda et al. (2005) demonstrated that differential absorption of pigments may lead to differences in skin or meat pigmentation. Garrett et al. (1999) stated that the pigment solubilization and subsequent absorption in the intestine occurs with the lipid phase, because soluble fibers negatively affect fat digestion and absorption of lipid soluble pigments. In contrast, diets with 2% DOP significantly (P < 0.05) increased the yellow and red color of broiler breast meat. The yellowness in breast chicken meat is a good indicator of the xanthophylls content (up to 90% of total carotenoids in some cultivars, Rodrigo et al., 2013) of the ingested feed (Pérez-Vendrell et al., 2001). Oranges compared to other *Citrus* are a good source of carotenoids and vitamin C (Rodrigo et al., 2013). Therefore, the more intense yellow tones of broiler meat from the DOP diet suggest a higher intake of yellow pigments, which may result from the intrinsic richness of it bioactive compounds peels. The addition of 2% DOP to the broilers diet improved the breast meat color while 2% DRGP had a visible total color difference ($\Delta E > 2$). Živković et al. (2017) stated that the use of 6% linseed in COBB 500 broilers diet had effect on the breast meat color (P < 0.05). Same authors stated that the sex of animals influenced the breast color when male chickens were compared with females, they had statistically significantly lighter breast meat.

The textural properties of the *Citrus* effect supplement on raw breast meat samples analyzed with Warner-Bratzler are presented in Figure 1. The Warner-Bratzler Shear Force values ranged from 40.95 N (DOP group) to 47.41 N (C group). Between C and DOP samples Warner-Bratzler, significantly (P < 0.05) decreased. As it can be observed from the Figure 1, the firmness (N) is corelated with the cutting/shearing. This aspect may be caused due to denaturation of both myofibrillar proteins and connective tissue from the samples (Wheeler et al., 1997). Usually firmness of meat decrease after 10 h, because of mechanical stability of raw meat (firmness) which is mainly influenced by its content of the connective tissues and by the length and integrity of the sarcomeres (Belk et al., 2001). According to Großklaus (1979), the content of connective tissues in broiler breast meat without the skin is almost negligible. Early changes in firmness could be caused by the state of contraction of the sarcomeres, which are changing the length in the rigor mortis stadium. Honikel et al. (1986) have demonstrated this phenomenon for meat from other species.

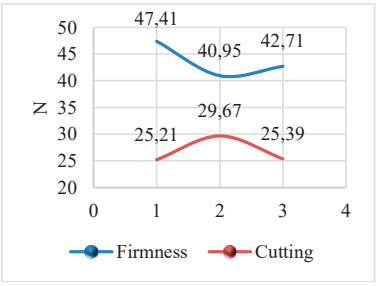

Figure 1. The textural properties of raw breast chicken meat samples after Warner-Bratzler test was applied

Regarding the TPA obtained after the tests were applied, it can be observed that in experimental groups (DOP and DRGP), the samples from raw breast meat had a significant (P < 0.05) higher level of hardness (firmness), compared to C group (Figure 2). The modifications for gumminess and cohesiveness (Figure 3) are insignificant, the variations of this parameters being very small. The values for springiness of raw breast meat samples were between 2.70 and 2.98. This parameter is important because is correlated with chewiness and has as a target older people. Furthermore, the resilience (Figure 4) had almost similar

values in all groups. The differences between the values of the groups were almost insignificant (P < 0.2027).

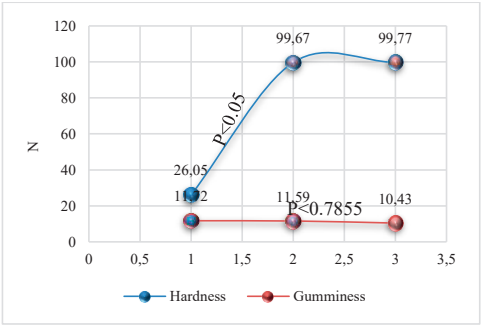

Figure 2. Hardness and gumminess of raw breast chicken meat (N)

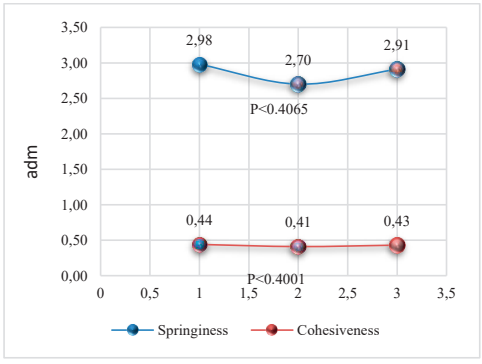

Figure 3. Springiness and Cohesiveness of raw breast chicken meat (adm)

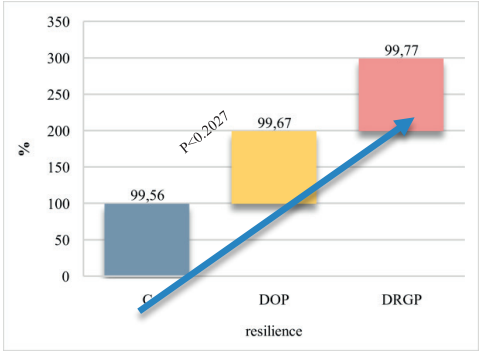

Figure 4. Resilience of raw breast chicken meat (%)

The quality of meat is determined by a number of factors that affect palatability (tenderness, juiciness and flavor). Such factors include the degree of maturity, color of lean, texture, and finally the degree and distribution of marbling (Caine et al., 2003). The determining factors for meat quality however, are multi-factorial and complex. This is because of the highly organized and complex structure of muscle tissue and the various processes the raw meat will undergo such as slaughter methods, storage time, storage temperature (freezing, chilling), amongst others all of which will affect the final texture. De Avilla et al. (2014) stated that meat hardness can be influenced by the fat content in the tissue muscles, which in our case the fat content in the samples was between 2.14 (C) and 3.20 (DRGP). Also, the springiness parameter in more likely in general to be affected. In our case the DOP group had slightly lower values compared to other two groups.

Tenderness can vary from animal to animal, from muscle to muscle within an animal, and from area to area within a muscle (Bourne, 1978), and is further affected by the processing treatment applied to the animals.

Fatty acid composition of raw breast meat from chickens fed diets containing 4% flax meal and 2% orange peel or 2% grapefruit peel at same level are presented in Table 4. The predominant fatty acids in chicken breast in all treatments were palmitic (16:0) and stearic (18:0) acids as SFA, oleic acid (18:1) as monounsaturated fatty acid (MUFA), and linoleic (18:2n-6) and arachidonic (20:4n-6) acids as PUFA. These effects could be caused by the use of supplements and the level of inclusion in diet. At 4% FM and 2% DCP incorporation rate, a significantly increase (P < 0.05) in the sum of MUFA in relation to the C was observed, whereas the levels of PUFA and SFA have maintained close values in relation to the C sample of raw breast meat.

Table 4. Fatty acid composition of raw breast meat from broilers fed DCP

Fatty acids	C	DOP	DRGP	SEM	P-value
	g/100 g total fatty acids				
C 8:0	0.06[a]	0.05[a]	0.03[b]	0.005	0.0569
C 10:0	0.02	0.03	0.03	0.002	0.2220
C 12:0	0.08[a]	0.03[b]	0.05[c]	0.006	0.0006
C 14:0	0.51	0.55	0.56	0.008	0.6339
C 14:1	0.08	0.11	0.12	0.008	0.1214
C 15:0	0.47	0.43	0.37	0.023	0.1991
C 15:1	0.15	0.20	0.17	0.017	0.5384
C 16:0	18.93	19.54	20.25	0.373	0.3736
C 16:1	2.87[a]	3.46	4.01[b]	0.203	0.0640
C 17:0	0.16	0.17	0.17	0.004	0.7747
C 17:1	0.15[a]	0.13	0.10[b]	0.009	0.0243
C 18:0	7.09[a]	6.42	6.06[b]	0.177	0.0433
C 18:1n9c	32.73[b]	34.98[a]	34.79[a]	0.412	0.0363
C 18:2n6	27.77	26.54	26.37	0.557	0.5603
C 18:3n6	0.15	0.15	0.14	0.008	0.6709
C 18:3n3	1.92	1.81	1.86	0.050	0.6995
C 18:2	0.25	0.21	0.19	0.014	0.2702
C 18:4n3	0.36[b]	0.55[a]	0.54[a]	0.026	0.0002
C 20:2n6	0.18	0.24	0.19	0.021	0.4583
C 20:3n6	0.60	0.46	0.47	0.035	0.2376
C22 (1n9)	0.07[a]	0.06	0.04[b]	0.004	0.3249
C 20:3n3	0.48[a]	0.37	0.31[b]	0.031	0.5643
C20:(4n6)	2.19[b]	1.38[a]	1.29[a]	0.160	0.0291
C22:(2n6)	0.24	0.26[a]	0.17[b]	0.018	0.0953
C22:(3n6)	0.17	0.12[a]	0.19[b]	0.014	0.0593
C 20:5n3	0.23	0.23	0.20	0.009	0.1685
C 24:0	0.26	0.26	0.23	0.011	0.2931
C 24:1n9	0.73[b]	0.45[a]	0.36[a]	0.059	0.0156
C22 (4n6)	0.16[b]	0.09[a]	0.08[a]	0.015	0.0389
C 22:5n3	0.35[a]	0.23[a]	0.24	0.025	0.0703
C 22:6n3	0.27[b]	0.18[a]	0.19[a]	0.16	0.0329
Others	0.17	0.13	0.10	0.015	0.1500
Raw breast meat fatty acids profile (% of total FAMEs)					
SFA	27.62	27.53	27.81	0.341	0.9520
MUFA	36.82[b]	39.41[a]	39.61[a]	0.545	0.0559
PUFA, from which:	35.38	32.90	32.47	0.789	0.2812
Ω:3	3.63	3.39	3.34	0.085	0.3398
Ω:6	31.50	29.29	28.94	0.708	0.2938
Ω:6/Ω:3	8.68	8.63	8.64	0.072	0.9706

Where: *Means within a row with no common superscript differ ($P < 0.05$).
**SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

The inclusion of 2% DCP had a marked effect on the fatty acid profile of broiler raw breast meat. The modification involves increasing of palmitic and oleic acids and a decrease of some n-3 and n-6 fatty acid precursors of PUFA especially in DRGP FM group. The α-linolenic fatty acid, had close values in all three groups and it wasn't influenced. These changes do not seem to be related with dietary contribution of both FM and DCP supplements. Both citrus peels have low fatty acid content, although the concentration of linolenic acid (in % of total fatty acids) is consistently low (about 6%) (Murao et al., 2008) and very variable in flax meals (30 to 60%) (Vlaicu et al., 2018; Turcu et al., 2019). But because FM was added at the same level (4%) in all three diets we did not find any differences between groups. Moreover, most of some structural lipids of DCP are probably not available for absorption, requiring fibrolytic fermentation, which is not possible in the upper compartments of the broiler digestive tract. Overall, the changes observed in fatty acid profile of chicken breast meat reported here most possibly result from the inhibition of de novo lipid synthesis (lower 16:0 and 18:1n-9) with an increase in proportion of exogenous fatty acids, mainly

linoleic acid, abundant in the broiler diets (Richards et al., 2003). Islam et al. (2012) stated that the citrus peel oil is rich in oleic acid (57.2 %), palmitic acid (38.6 %), stearic acid (4.1 %), which was reflected also in the meat samples from our study. According to Liu et al. (2012) the major fatty acid profiles in juice are identical in orange and grapefruit. Flax meal is a good source of n-3 fatty acids (Panaite et al., 2019; Vlaicu et al., 2019), but the levels of these fatty acids in breast meat from broiler chicken were not influenced among the treatments. Therefore, the n-6/n-3 ratio of chicken breast meat was not affected by the intake of DCP or FM. At the same time, the levels of the other nutritionally important n-3 fatty acids in meat, particularly of DPA and DHA, were not affected by the citrus peel or flax meal intake. López-Ferrer et al. (2001) stated that the levels of the above-mentioned fatty acids in broiler meat are much lower when compared with the percentages of the long-chain n-3 fatty acids reported in meat originated in birds supplemented with 2 to 4% of fish oil. Therefore, these results suggest that supplementation of broiler diets with DCP at a level of 2%, is unable to improve the n-3 fatty acids of raw broiler meat.

CONCLUSIONS

Supplementation with 2% dried citrus peel and 4% flax meal in diets for broiler chickens were effective in coloring the raw breast meat with more intense yellow tones. The results regarding the impact of flax meal used together with citrus peel, suggest that changed the levels of meat fatty acid profiles, by increasing MUFA and the palmitic acid and decreasing the predominance of total n-6 PUFA. Regarding the texture profile analysis, it seems that hardness and resilience of meat was higher compared to C group samples. These could be a positive effect, but usually it depends on the consumer preference which is usually based on different age of consumers.

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REFERENCES

Belk, K. E., George, M. H., Tatum, J. D., Hilton, G. G., Miller, R. K., Koohmaraie, M., Reagan, J. O., Smith., G. C. (2001). Evaluation of the tendertec beef grading instrument to predict the tenderness of steaks from beef carcasses. *Journal of Animal Science*, 79, 688–697.

Bourne, M. C. (1978). Texture profile analysis. *Food technology*, 32, 62–66.

Caine, W.R, Aalhus, Jl, Best, D.R, Dugan, M.E.R, Jeremiah, L. E. (2003). Relationship of texture profile analysis and Warner-Bratzler shear force with sensory characteristics of beef rib steaks, *Meat Science*, 64, 333–9.

Castaneda, M. P., Hirschler, E. M., Sams, A. R. (2005). Skin pigmentation evaluation in broilers fed natural and synthetic pigments. *Poultry Science,* 84, 143–147.

Chudnovskiy, R., Thompson, A., Tharp, K., Hellerstein, M., Napoli, J.L., Stahl, A. (2014). Consumption of clarified grapefruit juice ameliorates high-fat diet induced insulin resistance and weight gain in mice. *PloS One*, 9, 108408.

de Ávila, M. D. R., Cambero, M. I., Ordóñez, J. A., de la Hoz, L., Herrero, A. M. (2014). Rheological behaviour of commercial cooked meat products evaluated by tensile test and texture profile analysis (TPA). *Meat science*, 98(2), 310–315.

Elferink, E.V., Nonhebel, S., Moll, H.C. (2008). Feeding livestock food residue and the consequences for the environmental impact of meat. *Journal of Clean. Prod*, 16, 1227–1233.

Garrett, D. A., M. L. Failla, R. J. Sarama. (1999). Development of an in vitro digestion method to assess carotenoid bioavailability from meals. *J. Agric. Food Chem*, 47, 4301–4309.

Grigelmo-Miguel, N., Abadías-Serós, M. I., & Martín-Belloso, O. (1999). Characterisation of low-fat high-dietary fibre frankfurters. *Meat science*, 52(3), 247–256.

Großklaus, D. (1979). Geflu¨gelfleischhygiene. Parey, Berlin. Hillebrand, S. (1993). The sensory quality of turkey meat, with special reference to the effects of electrical stunning and chilling rate. *Ph.D. Thesis. Rijksuniversiteit Utrecht, Utrecht,* Netherlands.

Honikel, K. O., Kim, C. J., Hamm, R. (1986). Sarcomere shortening of pre-rigor muscle and its influence on drip loss. *Meat Science*, 16, 267–282.

Islam, M. R., Muslim, T., Rahman, M. A. (2012). Investigation on orange peel: derivatization of isolated cellulosic material and analysis of the fatty acids composition. *Dhaka University Journal of Science*, 60(1), 77–78.

Laufenberg, G., Kunz, B., Nystroem, M. (2003). Transformation of vegetable waste into value added products: (A) the upgrading concept; (B) practical implementations. *Bioresource Technologies*, 87, 167–198.

Liu, Y., Heying, E., Tanumihardjo, S. A. (2012). History, global distribution, and nutritional importance of citrus fruits. Comprehensive reviews in *Food Science and Food safety*, 11(6), 530–545.

Lo´pez-Ferrer, S., Baucells, M. D., Barroeta, A. C., Grashorn, M. A. (2001). n-3 Enrichment of chicken meat. 1. Use of very long-chain fatty acids in chicken diets and their influence on meat quality: Fish oil. *Poultry Science,* 80, 741–752.

Mirabella, N., Castellani, V., Sala, S. (2014). Current options for the valorization of food manufacturing waste: A review. *J. Clean. Prod*, 65, 28–41.

Mourão, J. L., Pinheiro, V. M., Prates, J. A. M., Bessa, R. J. B., Ferreira, L. M. A., Fontes, C. M. G. A., Ponte, P. I. P. (2008). Effect of dietary dehydrated pasture and citrus pulp on the performance and meat quality of broiler chickens. *Poultry Science*, 87(4), 733–743.

Niu, L., Wu, J., Liao, X., Chen, F., Wang, Z., Zhao, G., et al. (2008). Physicochemical characteristics of orange juice samples from seven cultivars. *Agricultural Sciences in China*, 7(1), 41–47.

NRC (1994). Nutrient requirements of poultry, Ninth Revised Edition, *National Academy Press*, Washington D.C.

Oluremi, O.I.A., Ngi, J., Andrew, I.A., (2007). Phytonutrients in citrus fruit peel meal and nutritional implication for livestock production. *Livest. Res. Rural Dev*., 19.

Panaite, T. D., Nour, V., Vlaicu, P. A., Ropota, M., Corbu, A. R., Saracila, M. (2019). Flaxseed and dried tomato waste used together in laying hens diet. *Archives of animal nutrition*, 73(3), 222–238.

Panouillé, M., Ralet, M.C., Bonnin, E., Thibault, J.F. (2007). Recovery and reuse of trimmings and pulps from fruit and vegetable processing. In Handbook of Waste Management and Co-Product Recovery in Food Processing; Waldron, K., Ed.; *Woodhead Publishing Limited: Cambridge*, UK, Volume 1, pp. 417–447.

Pe´rez-Vendrell, M., Herna´ndez, J. M., Llaurado, L., Schierle, J., Brufau, J. (2001). Influence of source and ratio of xanthophyll pigments on broiler chicken pigmentation and performance. *Poultry Science,* 80, 320–326.

Pfaltzgraff, L. A., Cooper, E. C., Budarin, V., Clark, J. H. (2013). Food waste biomass: A resource for high-value chemicals. *Green Chem*, 15, 307–314.

Rezzadori, K., Benedetti, S., Amante, E. R. (2012). Proposals for the residues recovery: Orange waste as raw material for new products, *Food and Bioproducts Processing*, 90(4), 606–614.

Richards, M. P., Poch, S. M., Coon, C. N., Rosebrough, R. W., Ashwellm, C. M., McMurtry, J. P. (2003). Feed restriction significantly alters lipogenic gene expression in broiler breeder chickens. *Journal of Nutrition,* 133, 707–715.

Rodrigo, M. J., Alquézar, B., Alós, E., Lado, J., Zacarías, L. (2013). Biochemical bases and molecular regulation of pigmentation in the peel of Citrus fruit. *Scientia Horticulturae*, 163, 46–62.

Turcu, R. P., Olteanu, M., Criste, R. D., Panaite, T. D., Ropotă, M., Vlaicu, P. A., Drăgotoiu, D. (2019). Grapeseed Meal Used as Natural Antioxidant in High Fatty Acid Diets for Hubbard Broilers. *Brazilian Journal of Poultry Science*, 21(2).

Vlaicu, P. A., Panaite, T. D., Cornescu, M. G., Ropota, M., Olteanu, M., Drăgotoiu, D. (2019). The influence of by-products on the production parameters and nutrient digestibility in fattening pigs diet (60-100 kg). *AgroLife Scientific Journal*, 8(1), 261–269.

Vlaicu, P. A., Panaite, T. D., Voicu, I., Turcu, R. P., Olteanu, M., Ropota, M. (2018). Determining the feeding value of some food industry by-products. *Scientific Papers: Animal Science and Biotechnologies*, 51(1), 62–69.

Vlaicu, P. A., Panaite, T. D., Dragotoiu, D., Ropota, M., Bobe, E., Olteanu, M., Criste, R. D. (2017). Feeding quality of the meat from broilers fed with dietary food industry by-products (flaxseed, rapeseeds and buckthorn meal, grape pomace). *Scientific Papers, Series D. Animal Science*, 60, 123–130.

Vlaicu, P. A., Dragotoiu, D., Panaite, T. D., Untea, A., Saracila, M., Mitoiu, M. (2017). Effect of rosehip addition to Ω3 PUFA-high layer diets on hen performance and egg quality. *Proceedings of the 21st European Symposium on Poultry Nutrition*

Waldron, K. (2007). *Waste minimization, management and co-product recovery in food processing: An introduction. In Handbook of Waste management and co-product recovery in food processing*. Waldron, K., Ed.; Cambridge, UK: Woodhead Publishing Limited, Volume 1, pp. 417–447.

Wheeler, T. L., Shackelford, S. D., Johnson, L. P., Miller, M. F., Miller, R. K., Koohmaraie, M. (1997). A comparison of Warner-Bratzler shear force assessment within and among institutions. *Journal of animal science*, 75(9), 2423–2432.

Živković, D., Lilić, S., Stajić, S., Vranić, D., Trbović, D., Stanišić, N. (2017). Effect of extruded flaxseed enriched diet on physico-chemical and sensory characteristics of broiler meat. *Biotechnology in Animal Husbandry*, 3(2), 221–231.

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APPLICATION OF HERBAL FEED ADDITIVE IN THE RATION TO GET ASUH MEAT OF SENTUL CHICKEN

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Abstract

Sentul chicken is a specific local chicken from the Ciamis region in West Java and can be used for egg and meat production. The continuous use of antibiotics for maintenance to prevent and treat diseases in Sentul chickens can cause residues in chicken meat. To reduce the use of antibiotics, it is necessary to find natural antibiotics derived from herbal plants such as mangosteen peel extract (MPE), containing xanthone compounds like antioxidants, which used to prevent free radicals. The study aimed to determine the effect of adding MPE in rations as a feed additive to get ASUH chicken meat. This study used 100 days-old Sentul chicks that were kept for 12 weeks in the litter system. The design used was CRD with 4 treatment levels of MPE, 41 ml/kg ration, 81 ml/kg ration and 122 ml/kg ration and repeated 5 times. The results showed that the addition of MPE in the ration could have a positive impact on the growth of Sentul chickens and internal organs to produce healthy chicken meat, low in cholesterol so that safe for consumption.

Key words*: feed additive, mangosteen peel extract (MPE), Sentul chicken, ASUH meat.*

INTRODUCTION

Sentul chicken is a specific local chicken from Ciamis region in West Java and a dual- purpose type that can utilized for eggs and meat production. They can adapt to the environment, and it remains productive even though their diets are low of quality. The fur is arranged neatly on its chest like dragon scales, and the colour of its scales is grey, white or yellow (Sartika and Iskandar, 2008; Widjastuti et al., 2017). In another way, this bird is very good to genetically improve chicken meat breeds, because has a compact body and white skin colour. Efforts can be made so that chickens can produce optimally, usually by using antibiotics. The use of antibiotics continuously for maintenance to prevent and treat disease in Sentul chicken can lead residues in chicken meat. One alternative method is the use of herbs, one of which is the mangosteen peel. Mangosteen skin is the biggest component of mangosteen fruit with a yield of 65% (Chaovanalikit et al, 2012). It contains a secondary metabolic mixture that is xanthones, and the most abundant content is alpha mangostin (Dermawan et al., 2019). The nutritional content contained in mangosteen peel is 62.05% water, 1.01% ash, 0.63% fat, 0.71% protein, 1.17% total sugar, and 35.61% carbohydrates. The inclusion of mangosteen peel in the diet is a problem because of its antinutrient content in the form of tannins. High tannin content will inhibit the absorption of feed and chicken growth. To reduce tannin levels in the mangosteen peel, extraction procedures must be carried out. Extraction is the process of separating solid or liquid materials with the help of solvents. The process of extracting the peel of mangosteen fruit to obtain antioxidant substances usually use a maceration process, which is a simple extraction method to extract simplified containing soluble chemical components in the solvent fluid (Do et al., 2014). Mangosteen peel contains xanthones of 107.76 mg per 100 g. In addition, mangosteen peel functions as an antitumoral, anti-inflammatory, antiallergic, antibacterial, antifungal and antiviral agent (Gondokesumo et al., 2019) as well as being able to improve the blood lipid profile (Watanabe et al., 2018). Xanthone compounds as antioxidants can be used to prevent free radicals. Free radicals are compounds which contain one or more unpaired electrons, so they are very reactive, and xanthone can improve the structures of intestinal villi in the process of nutrient absorption. In accordance with the

opinion of Velmurugan, and Citarasu (2010) antibacterial herbs are able to suppress the growth of pathogenic bacteria in the intestine. These free radicals can cause metabolite disorders and cause stress to livestock. The emergence of stress in poultry can be a trigger for the emergence of various diseases. This will affect the disruption of the consumption process and result in a decrease in meat quality. From some research results it can be calculated, the needs for antioxidant in the ration is considered based on the content of polyunsaturated fatty acids, every 1% of polyunsaturated fatty acids required 30 IU/kg of vitamin E rations as antioxidant or 30 ppm in the form (DL-α-Tocopheryl acetat) (Lesson and Summers, 2005). Based on the calculation of antioxidant requirements in the ration which is equivalent to vitamin E (DL-oc-Tocopheryl acetate) around 80 ppm, assuming xanthone content in mangosteen peel extract 97.68 ml/100 ml (Erlina, 2008), so the need for mangosteen peel extract in the ration is around 81 ml/kg ration (80 ppm xanthones).

The addition of MPE to Sentul Chicken ration is expected to improve health and growth in livestock, as well as produce high final body weight which can further produce ASUH chicken meat. The aim of the study was to determine the effect of adding MPE in rations as a feed additive to get ASUH chicken meat.

MATERIALS AND METHODS

Experimental design

This study was designed in Completely Randomized Design (CRD). The study used 100 days old chicks of Sentul divided into 4 groups and each group is repeated 5 times. Each cage contains 5 chickens which maintained until the age of 12 weeks. The coefficient value of variation of initial body weight of chicken equal to 9.47. The treatment consisted of the use of mangosteen peel extract (MPE), ie: P0 = 0 ml MPE/kg ration; P1 = 41 ml MPE/kg ration (40 ppm xanthones); P2 = 81 ml MPE/kg ration (80 ppm xanthones); and P3 = 122 ml MPE/kg ration (120 ppm xanthones) The feed and water were provided *ad libitum*. The local ingredients used to produce the diets and the energy metabolism and protein needs were formulated based on Widjastuti (1996) diet formulation for Sentul chicken. The feed ingredients of the ration comprised of yellow corn (56.00%), rice bran (21.50%), fish meal (9.25%), soybean meal (12.00%), bone meal (0.75%), and $CaCO_3$ (0.50%). Rations were prepared based on protein and metabolic energy requirements for the local chicken growth phase, ie. 17% protein and 2850 kcal/kg.

Chicken Slaughter Process

Each cage unit is randomly taken 1 chicken at the age of 12 weeks, so the total number of chickens is 20. Chickens to be slaughtered are fastened for 12 hours. Weigh the weight of chickens at the age of 12 weeks as life weight.

Processing of MPE (modification of Rismana et al., 2014)

Extraction of mangosteen peel is done by maceration with 96% ethanol solvent for 2 x 24 hours, the Maserat is then filtered and the obtained filtrate is concentrated with a rotary evaporator to get the thick mangosteen peel extract, then the sample is extracted dried using a freeze dryer to obtain mangosteen peel extract and as were done in the research Central laboratory of the University of Padjadjaran.

Small Intestine Morphometry

Jejunum samples were collected from the small intestine of Sentul chicken in each treatment group. The samples were washed in physiological NaCl, then fixed in Bouin solution for 2 days. The samples were dehydrated in alcohol with different concentrations for 30 min each i.e. 70, 80, 90 and 100%. Afterward, the samples were cleaned using xylol and alcohol 100% 3 times for 5 and were then infiltrated in an oven at please explain using xylol and paraffin solutions in the ratio of 3:1. 1:1, 0:1 in sequences for 15 min. Samples were prepared using standard paraffin embedding procedures by sectioning at 6-8 cm thickness and kept for 1 day. The samples were then stained by Haematoxylin-Eosin. In the end, the sample was covered with a mounting agent and viewed under the microscope and then counted the number of villi, villi height and width.

Data analyses

Analysis of variance was applied to the data using statistical package program of SPSS version 19. Significantly different means were separated by a Duncan's multiple comparison

test at 0.05 levels. The variables observed were carcass weight, abdominal fat, meat cholesterol content, and effects on liver, gizzard and chicken intestine.

RESULTS AND DISCUSSIONS

The effect of adding mangosteen peel extract (MPE) in the ration on final weight, carcass weight, abdominal fat, and cholesterol of Sentul chicken meat can be seen in Table 1 and Figure 1.

Table 1. The average of final weight, carcass weight, abdominal fat weight, and meat cholesterol

Variable	P0	P1	P2	P3
Final weight (g)	713.25 a	818.00 b	836.75 b	808.25 b
Carcass weight (g)	405.50 a	540.25 b	579.50 b	561.75 b
Abdominal fat weight (g)	2.20 a	2.38 a	2.50 a	2.55 a
Meat cholesterol (mg/100g)	63.47 a	59.09 a	59.20 b	54.35 b

Note: The same letter to the line shows no significant difference.

Final Weight

The results of statistical analysis (Table 1) show the final weight that the addition of mangosteen peel extract increases the final weight, this is because the xanthones content in MPE can work optimally as an antioxidant and prevent free radicals in Sentul hen's body. In accordance with the opinion of Zaboli et al. (2013) antioxidants convert free radicals into relatively stable compounds and stop the chain reaction from free radical damage that will have an impact on the growth rate of chickens. MPE supplementation at optimal dosages can help in the digestion process by improving the structure of intestinal villi in the process of absorption or absorption of feed nutritive substances and able to suppress the growth of pathogenic bacteria in the intestine, according to the opinion of Velmurugan and Citarasu (2010) which states that mangosteen skin contains xanthone compounds as antioxidants, antiviral, antifungal and antimicrobial which is thought to be able to improve the structures of intestinal villi in the process of absorption of nutrients and able to suppress the growth of pathogenic bacteria in the intestine so as to increase body weight growth. This condition causes the surface area of the small intestine villi to become wider so that the absorption of nutrients can take place well. The anti-bacterial properties of MPE can affect the walls and cell membranes of pathogenic bacteria undergoing protein denaturation and eventually their growth is inhibited. The growth of pathogenic bacteria which is blocked will increase the bacterial population so that the digestibility and absorption of nutrients becomes more maximal which in turn will increase the final weight

Carcass Weight

Average carcass weights can be seen in Table 1. The results of the analysis showed that the MPE in the ration were significant difference ($P<0.05$) to the carcass weight. This is in line with the Final weight which is also significantly affected by the increased use of MPE in the ration. Widjastuti et al. (2019) stated that the carcass production closely related to the final weight, the more the life weight increased, the carcass production also increased. Antioxidant compounds in MPE can convert free radicals into compounds that are relatively stable and can stop chain reactions from damage caused by free radicals that will have an impact on the growth rate of chickens, ultimately increasing the final weight and carcass weight (Zaboldi et al., 2013).

Abdominal fat

The average abdominal fat can be seen in Table 1. The average abdominal fat Sentul chicken research results ranged from 2.20 grams to 2.55 grams. The results of the analysis showed that the addition of MPE in the ration did not significantly affect abdominal fat ($P>0.05$). The resulting abdominal fat weight is related to ration consumption. Statistical results showed that the addition of MPE in the ration did not have a significant effect ($P>0.05$) on feed consumption. The addition of mangosteen peel extract does not reduce palatability, this is due to the existence of MPE extraction treatment with ethanol solvent which can reduce tannin levels so that the bitter taste and distinctive odor of mangosteen peel decreases. Consumption of the same ration between treatments proved that energy consumption and crude fat content consumed were relatively the same, so there was no excess energy accumulated in the form of abdominal fat.

Another reason that causes abdominal fat content is relatively the same is because Sentul chicken aged 12 weeks in a period of rapid growth. At this age, fat has not formed much because the absorbed food substances are still used first for pure growth, or in other words all the protein consumed is still concentrated for growth so that very little protein is piled up as abdominal fat.

Meat cholesterol

From Table 1 it can be seen that the content of Sentul chicken meat cholesterol decreases with the addition of MPE in the ration. Cholesterol reduction in the treatment of P1, P2 and P3 is desecrated. This is caused by bioactive xanthone and polyphenol compounds which can inhibit or suppress the HMG-CoA reductase enzyme which acts as a catalyst for the process of cholesterol biosynthesis and prevents increased secretion of bile salts thereby inhibiting cholesterol formation. According to Reynertson (2007) the addition of the right MPE can reduce inflammation and be able to capture free radicals or oxygen compounds effectively and will ultimately inhibit cholesterol synthesis. According to Adriani et al. (2014), compound xanthone is able to inhibit the process of cholesterogenesis in the squalene stage before it becomes cholesterol by inhibiting the synthesis of endogenous cholesterol and inhibiting the enzyme HMG Co-A reductase which acts as an intermediary for the synthesis of mevalonate which eventually becomes cholesterol. Polyphenol compounds in the addition of MPE can reduce total cholesterol levels by polyphenols bind to cholesterol so that cholesterol is absorbed slightly, while the remaining cholesterol that is not absorbed is secreted through feces (Yokozawa et al., 2002). The saponin content in MPE is also lipophilic which is able to dissolve fat and emulsion which can reduce chicken blood cholesterol due to hypercholesterolemia (Adriani et al., 2018).

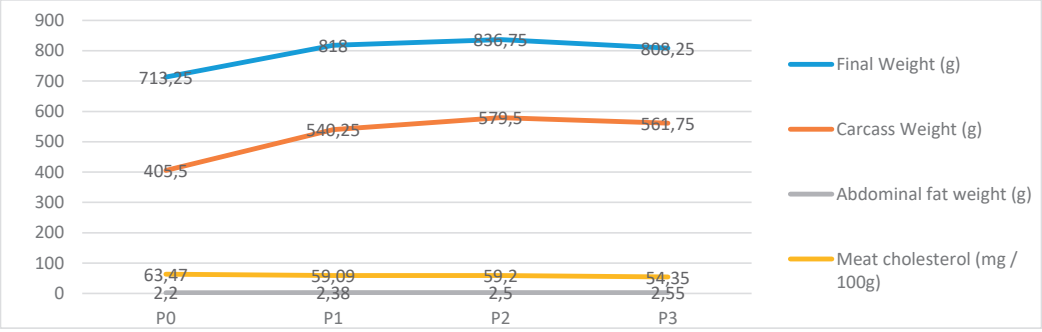

Figure 1. Average of final weight, carcass weight, abdominal fat weight, and meat cholesterol

Effects of addition of MPE on average liver, gizzard and jejunal morphometry of Sentul chicken

The results of average liver, gizzard and jejunal morphometry are presented in Table 2 and Figure 2.

Table 2. Effect of MPE on liver, gizzard and jejunal morphometry in Sentul chicken

Variable	P0	P1	P2	P3
Gizzard weight (g)	23.25 a	23.55 a	26.75 a	28.00 a
Heart weight (g)	28.25 a	26.22 a	25.95 a	25.35 a
Number of villi (unit)	31 a	35 b	40 b	42 b
Villi height (µm)	400.32a	493.27b	467.97b	486.02b
Top width (µm)	133.30a	153.09b	171.02	177.78b
Bottom width (µm)	143.04a	179.45b	253.26b	200.58b

Note: The same letter to the line shows no significant difference.

There was no significant (P>0.05) effect of using MPE as feed additive in the diet of Sentul chicken on liver weight and gizzard weight. This is because the levels of crude fiber and tannin on the mangosteen peel after undergoing

the extraction process decreases the crude fiber thereby reducing the work of the gizzard and the liver. According to Dedi Setiadi et al. (2012), the size of the gizzard is influenced by its activity. Gizzard muscle activity will occur when foods containing high crude fiber into it. The results of the study of adding MPE in the basal ration had a significant effect (P<0.05) on the number of intestinal villi. The P2 and P3 treatments had the most number of villi. This shows that there is a widening in the width of the upper and lower surfaces of the villi, thus indicating that the performance of the villi in the small intestine tends to be active which is found in the treatment group receiving MPE at the level of 81-122 ml/kg ration. The condition is caused by xanthones in P2 and P3 treatments which have an effective role in stimulating the development of intestinal villi size so that it affects the process of intestinal activation in digestion and absorption of nutrients. Increasing the villus width and the number suggests an increased surface area capable of greater absorption of available nutrients.

In accordance with the opinion of Natsir et al. (2016) and Rahmawati (2016), poultry feed containing herbs will affect the height and number of intestinal villi.

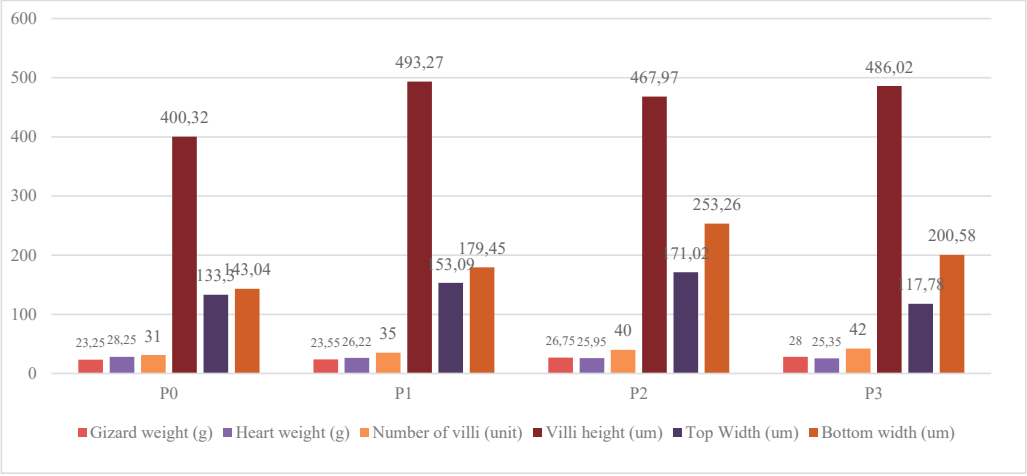

Figure 2. Effect of MPE on liver, gizzard and jejunal morphometry

CONCLUSIONS

The addition of MPE until 122 ml/kg in basal ration significantly affected the quality carcasses of Sentul chicken at 12 weeks and MPE can be natural antibiotics from herbal for Sentul chicken.

The addition of MPE in the ration could have a positive impact on the growth of Sentul chickens and internal organs so as to produce healthy chicken meat, low in cholesterol, so that it is safe for consumption.

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REFERENCES

Adriani, L., Hermawan, E., Hidayat, U. (2014). Decreasing cholesterol and triglyceride Level on blood by adding orange (*Citrus sinenis*) waste on Padjadjaran I sheep. *Science Papers. Series D. Animal Science*, 57, 70 -73.

Adriani, L., Widjastuti, T., Wiradimadja, R. (2018). Effect of mangosteen pell extract (*Garcinia mangostana* L.) on blood lipid of Sentul chicken growth phase. *The 3rd International Conference of Integrated Intellectual Community, Hanower.*

Chavanalikit, A., Mingmuang, A., Kitbun Leuwit, T. (2012). Anthocyanin and total phenolic content of mangosteen and effect of processing on the quality of mangosteen product. *International Food Research* Journal, 19, 1047-1053.

Dedi, Setiadi., Khaira, Nova, Syahrio, Tantalo (2012). Perbandingan Bobot Hidup, Karkas, Giblet, Dan

Lemak Abdominal Ayam Jantan Tipe Medium Dengan Strain Berbeda Yang Diberi Ransum Komersial Broiler. *Fakultas Pertanian. Universitas Lampung*.

Dermawan, Abdurraafi', Maududi, Julianti, Elin, Putra, Masteria Yunovilsa (2019). Identification and Evaluation of Antibacterial Compounds from the Vibrio sp. associated with the Ascidian *Pycnoclavella diminuta*. *Pharmaceutical Sciences & Research*., 6(3).

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetarredjo, F. E., Ismadji, S., Ju, Y. H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content and antioxidant activity of Limnophila aromatic. J. *Food Drug Anal*. 22(3), 296-302.

Erlina, N. P. (2008). Ekstraksi Xanthone dari Kulit Manggis (*Garcinia Mangostana* L.) dan Aplikasinya dalam Bentuk Sirup. *Fakultas Teknologi Pertanian IPB, Bogor.*

Gondokesumo, M.E., Pardjianto, B., Sumitro, S.B., Widowati, W., Handono, K. (2019). Xanthones analysis and antioxidant activity analysis (Applying ESR) of six different maturity levels of mangosteen rind extract (*Garcinia mangostana* Linn.). *Pharmacog J*., 11(2), 369-373.

Indrawati, Y., Asmara, Widjastuti, T., Setiawan, I. Abun, Partasasmita, R. (2018). The Growth Performances and The Gut Health parameters of Sentul Chicken Supplemented with a various dosage of Turmeric powder. *Nusantara Bioscience. ISCA Journal of Biological Science*, 10(3), 2087-3948.

Leeson, S, Summers, D. J. (2005). *Commercial Poultry Nutrition*. Third Edition. Canada: University Books Guelph. Publishing House.

Natsir, M. H., Widodo, E., Muharlien (2016). Penggunaan kombinasi tepung kunyit (*Curcuma domestica*) dan jahe (*Zingiber officinale*) bentuk enkapsulasi dan tanpa enkapsulasi terhadap karakteristik usus ayam pedaging. *Buletin Peternakan*, 40(1), 1-10.

Ngamsaeng, A. (2004). Effects of mangosteen peel (*Garcinia mangostana* L.) supplementation on rumen ecology, microbial protein synthesis, digestibility and voluntary feed intake in beef steer. *Tropical Feed Resources Research and Development Center, Department of Animal Science, Thailand*.

Permana, A.W. (2010). Kulit Buah Manggis Dapat Menjadi Minuman Instan Kaya Antioksidan. *Warta Peneltian dan Pengembangan Pertanian*, 32(2).

Rahmawati, N., Sudjarwo, E., Widodo, E. (2016). Uji aktivitas antibakteri ekstrak herbal terhadap bakteri *Escherichia coli*. *Jurnal Ilmu-Ilmu Peternakan*, 24, 24-31.

Reynertson, K. A. (2007). Phytochemical Analysis of Bioactive Constituens From Edible Myrtaceae Fruit. *[Dissertation]. The City University of New York, New York*.

Rismana, E., Kusumaningrum, S., Bunga, O., Nizar, Marhamah (2014). Pengujian Aktivitas Antiacne Nanopartikel Kitosan – Ekstrak Kulit Manggis (Garcinia mangostana). *Media Litbangkes*, 24(1), 19-21.

Widjastuti, T., Abun, Wiradimadja, R., Setiyaman, H., Rusmana, D. (2018). The effect of ration containing mangosteen peel meal (*Garcinia mangostana*) on final body weight, carcass composition and cholesterol content of Sentul chicken. *The 3nd International Comference of Integrated Intellectual Community, Jerman.*

Velmurugan, S., Citarasu, T. (2010). Effect of herbal antibacterial extract on the gut floral change in indian white shrimp Fenneropenaeus indicus. *Rom. Bioech. Left*., 15, 5709-5717.

Widjastuti, T. (1996). Penentuan Efisiensi Penggunaan Protein, Kebutuhan Protein Dan Energi Untuk Pertumbuhan dan Produksi Telur Ayam Sentul Pada Kandang Sistem Cage Dan Sistem Litter. *Disertasi, Universitas Padjadjaran, Bandung.*

Widjastuti, T., Setiawan, I., Abun Abun, Asmara Indrawati, Y., (2019). The effect of diet containing mangosteen peel extract (*Garcinia mangostana* L.) and supplemented with zinc and cooper on the quality of Sentul chicken carcass. *Proceedings of The International Scientific Congress "Life Sciences, A Challenge For The Future". Iasi, Romania.*

Widjastuti, T., Abun, I. S. (2017). The use of turmeric (*Curcuma domestica* Val) meal in the dietal feed additive on hen-day production and egg quality of Sentul chicken. *Scientific Papers Series D. Animal Science,* LX, 131-135.

Widjastuti, T., Sujana, E., Darana, S. (2012). The yielding characteristic of Sentul chickens fed diet containing papaya leaves meal (*Caraca papaya* L. Less). *Scientific Papers, Animal Science, Series D*., LV (4).

Yokozawa, T., Kim, H.Y., Cho, E.J., Choi, J.S., Chung, H.Y. (2002). Antioxidant effects of isorhamnetin 3,7-di-O-beta-D-glucopyranoside isolated from mustard leaf (*Brassica juncea*) in rats with streptozotocin-induced diabetes. *J Agric Food Chem*., 50(19), 5490.

Zaboli, G. Z., Bilondi, H. H., Miri, A. (2013). The Effect of Dietary Antioxidant Supplements on Abdominal Fat Deposition in Broilers. *Life Sci. J.,* 10, 328-333.

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THE EFFECT OF GIVING MANGOSTEEN (*Garcinia mangostana* L.) EXTRACT WITH MINERAL SUPLEMENTATION ON BLOOD AND YELLOW EGG CHOLESTEROL LEVELS OF CHICKEN PHASE LAYER

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Abstract

The research was conducted to know the effect of mangosteen peel extract supplemented Cu and Zn on blood cholesterol and egg yolk cholesterol of Sentul chicken. The research was done from Agustus until November 2019. Samples test was held in Physiology and Biochemistry Laboratory, Animal Husbandry Faculty, Padjadjaran University, Sumedang. The methods used experimental with a Completely Randomized Design (CRD) and the effect of treatment using Analysis of Variance (ANOVA) followed by Duncan and Orthogonal Polynomial. The treatment consisted of five kind with five repetitions, P0 = basal ration, P1 = basal rations + 60 mg/kg mangosteen peel extract + Cu 0.3 mg and Zn 2.4 mg, P2 = basal rations + 120 mg/kg mangosteen peel extract + Cu 0.6 mg and Zn 4.8 mg, P3 = basal rations + 180 mg/kg mangosteen peel extract + Cu =0.9 mg and Zn 7.2 mg, P4 = basal rations + 240 mg/kg mangosteen peel extract + Cu 1.2 mg and Zn 9.6 mg. The result showed that the effect of giving 120 mg/kg mangosteen peel extract supplemented Cu 0.6 mg and Zn 4.8 mg was the significant effect ($P<0.05$) decreasing blood cholesterol levet but non-significant ($P>0.05$) decreasing egg yolk cholesterol levels of Sentul chicken.

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Key words*: blood cholesterol, Cu (copper), egg yolk cholesterol, mangosteen peel extract, Zn (zinc).*

INTRODUCTION

Sentul chicken is one type of local chicken typical of the Ciamis region of West Java which has gray feather characteristics. The advantage of sentul chicken compared to other native chickens, which has relatively rapid growth (Kurnia, 2011). Sentul chickens are categorized as dual-purpose chickens, which are capable of producing meat and eggs. Sentul chicken meat and eggs can be used as an alternative to meet the needs of community animal protein.

Lately, some people are more selective because health awareness is getting higher. People crave food from animals, especially poultry with low fat content such as cholesterol (Pogurschi et al., 2019). According to Rasyaf (1995), native chicken meat has a low-fat content, but with increasing age of the chicken, the deposition of abdominal fat is increasing as well as with subcutaneous fat. The high-fat content is identical to the high cholesterol content.

Cholesterol content in eggs is influenced by several factors such as age, genetics, nutrients, and drugs (Ketaren, 2010). Effect of fat in the feed (vegetable oil, animal oil, cholesterol, and B sitosterol) can increase liver cholesterol, serum, and egg yolks in laying hens (Han et al., 1993). Cholesterol content in egg yolks can change up to 25% by cholesterol derived from feed and fat (Hargis, 1988). Egg yolk cholesterol is higher than meat cholesterol, this is because the egg is the end of the distribution of vitellogenin which is composed of cholesterol, triglycerides, phospholipids, and proteins (Watson, 2002).

Efforts to reduce cholesterol levels in the blood and eggs can be done by using rations mixed with herbal plants, one of which is mangosteen peel extract (*Garcinia mangostana* L.). Mangosteen peel extract contains xanthone compounds as antioxidants. Xanthones are natural chemical substances that are classified as phenol or polyphenolic compounds. Mangosteen peel extract contains a lot of antioxidants of 84.6-86.3% and xanthone content is more than 90% (Diwyanto et al., 2011). Xanthones can inhibit the process of cholesterogenesis. Effect of mangosteen peel

extract can decrease Low Density Lipoprotein also increased High Density Lipoprotein (Lovita et al., 2018).

Mangosteen peel extract also contains flavonoid compounds that can reduce blood cholesterol levels by reducing the absorption of cholesterol and bile acids in the small intestine which causes increased excretion through feces. Liver cells increase the formation of bile acids from cholesterol so that they can reduce fat due to being converted into energy (Sucipto, 2008).

Miryanti et al. (2011) presented mangosteen peel extract results from the analysis of Gas Chromatography Mass Spectrometry (GCMS) that mangosteen peel extract contains methyl esters of unsaturated organic acids that are easily oxidized. Supplementation of Cu and Zn plays a role to temporarily activate the bioactive contained in the mangosteen peel extract which is reactive, thus making the ionization in the digestive tract higher and can be optimally utilized right on target. Copper (Cu) is an essential mineral micro element that is cationic. Copper is one of the mineral elements that are needed in the process of metabolism, hemoglobin formation and physiology in the animal's body (Burns, 1981). The form of copper metal that is given into the feed mixture is in the form of copper salt compounds, such as copper sulfate, copper oxide, copper carbonate, and copper proteinate. Usually sulfate and copper oxide are often added to ruminant feed (Baker et al., 1991; Johnson and Engle, 2003).

MATERIALS AND METHODS

The material used in this study was 40-tail chicken, maintained from the age 28 weeks to 35 weeks. Chicken is divided into 5 treatments and each treatment is repeated 4 times, each cage contains 1 tail.

Sample Collection Stage

a. Blood sampling

Blood was taken at the end of the study, and eggs samples were taken from one chicken for each test in each treatment. Total blood samples were taken in the last week are 20 samples. The sample selection is based on the average body weight close to the same. Blood is drawn through the 2 cc chicken wing pectoralis vein. Blood samples were taken using a 5 ml syringe, and were collected in vaccumtube Ethylene Diamine Tetra Acetyl Acid (EDTA).

b. Chicken egg sampling

Egg sampling was carried out at the end of maintenance ie from each treatment 2 egg samples were taken from each cage, so that 10 egg samples were obtained from 5 treatments and 4 replications. Each egg is broken and then separated between egg whites and yolks. Egg yolks that have been separated and then placed in a plastic container, then roasted until dry, then ready to be tested for cholesterol levels. Chicken weighed initially according to treatment. The procedure for making mangosteen peel extract, namely, a sample of 7,000 g of fresh mangosteen peel, dried and cut into small pieces. The dry sample weighed 5,000 g. Then macerated with 96% ethanol for 24 hours. 96% ethanol extract from maceration was filtered with filter paper and the filtrate was collected. The filtrate was then evaporated using a rotary evaporator at ± 62°C in order to obtain concentrated ethanol extract 4.471 g. After that, the thick material is taken to the oven with a temperature of 600^0C and the mangosteen peel extract powder is obtained at 3.621 g. Mangosteen peel extract is then supplemented using Cu and Zn.

Based on the composition of the ration, the nutrient content and metabolic energy of the basal ration are presented in Table 1.

Table 1. Nutrient content and basal energy metabolism of ration

Nutrient Content and Metabolism Energy	Amount	Nutritional Needs of Local Chicken
Matabolizable Energy (ccal/kg)	2757	2750
Protein (%)	15.63	15-16
Fat (%)	5.14	8**
Crude Fiber (%)	4.16	8**
Calcium (%)	3.28	3.25-4.25
Phosphor (%)	1.39	0.3**
Lysine (%)	1.06	0.9**
Methionin (%)	0.37	0.35**

Source: Widjastuti (1996);
**NRC (1994) for light type laying hens

Observed variables include:
a. Chicken Blood Cholesterol Levels Layer Phase;
b. Chicken Egg Yolk Cholesterol Levels Layer Phase.
This study uses an experimental method with a Completely Randomized Design (CRD). There are 20 treatment units consisting of 5 treatments and 4 replications. The experimental diet consisted of P0 = basal ration, P1 = 60 mg/kg ration of mangosteen peel extract + Cu 0.3 mg and Zn 2.4 mg, P2 = 120 mg/kg mangosteen peel extract ration + Cu 0.6 mg and Zn 4.8 mg, P3 = 180 mg/kg of mangosteen peel extract + Cu 0.9 mg and Zn 7.2 mg, and P4 = 240 mg/kg of mangosteen peel extract + Cu 1.2 mg and Zn 9.6 mg. The data obtained were analyzed by ANOVA variance test and the effect of treatment using Duncan's and Polynomial Orthogonal test.

RESULTS AND DISCUSSION

Effect of Treatments on Chicken Blood Cholesterol Layer Phase

Sentul chicken blood cholesterol content which has been treated with mangosteen peel extract which has been supplemented with Cu and Zn, the results of laboratory analysis are presented, presented in Table 2.

Table 2. Blood cholesterol and egg yolk levels of chicken layer phase

Parameter	Treatment				
	P0	P1	P2	P3	P4
Blood cholesterol (mg/dl)	206.28^{a}±26.25	160.42^{a}±14.43	119.31^{b}±17.44	114.81^{b}±12.11	112.67^{b}±26.74
Egg yolk cholesterol (mg/100 g)	122.25±30.51	125.62±35.38	109.48±45.93	149.48±11.54	119.23±18.63

Based on Table 2 that the highest blood cholesterol level in P0 is 206.28 mg/dL, followed by P1 which is 160.42 mg/dL, then P2 has a value of 119.31 mg/dL, then P3 is 114.81 mg/dL, and P4 has a value of 112.67 mg/dL. The results of the analysis of variance showed that the administration of mangosteen peel extract supplemented with Cu and Zn had a significant effect ($P<0.05$) on the level of layer chicken blood cholesterol levels.
The treatment of P1 (60 mg/kg ration) was not significantly different from the treatment of P0 (basal ration). P2 (120 mg/kg ration) was not significantly different ($P>0.05$) with P3 (180 mg/kg ration), and P4 (240 mg/kg), but significantly different ($P<0.05$) with P0 treatment (0 mg/kg ration) and P1 (60 mg/kg ration). These results indicate that treatment P2, P3, and P4 has the effect of reducing blood cholesterol levels
Increasing the dose of mangosteen peel extract supplemented with Cu and Zn in the ration can reduce blood cholesterol levels. Mangosteen peel extract dosage 120-240 mg/kg ration gives real results in reducing blood cholesterol chicken layer phase. This is because the active

compound contained in the mangosteen peel extract is xanthone which is able to inhibit the process of cholesterol synthesis. The process of cholesterol synthesis starts from acetyl CoA which is the result of carbohydrate or fat metabolism. The change in acetyl co-A to mevalonate until cholesterol is mediated by the enzyme HMG CoA reductase. Xanthones work through a mechanism of inhibiting the activity of the HMG CoA reductase enzyme, which can cause inhibition of cholesterol biosynthesis (Botham and Mayes, 2015; Lovita, et al., 2005). Xanthones play a role in the preparation of xanthydrol which is useful in controlling blood cholesterol oxidation Low Density Lipoprotein (LDL) (Jung et al., 2006). Xanthones reduce the concentration of cholesterol in hepatocytes and increase the performance of LDL receptors which are closely related to the components of very low density lipoprotein (VLDL) which causes cholesterol to be reduced (Grundry, 1988).

Decreased levels of sentul chicken blood cholesterol are also affected by another active compound found in mangosteen peel extract, namely flavonoids. Flavonoids contained in mangosteen peel extract can increase the activity of the lipoprotein lipase enzyme. The increase in the enzyme VLDL lipoprotein which carries triglycerides will undergo hydrolysis to fatty acids and glycerol. The released fatty acids are then absorbed by muscles and other tissues that are oxidized to produce energy and by adipose tissue and stored as energy reserves (Marks et al., 2000). Flavonoids can also act as cofactors of cholesterol esterase enzymes and inhibitors of food cholesterol absorption by inhibiting the formation of micelles so that cholesterol absorption is inhibited (Olivera et al., 2007).

The quality of rations supplemented with Cu can improve the metabolic system and physiological processes that are in the body of chickens (Scott et al., 1982). High concentrations of Cu are closely related to high cholesterol levels. This is consistent with Klevay's (1980) study which states that Cu minerals and cholesterol are negatively correlated with plasma cholesterol concentrations. The lack of Cu minerals does not increase cholesterol concentrations in the liver but the bile acids increase and the effect of the increase is not large to reduce blood cholesterol levels. Zn Mineral has various functions in the body, especially for the digestion process. Zn is involved in several enzyme activities and is also a cofactor of more than 70 kinds of enzymes. Minerals Cu and Zn act as protective compounds for mangosteen peel extract, which causes ionization in the digestive tract to increase.

To find out the pattern of the relationship between the effect of using mangosteen peel extract supplemented with Cu and Zn on blood cholesterol levels, an orthogonal polynomial test was performed. The results of the Orthogonal Polynomial Test showed a significant difference ($P<0.05$) in linear regression with the equation $Y = -23.282x + 212.55$, and the coefficient of determination was 0.8234 ($R2 = 0.8234$). The average blood cholesterol level drops with increasing concentration of mangosteen peel extract supplemented with Cu and Zn. The results of the analysis of the coefficient of determination (R2) showed the percentage contribution of free variables (level of mangosteen peel extract supplemented with Cu and Zn) to the dependent variable (blood cholesterol level) was 82.34%.

Effect of treatments on egg yolk cholesterol chicken layer phase

The highest egg yolk cholesterol level in P3 is 149.46 mg/100 g, followed by P1 which is 125.62 mg/100 g, then P0 is 122.25 mg/100 g, P4 is 119.23 mg/100 g, and P2 is 109.48 mg/100 g. The results of the analysis of variance showed that the administration of mangosteen peel extract supplemented with Cu and Zn was not significantly different ($P>0.05$) on egg yolk cholesterol levels.

This study was not y significantly different, but showed an improvement decrease in egg phase yolk cholesterol levels, in P2 is 17.48% compare to control. This is because the active compound extract of flavonoid mangosteen peel can inhibit cholesterol synthesis. According to Metwally et al. (2009) flavonoids reduce cholesterol synthesis by inhibiting the activity of the enzyme acyl-CoA cholesterol acyl transferase (ACAT) which plays a role in

decreasing cholesterol esterification in the intestine and liver. Flavonoids are antioxidants that can reduce cholesterol levels in the blood, the mechanism by which flavonoids inhibit cholesterol synthesis through HMG CoA reductase inhibitors (Chen et al., 2006).

Egg cholesterol is synthesized in the liver, then carried by the blood in the form of lipoproteins and stored in growing follicles and passed on to the ovaries (Hammad et al., 1996). The active compound contained in mangosteen peel extract, namely flavonoids, acts as a phytoestrogen which triggers the biosynthesis of vitellogenin in the liver. Vitellogenin is composed of cholesterol, triglycerides, phospholipids, and proteins (Watson, 2002). Phytoestrogens will stimulate the formation of follicles in the ovary which causes the number of follicles to increase so that the distribution of fat and cholesterol for the development of more follicles which in turn causes a decrease in egg cholesterol. Cholesterol levels in the blood cause the amount of cholesterol that enters the ovaries to be lower.

CONCLUSIONS

1) The administration of mangosteen peel extract supplemented with Cu and Zn minerals has can reduce blood cholesterol, but can not reduce the egg cholesterol chicken phase layer.
2) The dose of mangosteen (*Garcinia mangostana* L.) peel extract as much as 120 mg/kg of rations supplemented with Cu and Zn minerals is the best concentration.

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REFERENCES

Adriani, L., Ana, R, An-An, Y, Andi, M, Nenden, I. (2005). Profil Serum Glutamate Oxaloacetat Transaminase (SGOT) dan Tingkat Glutamat Pyruvate Transaminase (SGPT) Broiler yang Diberikan Juice Noni (*Morinda citrifolia*) dan Gula Sawit (Arengapiata). Universitas Padjadjaran, Indonesia.

Adriani, L., Widjastuti, T., An-An, Y. (2018). Comparative Effect of Mangosteen (*Garcinia mangostana* L.) Peel Extract and Meal Usage In Sentul Chicken Ration, On Blood Biochemistry. Jurnal, Fakultas Peternakan Universitas Padjadjaran, Sumedang.

Baker, D.H., Odle, J., Frank M.A., Wieland, T.M. (1991). Bioavailability of coppr in cupric oxide and in a copper-lysine complex. *Poult. Sci*., 70, 177.

Botham, K.M., Mayes, P.A. (2015). Harper's Illustrated Biochemistry, 30th ed. New York, USA: McGraw Hill Medical Publishing House.

Burns, M.J. (1981). Role of copper in physiological process. *Auburn Vet. J*., 38(1), 12-13.

Chen, H., Yan, X., Zhu, P., Lin, J. (2006). Flavonoids as Dietary Regulators of Nuclear Receptor Activity, US, pp831-844.

Diwyanto, K., Zainuddin, D., Sartika, T., Rahayu, S., Djufri, Arifin, C., Cholil (2011). *Model pengembangan peternakan rakyat terpadu berorientasi agribisnis: komoditas ayam lokal*, Laporan Kerjasama Direktorat Jenderal dengan Balitnak Ciawi, Bogor.

Grundy, S.M. (1998). Hypertriglicridemia, atherogenic dyslipidemia, and the metabolic syndrome. *Am J Cardiol*., 81, 18-25.

Hammad, S. M., Siegel H.S., Marks, H.L. (1996). Dietary cholesterol effects on plasma and yolk cholesterol fraction in selected lines of Japanese-Quail. *Journal Poultry Sci*., 75, 933-942.

Han, C.K., Sung, K.S., Yoon, C.S., Lee, N., Kim, C.S. (1993). Effect of dietary lipids on liver, serum and egg yolk cholesterol contens on laying hens. *AJAS*, 6 (2), 243-248.

Hargis, S.P. (1988). Modifying egg yolk cholesterol in the domestics fowl-a review World poultry. *Science journal*, 44, 17-29.

Johnson, L.R., Engle, T.A. (2003). The effects of copper source and concentration lipid metabolism in growing and finishing angus steers. *Asian Aust. J. Anim. Sci*., 16(8), 1131-1136.

Jung, H. A., Su, B. N., Keller, W. J., Mehta, R. G., Kinghorn, A. D. (2006). Antioxidant Xanthones from The Pericarp of *Garcinia mangostana* (Mangosteen). *J Agric. Food. Chem.*, 54, 2077-2082.

Ketaren, P. (2010). Kebutuhan gizi ternak unggas di Indonesia. *Wartazoa*, 20(4), 172-180.

Klevay, L. M. (1980). The influence of copper and zinc on the occurrence of ischemic heart disease. *J. Environ. Pathol. Toxicol*., 4, 281-287

Kurnia, Y. (2011). *Morfometrik Ayam Sentul, Kampung dan Kedu pada Fase Pertumbuhan dari Umur 1-12 Minggu*. Skripsi. Program Alih Jenis. Departemen Produksi dan Teknologi Peternakan, Fakultas Peternakan, Institut Pertanian Bogor, Bogor.

Marks, D.B., Marks, A.D., Smith, C.M. (2000). Biokimia kedokteran dasar. Jakarta, EGC.

Metwally, M.A.A., El-Gellal., A.M., El-Sawaisi, S.M. (2009). Effect of silymarin on lipid metabolism in rats. *World Appl. Sci. J*., 6(12), 1634-1637.

Miryanti, A, Sapei, L., Budiono, K., Indra, S. (2011). Ekstraksi Antioksidan dari Kulit Manggis (*Garcinia*

mangostina L). Lembaga Penelitian Pengabdian masyarakat, Universitas Katolik Parahyangan Bandung.

National Research Council (NRC) (1994). Nutrient Requirement of Poultry, 9th Revised Edition. Washington DC, USA: National Academy Press Publishing House.

Olivera, T., Ricardo, K.F.S., Almeida, M.R., Costa, M.R., Nagem, T.J. (2007). Hypolipidemic effect of flavonoids and cholestyramine in Rats Tania. *Latin American Journal of Pharmacy*, 26(3), 407-410.

Pogurschi, E.N., Sârbu, N.D., Nicolae, C.G., Zugravu, C.A., Vlad, I., Maftei, M., Marin, M.P., Munteanu, M.F. (2019). Beneficial uses of chromium in laying hens nutrition: a review Scientific Papers. Series D. Animal Science, LXII(2), 65-70.

Rasyaf, A. (1995). Beternak Ayam Pedaging. Cetakan ke 13, Penebar Swadaya, Jakarta.

Scott, M.L. (1982). Nutrition of the Chickens. Second Ed. Ithaca, New York, USA: M.L. Scott and Associates Publishing House.

Sucipto, A. (2008). Kedelai dan Kesehatan. Diakses pada 20 Januari 2020, dari http:/naksara.net/About-Life/Health/kedelai dan kesehatan.html.

Watson, R.R. (2002). Eggs and Health Promotion. USA: Iowa State Press Publishing House.

Widjastuti, T. (1996). Penentuan Efisiensi Penggunaan Protein, Kebutuhan Protein dan Energi untuk Pertumbuhan dan Produksi Telur Ayam Sentul pada Kandang Sistem Cage dan Sistem Litter. Disertasi. Program Pascasarjana Unpad. Bandung.

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RESEARCH ON PERCENTAGE VARIATION CONCERNING COW'S MILK PROTEIN, LACTOSE AND FAT, DEPENDING ON THE SEASON

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Abstract

This research paper consisted in carrying out a study that elucidates how the quality of cow's milk varies depending on the time of lactation initiation (calendar month in which the cows gives birth and the lactation starts). Thus, there were examined milk samples from two groups of cows that gave birth in different seasons (autumn and spring). The determinations concerned were the following: the percentage of protein, the percentage of lactose and the fat percentage in milk. After analyzing the results, compared to the cows that gave birth in autumn, we observed that the percentage of protein increased in the milk from the cows that gave birth in spring, whereas the percentage of fat decreased. The percentage of lactose varied within the similar limits for both experimental groups.

Key words*: composition, cow, milk, season.*

INTRODUCTION

Milk has a high biological value and that is why it is the only consumed product by both human and mammal babies in the first period of their lives. It is the most complete food because it provides all the necessary nutrients for growth and development of the newborn. Having a rich and varied chemical composition, milk ensures most substances that are necessary for living tissue and for maintaining metabolic processes in the organism (Cotor et al., 2012; Codreanu et al., 2012; Mihai et al., 2019; Nistor et al., 2019). Cow milk is an important food for people (due to its special chemical composition and nutritional value) and it also represents a major row material for the manufacture of dairy products (Vidu et al., 2014; Oprea et al., 2019). We should mention that milk and dairy products maintain their freshness and physical-chemical characteristics only if they are properly packaged and stored (Petcu, 2014a; Petcu, 2014b; Visoescu et al., 2015). It is well-known that there are many factors which influence milk production, including: breed, age, individual, ways of feeding and watering, housing conditions, milk management, health status and individual hormonal status etc. (Cotor et al., 2011; Codreanu et al., 2018). Studying the literature, we came across very few data regarding the variation of milk production and its chemical composition during the lactation period, depending on the moment when the lactation started.

This study is important mainly for farmers (cow's milk breeders), as they have the opportunity to organize their farm so that they can obtain raw material for the industry during the whole year (Savu et al., 2002; Petcu, 2006). However, they should take into consideration how the production varies (quantitative and qualitative), depending on the season in which cows gave birth (Tapaloaga et al., 2016; Tapaloaga, 2018).

MATERIALS AND METHODS

We used 20 cows (Holstein breed) in order to carry out this research. They were divided into two experimental groups: first group (n = 10) included cows that gave birth at the beginning of autumn (September-October) and the second group (n = 10) included cows that gave birth at the beginning of spring (February-March).

To evaluate the parameters taken into account, milk samples were collected at different times of lactation.

Regarding the first group, the milk samples were analysed at the end of the first lactation month (October), in the middle of the third month of lactation (December), in the middle of the fifth month of lactation (February) and in the middle of the seventh month of lactation (April).
Regarding the second group, the milk samples were analysed at the end of the first lactation month (March), in the middle of the third month of lactation (May), in the middle of the fifth month of lactation (June) and in the middle of the seventh month of lactation (August).
The evaluated parameters were percentage of protein, the percentage of lactose and the percentage of fat. All the determinations were performed within the laboratory of the milk processing unit.
The protein percentage determination was completed using the protein titration method, the lactose percentage was achieved with the potassium ferricyanide method (Savu et al., 2002) and the fat percentage determination was done by the Gerber method (Ghiță, 2008).
The statistical analysis and the relevance of the assessment concerning the differences between the obtained sets of values, were calculated using the t test (Student).

RESULTS AND DISCUSSIONS

The results are presented in tables and figures for each determined parameter (percentage of protein, percentage of lactose and percentage of fat), being accompanied by interpretations and discussions.

Results and discussions regarding the percentage of milk protein

The evolution of the milk protein percentage during the entire lactation is presented in dynamics in Table 1 and Figure 1, for both experimental groups.

Table 1. The dynamical evolution of the milk protein percentage, for each experimental group

Lot category	Month I	Month II	Month V	Month VII
Lot1	3.70	3.58	3.52	3.80
Lot 2	3.68	3.78*	3.92*	4.06*

*(P<0.05)

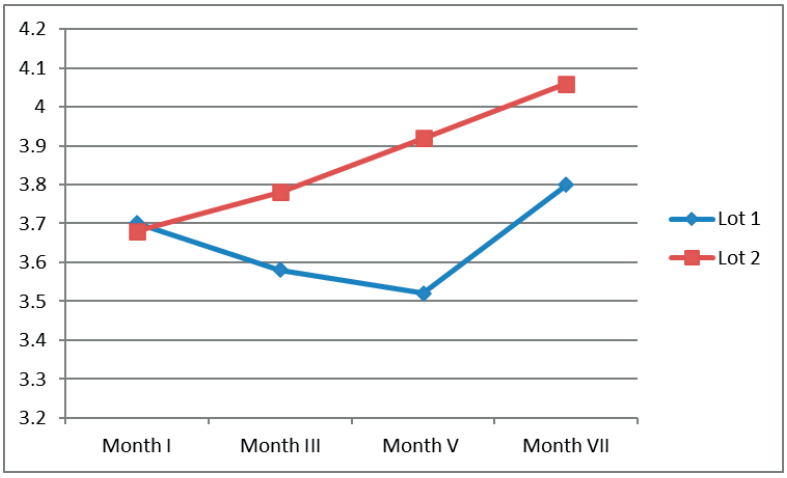

Figure 1. The evolution of the percentage of milk protein in the case of the two experimental groups (red - cows that gave birth in spring; blue - cows that gave birth in autumn)

From the data presented in Table 1 and Figure 1, it is observed that in the case of the cows which gave birth in spring (group 2), the milk protein percentage had continuously increased throughout the lactation with a bimonthly growth rate of 2.7-4.8%. On the other side, the

milk from the cows that gave birth in autumn (group 1), had an initial decrease in the protein percentage until the 5th month, after which, it registered an increase until the 7th month, when exceedances from the initial values by even 2.7%, could be observed. The differences between the two experimental groups in the 3rd, 5th and 7th months of lactation were statistically significant (P<0.05).

These results could be explained by the animal's diet because in the summer, their food provides a higher nutritional contribution, from a qualitative point of view because the green fodder contains consistent amounts of protein, compared to the dry fodder and the silo (that are used as food source in the winter).

These results are similar to those reported in literature by Yang et al. in 2013 and by Bertocchi et al. in 2014.

Results and discussions regarding the milk lactose percentage.

The evolution of the milk lactose percentage during the entire lactation is dynamically presented in Table 2 and Figure 2, for both experimental groups.

Table 2. The dynamical evolution of the milk lactose percentage, for both experimental groups

Lot category	Month I	Month III	Month V	Month VII
Lot 1	4.72	4.70	4.68	4.70
Lot 2	4.70	4.64	4.62	4.68

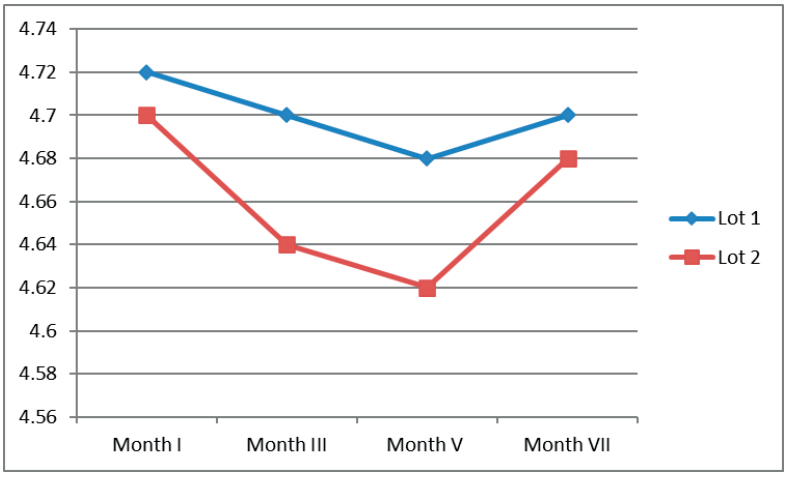

Figure 2. The evolution of the milk lactose percentage, for both experimental groups (red - cows that gave birth in spring; blue - cows that gave birth in autumn)

From the presented data in Table 2 and Figure 2, it is observed that the dynamic of evolution is almost the same in the case of the two experimental groups (the obtained values are similar). The statistical analysis showed that there are no significant differences between the two groups (P>0.05), regarding this parameter. It is obvious that the lactose percentage is not influenced by the diet.

Similar results were obtained in other studies conducted by Cotor et al. in 2015 and by Van Laer et al. in 2015.

Results and discussions regarding the percentage of milk fat

The evolution of the milk fat percentage of during the entire lactation is dynamically presented in Table 3 and Figure 3, for both experimental groups.

Table 3. The dynamical evolution of the milk fat percentage, for both experimental groups

Lot category	Month I	Month III	Month V	Month VII
Lot 1	3.78	4.34	4.51*	4.42*
Lot 2	3.98	3.86	3.74	4.11

*(P<0.05)

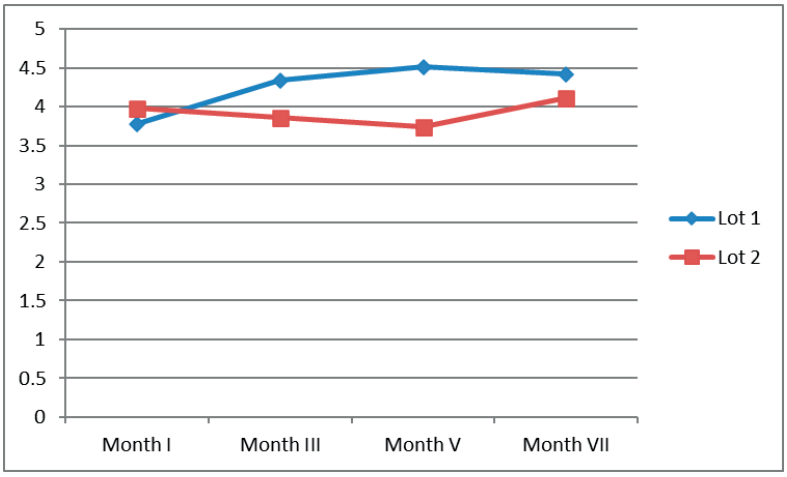

Figure 3. The evolution of the milk fat percentage, for both experimental groups (red - cows that gave birth in spring; blue - cows that gave birth in autumn)

From the presented data, regarding the cows that gave birth in autumn (lot 1), a continuous increase of the milk fat percentage can be observed, with bimonthly rates between 14.82% (3rd month) and 3.92% (5th month).

Regarding the cows that gave birth in spring (lot 2), we initially found a decrease in milk fat percentage until the 5th month, when a decrease of 3.11% was registered, compared to the 3rd month. After that, a huge increase of this parameter was noticed until the 7th month of lactation, at which point an increased value of 9.89% was registered, compared to the 5th month. The differences between the two groups were statistically significant (P<0.05) for the 5th and 7th months of lactation.

These differences are due to the diet. Regarding the cows that gave birth in spring, their lactation took place during the hot season, having had the benefits of succulent fodder, which induced a volumetric increase of milk production, based on the hydric component of milk secretion. This fact led to a dilution of the hydric component and an implicit decrease in fat percentage. At the end of lactation (October), an increase of the analysed parameter is noticed. Our results regarding this parameter, are similar to those obtained by other authors cited in literature (Cotor et al., 2009).

Concerning the cows that gave birth in autumn, their lactation took place in the cold season, so their diet was predominantly represented by coarse fodder. Studying the literature, we noticed that there is a positive correlation between the size of fat globules of milk and the percentage of cellulose from fodder (the main source of volatile fatty acids) (Alstrup et al., 2016). This explains the higher values of this parameter in the case of this group.

CONCLUSIONS

Unlike the ones that gave birth in autumn, the cows that gave birth in spring registered an increase in milk protein percentage during the lactation, with significant differences (P<0.05) for the 3rd, 5th and 7th months.

The milk lactose percentage varied within the same limits for both experimental groups. The differences that we found were not statistically significant (P>0.05).

Unlike the cows that gave birth in spring, the cows that gave birth in autumn registered an

increase in milk fat percentage during the lactation, with significant differences ($P<0.05$) for the 5th and 7th months.

REFERENCES

Alstrup, L., Søegaard, K., Weisbjerg, M.R. (2016), Effects of maturity and harvest season of grass-clover silage and of forage-to-concentrate ratio on milk production of dairy cows. *J. Dairy Sci*., 99(1), 328-40.

Bertocchi, L., Vitali, A., Lacetera, N., Nardone, A., Varisco, G., Bernabucci, U. (2014), Seasonal variations in the composition of Holstein cow's milk and temperature-humidity index relationship. *Animal*, 8(4), 667-674.

Codreanu, I., Dogaru, M., Goran, G.V., Codreanu, M. D. (2012), Observation regarding hematological and biochemical investigation in ruminal acidosis in cattle. *Lucrări Ştiinţifice - Medicină Veterinară, Universitatea de Ştiinţe Agricole şi Medicină Veterinară "Ion Ionescu de la Brad" Iasi*, 55(1), 268-272.

Codreanu, I., Călin, S. (2018). Investigations regarding the influence of the physiological status on some electrolytic, enzymatic and biochemical blood parameters in ewes. *Rev Rom Med Vet*, 28(4), 5-13.

Cotor, G., Gâjâilă, G., Gâjâilă, I., Dobrea, M., Ghiţă, M. (2009). The effect of ambiental temperatures, during the summer season, on the milk fat globule size, in dairy cows. *Scientific Works-University of Agronomical Sciences and Veterinary Medicine, Bucharest Series C, Veterinary Medicine*, 55(3), 88-91.

Cotor, G., Ghiţă, M. (2012). *Theoretical and practical notions of animal biology/Noţiuni teoretice şi practice de biologie animală*. Bucharest, RO: Printech Publishing House.

Cotor, G., Pop, A., Ghiţă, M. (2011). The effect of ovine placenta extract on mammogenesis, lactogenesis, and galactopoiesis in sheep. *Turk. J. Vet. Anim. Sci.,* 35(1), 137-142.

Cotor, G., Gâjâilă, G., Viţălaru, A.B., Ghiţă, M., Brăslaşu, C. (2015). The effect of environmental temperature variation, on milk yield and composition, in dairy cows. *Journal of Biotechnology*, 208, S39.

Ghiţă, M. (2008). *Animal biology (Fundamentals of physiology of animal production with food relevance) - Practical Workbook/Biologie animală (Elemente fundamentale de fiziologie a producţiilor animaliere cu relevanţă alimentară) - Caiet de lucrări practice*. Bucharest, RO: Printech Publishing House.

Mihai, R., Mihalascu, C., Mărginean, G.E., Marin, M.P., Cărătuş, M.A., Vidu, L. (2019). The dynamics of milk production in Montbeliarde breed on a farm in southern Romania. *Scientific Papers. Series D. Animal Science*, LXII (2), 165-169.

Nistor, C.E., Bacila, V., Avram, P., Usturoi, A., Avarvarei, B.V. (2019). Evaluation of raw milk quality gathered from north east area of Romania. *Scientific Papers. Series D. Animal Science*, LXII(2), 289-295.

Oprea, O.D., Petcu, C.D., Ciobotaru-Pîrvu, E. (2019). A study concerning quality assessment and processing particularities in certain dairy products. *Scientific Works. Series C. Veterinary Medicine*, LXV(1), 121-126.

Petcu, C.D. (2006). *HACCP-Food safety guarantor*, Bucharest, RO: Idea Design Publishing House.

Petcu, C.D. (2014a). *Packaging used in the food industry/Ambalaje utilizate în industria alimentară*. Bucharest, RO: Granada Publishing House.

Petcu, C.D., Şulea, C., Dumitrache, M. (2014b). *Audit of Producers/Users of Compressed Air and other Industrial Gases used in the Food Industry*, Quality-Access to Success, 15 (130).

Savu, C., Petcu, C.D. (2002). *Hygiene and control of products of animal origin/Igiena şi controlul produselor de origine animală*. Bucharest, RO: Semne Publishing House.

Tapaloaga, P.R., Tapaloaga, D., Sonea, A., Marin, M. P., Petcu, C.D. (2016), Reproduction activity planning in a beef cow herd in the South of Romania, *Journal of Biotechnology*, 231, S67-S67.

Tăpăloagă, D., Tăpăloagă P. (2018). Sexed semen and the reproductive management of a dairy farm. *Journal of Biotechnology*, 280, S4-S5.

Van Laer, E., Tuyttens, F.A., Ampe, B., Sonck, B., Moons, C.P., Vandaele, L. (2015). Effect of summer conditions and shade on the production and metabolism of Holstein dairy cows on pasture in temperate climate. *Animal*, 9(9), 1547-58.

Vidu, L., Băcilă, V. Udroiu, A., Popa, R., Popa, D, Stanciu, M., Tudorache, M., Custură, I. (2014). Study regarding the production performance of Montbeliarde dairy cows in the southern area of Romania. *Scientific Papers: Series D, Animal Science,* LVII, 216-220.

Visoescu, D.I., Petcu, C.D., Tapaloaga D. (2015). Researches regarding the influence of packaging on the quality of some dairy products. *Journal of Biotechnology*, 208, S19-S19.

Yang, L., Yang, Q., Yi, M., Pang, Z.H., Xiong, B.H. (2013). Effects of seasonal change and parity on raw milk composition and related indices in Chinese Holstein cows in Northern China. *J. Dairy Sci*., 96(11), 6863-6869.

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STUDY ON THE EFFECTIVENESS OF SELF-CONTROL PROGRAMS FOR MYCOTOXINS IN COMPOUND FEED MANUFACTURING

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Abstract

The self-control program is evidence of one's own supervision in the factory, and may include a variety of analyzes (mycological, mycotoxicological, chemical, spectrophotometric). The aim of the paper is to carry out an analysis of the effectiveness of self-control programs for mycotoxins, between January and June 2019, for a factory laboratory in a feed mill from Romania. Methodologically, the data were processed, analyzed and synthesized in the form of graphs and tables. The results show that during the analyzed period, the self-control program for mycotoxins in the factory laboratory provided a total number of 1080 analyzes to be performed, of which 720 for raw materials, and 360 for compound feed; in fact, 455 analyzes were performed (42.1% of the total), of which 255 for raw materials (35.4%) and 200 for compound feed (55.5%). As a result of the study carried out in the feed mill, it is found that the monitoring of the level of mycotoxin contamination should be increased by increasing the degree of accomplishment of the analyzes proposed in the self-control program.

Key words*: compound feed, mycotoxins, raw materials, self-control program.*

INTRODUCTION

Compound feed production in the European Union - 28 increased by 1.8% in 2018, to 163.3 million tons; the compound feed for poultry has increased production by 1.7% (FEFAC, 2019). The efficient and intensive production of meat, milk, eggs and other foods requires mixed and balanced compound feed. Safe feed allows farmers to ensure food safety, reduce production costs, maintain or increase food quality and consistency, and increase animal health and welfare; they can also reduce the potential for pollution caused by animal waste by providing a certain amount of bioavailable nutrients (FAO and IFIF, 2010).

The compound feed and raw materials can be contaminated with unwanted substances, which can come from the environment and/or from the production process. When animals consume such contaminated feed, contaminants can be transferred to food of animal origin, such as milk, meat and eggs (EFISC, 2014).

Mycotoxins are chemical compounds produced by molds. There are literally hundreds of mycotoxins, some of which are used as antibiotics and are known to us, such as penicillin, others are very dangerous, such as aflatoxins, one of the most powerful carcinogens known (Richard, 2015). FAO (Food and Agriculture Organization) estimated that up to 25% of food in the world is significantly contaminated with mycotoxins (Smith et al., 1994). The emergence of the current mycotoxicology began with the discovery of aflatoxins in peanut flour incorporated in the feed of several animal species, including turkeys and poultry, in England in 1961 (Richard, 2007). More than 350 types of mycotoxins have been identified in nature. They differ in terms of their chemical structure and their biological activity; their action may be: carcinogenic (aflatoxin B_1, ochratoxin A, fumonisin B_1), estrogenic (zearalenone), neurotoxic (fumonisin B_1), nephrotoxic (ochratoxin), dermatotoxic (trichothecenes), immunosuppressive (aflatoxin B_1, ochratoxin A, T-2 toxin) (Pop, 2006).

Given the unavoidable presence of mycotoxins, systematic monitoring of raw materials and

finished products intended for human and animal nutrition should be carried out systematically (Psomas and Kafetzopoulos, 2015). Food processing and production include several key points capable of affecting mycotoxin synthesis. Therefore, each technological process for food production and storage should be guided in accordance with the principles of Good Agricultural Practice (GAP), Good Manufacturing Practice (GMP) and systems analysis risks and critical control points (HACCP - Hazard Analysis and Critical Control Points) (Pleadin et al., 2019).

In accordance with Regulation (EC) 183 of 2005 of the European Parliament and of the Council of 12 January 2005 laying down the requirements for feed hygiene, feed companies must establish a self-control procedure and implement self-control programs for their productive activity. The self-control program is the evidence of one's own supervision in factory and may include a variety of analyzes (mycological, mycotoxicological, chemical, spectrophotometric etc.). The self-control program involves the collection of samples by specialized personnel, from the raw materials, from the manufacturing batches, the collection of sanitation tests for the control of the hygiene status of the factory, samples from the water used in the technological process, and their analysis.

The effects of an inappropriate product whose non-conformities have not been identified can be disastrous if they enter the technological process, both for the budget of the organization and for the safety of animals and consumers, which can lead to legal incidents (Pop, 2007).

The aim of the paper is to carry out an analysis of the effectiveness of self-control programs for mycotoxins, between January and June 2019, for a factory laboratory in a feed mill from Romania.

MATERIALS AND METHODS

Methodologically, the self-control programs for mycotoxins from the factory laboratory of a feed mill from Romania were processed, analyzed and synthesized. The self–control programs analyzed corresponded to the period January-June 2019, and the following elements were: mycotoxins to be analyzed, the total number of analyzes proposed to be performed, the number of analyzes to be performed for all raw materials, the number of analyzes to be performed for all types of compound feed; the degree of accomplishment of the analyzes proposed within the mycotoxicological self-control program was monitored for each month from January to June 2019.

To assess the effectiveness of self-control programs for mycotoxins, we followed the results of the analyzes for some raw materials (corn, wheat, soybean meal, sunflower meal) and for the compound feed for broiler in different breeding stages (starter, grower, finisher). The number of analyzes performed and their results were identified, for each month in the set time frame, for each raw material and type of finished product taken into the study.

Quantitative determinations of contamination with: aflatoxin B_1, deoxynivalenol, fumonisin B_1+B_2, toxin T-2, zearalenone, were analyzed, processed, synthesized and interpreted; the quantitative determination of mycotoxins was performed using the ELISA (Enzyme-Linked Immunosorbent Assay) technique.

The results obtained were compared with the maximum level allowed by the European Union legislation.

The interpretation of the data has led to the formulation of conclusions aimed at the elaboration and observance of self–control programs for mycotoxins in compound feed manufacturing.

RESULTS AND DISCUSSIONS

In the production of compound feed, self–control represents the activity carried out by the quality manager, together with all other responsible factors, in order to prevent the introduction of inappropriate raw materials or auxiliaries into the technological process. Within the mycotoxicological self-control program (Table 1), from January to June 2019, for the raw materials and for the compound feed, were determined quantitatively contaminations with aflatoxin B_1, deoxynivalenol (DON), fumonisin B_1+B_2, ochratoxin A (OTA), T-2 toxin (T2), zearalenone (ZEN). For each mycotoxin, a total of 30 analyzes were proposed for each month,

of which 20 for raw materials, and 10 for compound feed. Cumulatively, for each month, a number of 180 mycotoxicological analyzes were proposed, of which 120 for raw materials and 60 for compound feed. In January, 58.3% of the 180 proposed analyzes were performed; out of 120 analyzes for raw materials, 54 were performed, and out of 60 analyzes for finished products, 51. In February, 87.2% of the planned analyzes were performed; 71 analyzes were performed for the raw materials, and 86 for compound feed.

Table 1. Mycotoxicological self-control program (factory laboratory)

Month	Mycotoxins analyzed	Proposed analyzes	Analyzes performed %	Analyzes by product category			
				Raw material	Analyzes performed	Compound feed	Analyzes performed
January 2019	Aflatoxin B_1	30	58.3	20	14	10	6
	Deoxynivalenol	30		20	9	10	11
	Fumonisin B_1+B_2	30		20	10	10	9
	Ochratoxin A	30		20	0	10	0
	T-2 toxin	30		20	16	10	19
	Zearalenone	30		20	5	10	6
Total		**180**		**120**	**54**	**60**	**51**
February 2019	Aflatoxin B_1	30	87.2	20	18	10	28
	Deoxynivalenol	30		20	16	10	20
	Fumonisin B_1+B_2	30		20	10	10	9
	Ochratoxin A	30		20	0	10	0
	T-2 toxin	30		20	10	10	9
	Zearalenone	30		20	17	10	20
Total		**180**		**120**	**71**	**60**	**86**
March 2019	Aflatoxin B_1	30	14.4	20	12	10	14
	Deoxynivalenol	30		20	0	10	0
	Fumonisin B_1+B_2	30		20	0	10	0
	Ochratoxin A	30		20	0	10	0
	T-2 toxin	30		20	0	10	0
	Zearalenone	30		20	0	10	0
Total		**180**		**120**	**12**	**60**	**14**
April 2019	Aflatoxin B_1	30	45.5	20	15	10	7
	Deoxynivalenol	30		20	15	10	7
	Fumonisin B_1+B_2	30		20	13	10	6
	Ochratoxin A	30		20	0	10	0
	T-2 toxin	30		20	0	10	0
	Zearalenone	30		20	13	10	6
Total		**180**		**120**	**56**	**60**	**26**
May 2019	Aflatoxin B_1	30	35.5	20	10	10	4
	Deoxynivalenol	30		20	10	10	6
	Fumonisin B_1+B_2	30		20	6	10	0
	Ochratoxin A	30		20	0	10	0
	T-2 toxin	30		20	14	10	0
	Zearalenone	30		20	10	10	4
Total		**180**		**120**	**50**	**60**	**14**
June 2019	Aflatoxin B_1	30	11.6	20	12	10	0
	Deoxynivalenol	30		20	0	10	9
	Fumonisin B_1+B_2	30		20	0	10	0
	Ochratoxin A	30		20	0	10	0
	T-2 toxin	30		20	0	10	0
	Zearalenone	30		20	0	10	0
Total		**180**		**120**	**12**	**60**	**9**
Grand total		**1080**	**42.1**	**720**	**255**	**360**	**200**

In March, 14.4% of the total analyzes were performed; analyzes were performed for aflatoxin B_1 only (12 for raw materials and 14 for compound feed). During April 45.5% of the proposed analyzes were performed; 56 analyzes for raw materials, and 26 for compound feed.

In May, the degree of analysis was 35.5%; 50 analyzes were performed for raw materials, and 14 for compound feed. In June, a percentage of 11.6% of the total proposed analyzes was achieved; 12 analyzes were performed for the quantitative determination of aflatoxin B_1 contamination of raw materials, and 9 analyzes

for the determination of DON contamination of the compound feed. During the studied time period, no analysis was performed to determine the content of ochratoxin A, which led to a decrease in the percentage of planned analyzes. During the six months taken in the study, the degree of analysis (Figure 1) was fluctuating, and only in two months it exceeded the threshold of 50%.

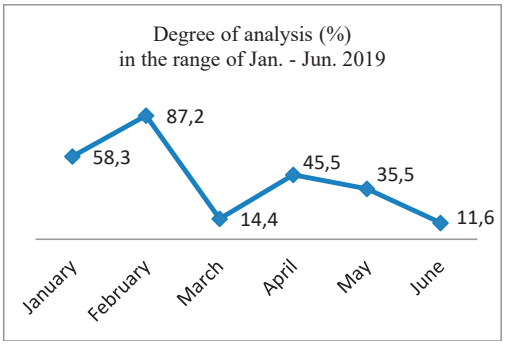

Figure 1. Monthly degree of mycotoxicological analysis from self-control program (%)

In January the level of analysis was 58.3%, followed by an increase in February to 87.2%; in March there was a decrease to 14.4%, followed again by an increase to 45.5% in April; in May and June there were decreases in the number of analyzes, reaching 35.5%, respectively 11.6%.

Between January-June 2019, a total of 455 mycotoxicological analyzes were carried out, of which 255 for raw materials and 200 for compound feed. According to the self-control plan, the total number of analyzes proposed was 1080, of which 720 for raw materials and 360 for compound feed. Considering the data presented above, a general degree of 42.1% of the mycotoxicological analyzes from the self-control program results for the study period.

The results of the analyzes were interpreted and compared with reference to the European Union legislation (Table 2) regarding the maximum permitted limits of mycotoxins in the raw materials and compound feed. The results of the quantitative determinations of mycotoxin contamination (Table 3) for the raw materials (maize, wheat, soybean meal, sunflower meal) and the compound feed for broiler (starter, grower, finisher) indicate that all values are under the limits required by law.

Table 2. Allowed limits for mycotoxins (ppm)

Micotoxyns	Products for animal feed	Maximum permissible limit (ppm)	Normative
Aflatoxin B_1	Feed raw materials	0.02	Reg. EU no. 574/2011
	Compound feed for pigs and poultry, with the exception of young animals	0.02	
DON	Cereals and cereal By-products	8	Rec. EU no. 576/2006
	Maize by–products	12	
	Compound feed for broiler	5	
Fumonisin B_1+B_2	Maize and maize By-products	60	Rec. EU no. 576/2006
	Compound feed for broiler	20	
OTA	Cereals and cereal products	0.25	Rec. EU no. 576/2006
	Compound feed for broiler	0.1	
T-2 Toxin	Cereal based products	0.5	Rec. EU 165/2013
	Compound feed for broiler	0.25	
ZEN	Cereals and cereal products	2	Rec. EU no. 576/2006
	Maize by-products	3	
	Compound feed for pigs	0.25	

For the raw materials studied, 223 mycotoxicological analyzes were performed during the Jan.-June 2019. For the maize 76 analyzes were performed, representing 34% of the total; for 10 analyzes (13.1%) the content of mycotoxins was undetectable. There were 63 analyzes for grain (28.2% of the total); for 7 analyzes (11.1%) the results were non-quantifiable. During the period studied, 53 analyzes (23.1 % of the total) for soybean meal were performed; for 8 analyzes (15%) the content of mycotoxins was undetectable. During the study period, 31 mycotoxicological analyzes were performed for sunflower meal (13.9% of the total); two analyzes (6.4%) had undetectable results.

For compound feed for broiler (starter, grower, finisher) taken in the study, between Jan.-June 2019, 116 mycotoxicological analyzes were performed. During the study period, 33 analyzes (28.4% of the total) were performed for starter compound feed; for 3 analyzes (9%) the content of mycotoxins was undetectable. There were 41 analyzes (35.3% of the total) for grower compound feed; for 5 analyzes (15.1%) the results were non-quantifiable. For the finisher compound feed, 42 mycotoxicological analyzes were performed (36.2% of the total); undetectable results were recorded for 6 analyzes (14.2%).

Table 3. Results of mycotoxicological analyzes (min.-max.)

Product	Month	No. of analyzes	Micotoxyns (ppm)				
			Aflatoxin B_1	DON	Fumonisin B_1+B_2	T-2 Toxin	Zearalenone
Raw materials							
Maize	Jan.	19	0.0010-0.0035	0.035-0.147	0.230-0.310	0.007-0.076	0.015-0.051
	Feb.	12	0.0010-0.0140	0.122	undetectable	undetectable	0.010-0.039
	Mar.	8	0.0007-0.0009	–	–	0.005-0.023	–
	Apr.	18	<0.0006	0.110-0.355	0.053-0.116	–	0.0015-0.0605
	May	15	0.0004-0.0013	0.077	–	0.0076-0.0455	–
	Jun.	4	0.0007-0.0021	–	–	–	–
Wheat	Jan.	10	0.0013-0.0029	0.080-0.095	0.343	0.013-0.410	0.028
	Feb.	8	0.001-0.008	0.072-0.192	0.020	undetectable	0.002-0.660
	Mar.	5	0.0005	–	–	0.007-0.008	–
	Apr.	4	<0.0006	0.144	0.063	–	0.0169
	May	31	0.0005-0.0025	0.002-0.052	undetectable	0.0125-0.0811	0.0021-0.0065
	Jun.	5	0.0002-0.0007	–	–	–	–
Soybean meal	Jan.	7	–	0.064	0.170-0.236	0.014-0.055	–
	Feb.	24	0.0015-0.0089	0.042-0.063	undetectable	0.025-0.048	0.045-0.080
	Mar.	5	0.0008	–	–	0.040-0.043	–
	Apr.	12	<0.0006	0.185-0.262	0.010-0.090	–	0.0031-0.0454
	May	4	0.0014	0.074-0.313	–	–	0.0312
	Jun.	1	0.001	–	–	–	–
Sunflower meal	Jan.	2	–	0.012	–	0.016	–
	Feb.	14	0.0019-0.0060	0.041-0.100	undetectable	0.006-0.010	0.013-0.029
	Mar.	2	0.0019	–	–	0.019	–
	Apr.	12	0.0006-0.0029	0.100	0.001-0.058	–	0.0019-0.0630
	May	0	–	–	–	–	–
	Jun.	1	0.0033	–	–	–	–
Total		**223**					
Compound feed							
Starter	Jan.	11	0.0028-0.0033	0.105-0.111	0.120-0.150	0.009-0.086	0.058-0.061
	Feb.	13	0.0013-0.0080	0.066-0.070	0.020	0.050-0.053	0.021-0.093
	Mar.	2	undetectable	–	–	0.016	–
	Apr.	4	0.0007	0.170	0.008	–	0.0185
	May	3	0.0011	0.070	–	–	0.0122
	Jun.	0	–	–	–	–	–
Grower	Jan.	6	–	0.126-0.154	0.231	0.013-0.051	–
	Feb.	18	0.0007-0.0050	0.057-0.083	undetectable	0.008-0.088	0.023-0.083
	Mar.	7	0.0006	–	–	0.010-0.018	–
	Apr.	6	0.0006-0.0010	0.168-0.224	0.029	–	0.205
	May	4	0.0011	0.090-0.094	–	–	0.0065
	Jun.	0	–	–	–	–	–
Finisher	Jan.	10	0.0031	0.134-0.156	0.244-0.665	0.007-0.051	0.057
	Feb.	24	0.0006-0.0040	0.008-0.080	0.012	undetectable	0.017-0.076
	Mar.	3	0.0012	–	–	0.020	–
	Apr.	4	<0.0006	0.152	0.096	–	0.0297
	May	1	–	0.072	–	–	–
	Jun.	0	–	–	–	–	–
Total		**116**					

CONCLUSIONS

Effectiveness represents the degree of accomplishment of the planned activities. In this study, effectiveness refers to the degree of accomplishment of the activities planned in the self-control program for mycotoxins in the factory laboratory from a feed mill.

In terms of contamination with aflatoxin B_1, deoxynivalenol, fumonisin B_1+B_2, T-2 toxin, and zearalenone, all quantitative determinations of raw materials and compound feed, had

results that met the parameters specified in Rec. 576/2006, Reg. 574/2011, and Rec. 165/2013.

The self-control program for mycotoxins from the factory laboratory provided for the period January-June 2019 a total of 1080 analyzes, of which 720 for raw materials, and 360 for compound feed. Out of the total analysis proposed, 455 were carried out, of which 255 for raw materials and 200 for compound feed. As a percentage, 42.1% of the total proposed analyzes were performed, 35.4% of the proposed analyzes for the raw materials, and 55.5% of the proposed analyzes for the compound feed.

During the period January-June 2019, for the quantitative determination of contamination with the five types of mycotoxins, for the four raw materials studied, 223 analyzes were performed (87.4% of the total raw materials analyzed). For the three types of compound feed for broiler, 116 analyzes were performed to determine the five types of mycotoxins (58% of the total compound feed analyzed).

In the present study, at the reception of the four raw materials analyzed, for mycotoxins were identified values that fall under the limits allowed by the legislation; taking into consideration the homogenization of the raw materials for obtaining the compound feed, the mycotoxins can accumulate and can reach or exceed the maximum values established in the regulations.

As a result of the study carried out in the feed mill, it is found that the monitoring of the level of mycotoxin contamination should be increased by increasing the degree of accomplishment of the analyzes proposed in the self-control programs. The degree to which self-control programs for mycotoxins are fulfillment is of major importance, as an improperly identified unidentified product can have adverse effects on the health of animals and consumers.

REFERENCES

EFISC (2014). *European Guide to good practice for the industrial manufacture of safe feed materials 3.1*. Brussels.

FAO and IFIF (2010). *Good practices for the feed industry - Implementing the Codex Alimentarius Code of Practice on Good Animal Feeding*. FAO Animal Production and Health Manual Rome, 9, 8.

Fédération Européenne des Fabricants d'Aliments Composés (FEFAC), (2019). *Annual Report 2018-2019*. Bruxelles.

Pleadin, J., Frece, J., Markov, K. (2019). Advances in Food and Nutrition Research, Vol 89. Academic Press (Ed.), *Mycotoxins in food and feed* (pp. 297–345). London, UK: Elsevier Publishing House.

Pop, C. (2007). *Quality management/Managementul calității*. Iasi, RO: Alfa Publishing House.

Pop, I. M. (2006). *Feed additives/Aditivi furajeri*. Iasi, RO: TipoMoldova Publishing House.

Psomas, E.L., Kafetzopoulos, D.P. (2015). HACCP Effectiveness Between ISO 22000 Certified and non-Certified Dairy Companies. *Food Control*, 53. 134–139.

Recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding (576/2006).

Recommendation of 27 March 2013 on the presence of T-2 and HT-2 toxin in cereals and cereal products (165/2013).

Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene.

Regulation (EU) No 574/2011 of 16 June 2011 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels for nitrite, melamine, Ambrosia spp. and carry-over of certain coccidiostats and histomonostats and consolidating Annexes I and II thereto.

Richard, J.L. (2007). Some major mycotoxins and their mycotoxicoses - An overview. *International Journal of Food Microbiology*, 119, 3–10.

Richard, J.L. (2015). Romer Labs' Guide to Mycotoxins. Richard, J.L. (Ed.), *Mycotoxins - an overview* (pp. 1–48). Leicestershire, England.

Smit, J.E., Lewis, C.W., Anderson, J.G., Solomons, G.L. (1994). *Mycotoxins in human nutrition and health*. Directorate General XII, Science, Research and Development. European Commission.

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EFFECT OF SUBSTITUTION OF GOROHO BANANA (*Musa acuminafe* sp.) STEM MEAL FERMENTED WITH *Trichoderma viridae* IN RATION ON BLOOD LIPID PROFILES AND MEAT QUALITY OF BROILER CHICKEN

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Abstract

The purpose of this research was to determine the substitution of goroho banana (Musa acuminafe sp.) stem meal fermented with Trichoderma viride on blood lipid profiles and meat quality of broiler chicken. One hundred and twenty day old chick CP 707 divided into 20 unit cages, each unit consisted of 6 chickens. Complatelly Randomised Design (CRD) was used in this research with 4 treatments and 5 replications. The treatments were: R0 = 100% corn without goroho banana stem meal fermented, R1 = 95% corn + 5% goroho banana stem meal fermented, R2 = 90% corn + 10% goroho banana stem meal fermented and R3 = 85% corn + 15% goroho banana stem meal fermented. Parameter measured were blood lipide profiles (triglyceride, total cholesterol, HDL, LDL) and meat cholesterol. The results showed that substitution of goroho banana stem meal fermented had a significant effect (P<0.05) on a decreased in blood trigliseride, blood cholesterol and meat cholesterol, R3 has significant different (P<0.05) lowest compared to the other treatments (R0, R1 and R2). On the other hand there were no significant effect (P>0.05) on blood HDL and LDL among the treatments. It can be concluded that goroho banana (Musa acuminafe sp.) stem meal fermented with Trichoderma viride can be substituted up to 15% corn meal in ration which improved the meat quality.

Key words*: blood lipids, broiler, goroho banana stem, meat cholesterol, Trichoderma viride fermented.*

INTRODUCTION

Goroho banana (*Musa acuminafe* sp.) is one of the typical types of bananas which is very popular with consumers, especially in the areas of the city of Manado and Minahasa so many places selling fried foods that use it because it has a distinctive taste and is consumed by diabetics. As a result, many banana stems are just thrown away as trash that disturbs the aesthetics of the environment. Utilization as a banana bar in general has not been popular, there is no information. The use of goroho banana stems is possible as a feed because in terms of composition the goroho banana stems contain enough nutrients needed by livestock. Chemical analysis shows that the banana goroho stem (*Musa acuminafe* sp.) contains protein (2.53%), fat (1.49%), ash (12.93%) and crude fiber (23.48%) and gross energy 3723 kcal (Laboratory analysis, 2012). Efforts to improve nutrition have been carried out through fermentation with a protein yield of 4.86%, crude fiber 22.03., Fat 0.94, Ca 0.42, P 0.18 and Gross Energy 3156.67 kcal/kg (Najoan et al., 2016). Even though the increase is only about 47.94% for protein and the decrease in crude fiber is only about 6.72% but it is hoped that biologically fermented products have a higher beneficial value in influencing the performance and quality of broiler carcasses, even as a low-fat feed and easy crude fibre exploited can affect the cholesterol content in broiler meat. This content lipid value could be conversed as energy value and accumulated in adipose tissues (Rumokoy, 2012)

Najoan et al. (2019) reported goroho banana stem meal fermented with *Trichoderma viride* have bioactive compounds such as flavonoid, vitamin C, vitamin E and tannin. The present study was conducted to investigate the effect of goroho banana stem meal fermented with *Trichoderma viride* in diet on blood fat profiles and meat cholesterol of broiler chicken.

MATERIALS AND METHODS

The experiment was carried out experimentally using a Completely Randomized Design (Steel and Torrie, 1995) with 4 treatments and 5 replications. Each test uses 5 chickens. The number of chickens used was 100 tails. Treatment consisted of: R0: Without replacement of corn, R1: Replacement of corn by 5% goroho banana stem meal fermented, R2: Replacement of corn by 10% goroho banana stem meal fermented and R3: replacement of corn by 15% goroho banana stem meal fermented.

Material goroho banana stem and fungi *Trichoderma viride* showed in Figures 1 and 2. Fermentation process can be seen in Figure 3. Parameters observed were serum cholesterol, triglycerides, LDL, and HDL and meat cholesterol. The composition of nutrients and metabolic energy ration constituent feed ingredients, feed composition ration treatment and nutrient composition of experiment can be seen in Table 1.

Table 1. Composition and nutrients contents of diet

Feed ingredients	Treatments			
	R0	R1	R2	R3
	----------%----------			
Yellow corn	57.00	54.15	51.30	48.45
Goroho stem meal Fermentation	0.00	2.85	5.70	8.55
Fine rice bran	5.00	5.00	5.00	5.00
Coconut meal	9.00	9.00	9.00	9.00
Soybean meal	15.00	15.00	15.00	15.00
Fish meal	12.00	12.00	12.00	12.00
Coconut oil	1.00	1.00	1.00	1.00
Top Mix	1.00	1.00	1.00	1.00
Nutrien	R0	R1	R2	R3
Crude Protein (%)	21.37	21.18	20.88	20.82
Crut fiber (%)	8.37	8.26	8.16	8.05
Crude fat (%)	4.12	4.73	5.34	5.96
Calcium (%)	1.24	1.23	1.23	1.22
Phospor (%)	1.03	1.29	0.99	0.98
Metabolizable energy (kcal/kg)	3101.23	3101.08	3100.3	3100.06

(*) Analyses of Laboratory Chemistry and Animal Feed of Faculty of Animal Science, Padjadjaran University, UNPAD (2016)

Blood samples were taken at the end of the study with 1 chicken in each unit of the Experiment. Blood samples taken through the brachial vein then analyzed to determine cholesterol, triglyceride, HDL and LDL level, and meat samples on the breast and thighs were analyzed to determine meat cholesterol.

Figure 1. Goroho Banana Stem

Figure 2. Fungi *Tricoderma viridae*

Figure 3. Fermentation process

RESULTS AND DISCUSSIONS

The results of the measurement of total cholesterol, triglyceride, HDL, LDL and meat cholesterol are presented in Table 2. The results of statistical analysis showed that GE addition in ration had significant effect ($P<0.05$).

Table 2. The level of LDL, HDL, and cholesterol

Parameters	Level of goroho banana stem meal			
	R0	R1	R2	R3
Triglyceride (mg/dl)	67.60	61.00	53.80	42.00
LDL (mg/dl)	37.20	36.40	36.00	35.00
HDL (mg/dl	77.20	78.00	78.60	79.20
Total cholesterol (mg/mg))	143.20	133.60	124.40	118.60
Meat cholesterol	124.60	100.20	89.20	68.80

Triglycerides

There was a significant ($P < 0.05$) decrease in the level of triglyceride in birds use banana goroho stem meal of treatment (Table 2). HDL level compared to R0, R1, R2, and R3. HDL is a lipoprotein which maintains the balance of cholesterol so that it is not accumulated in the cell. This balance is managed by the sterol slough off from membrane at the same rate with the number of cholesterol synthesis entering the liver (Hasanudin et al., 2013). Triglycerides can be found as grains of levels the body of broilers are less than 150 mg/dl. Triglyceride measured in this study was found in treatment R0 (67.60 mg/dl) and decrease with supplementation of goroho banana stem meal fermented levels 15% (R3) (42.00 mg/dl). The indicate goroho banana stem meal fermented is have a flavonoids content. Farida et al. (2009) reported that banana peel flavonoids can enhance the activity and levels lipoprotein lipase and there by lower triglyceride levels.

Besides decrease, the triglyceride levels in the blood caused the goroho banana stem meal fermented have saponin and tannins content that able to inhibit the absorption of triglycerides in the intestine so that reducing the number of triglycerides in the blood. This work is in line with research Noorrafiqi et al. (2013) state that the saponins can bind the bile salts and cholesterol to micelles forms that cannot be absorbed by the intestine. Also, the saponin will increase the cell regeneration that influences the decrease in triglycerides. Lipid substance in broiler meat could influence its quality (Rumokoy and Toar, 2014).

Low Density Lipoprotein (LDL)

Average serum LDL level of broiler chicken in the present study was presented in Table 2. There is a no significant ($P<0.05$) difference between R0, R1, R2 and R3. It showed that the LDL level in all treatment groups remained within in the normal range of ≤ 130 ml/dl. This indicates that chickens feather meal did not give significant effect to Broiler LDL blood level ($P > 0.05$). It was in line with others researchers who worked with different feed mixtures state that banana goroho stem meal fermented give no effect on LDL levels in broiler blood.

LDL content in this research tendency decrease in the levels of goroho banana stem meal fermented this indicates. Decreased blood cholesterol levels are followed bay LDL levels because there is a direct relationship between cholesterol and LDL (the higher the blood cholesterol level, the higher the LDL levels). This is reinforced by the opinion of Motgomery et al. (1993) that LDL plays a role in providing cholesterol in the body tissue, so LDL levels in the blood are influenced by cholesterol concentrations. LDL levels in the study range 35.00-37.20 mg/dl, above normal LDL < 130 mg/dl (Basmatioglu and Ergul, 2015).

Hight Density Lipoprotein (HDL)

Average serum HDL level of broiler chicken in the present study was presented in Table 2. There is a no significant difference ($P < 0.05$). HDL is a lipoprotein which maintains the balance of cholesterol so that it is not accumulated in the cell. HDL (High Density Lipoprotein) often named as a good cholesterol because it is a lipoprotein that transports lipid from periphery to hepar (Hasanudin et al., 2013). The function of HDL is to carry the remain cholesterol which is not being used into the liver. This remain cholesterol will be using as a precursor in the formation of bile salt and steroid hormones. The remain cholesterol which is not being used will be excreted (Rosidi I., 2003). HDL The normal HDL levels in the blood of broiler chickens is 66.5-97.7 mg/dl (Situmorang and Martha, 2014), whereas according to Basmaciglu and Ergul, 2005, is ≥ 22 mg/dl. In this study, HDL levels presented in Table 2. It showed HDL there is increasing tendency, but one-way ANOVA analysis of HDL level showed that there was no significant difference between control (R0). Guilaume et al. (2006) who reported the flavonoid can increase HDL. According to Muray et al. (2012), HDL is a lipoprotein that transports lipids from the periphery to the liver. The HDL molecule is relatively small compared to other lipoproteins, so it can pass through the vascular endothelial cells and enter the intima to transport back the cholesterol collected in the macrophages. In addition, HDL also has anti-oxidant properties that can prevent the occurrence of LDL oxidation

Total Cholesterol

Cholesterol is an essential structural component that forms the cell membrane and the lipoprotein externa layer. In the body, cholesterol is the precursor of all steroidal compounds. The quantification results of total cholesterol were presented in Table 2. The normal standard of total cholesterol of broiler chickens is 128-140 mg/dl according to Silva et al. (2007). It showed that the total cholesterol in this study range (118.40-143.20 mg/dl) as the normal range.

The lowest levels of total cholesterol in treatment were due to a goroho banana stem meal fermented contents a bioactive compounds such as flavonoids, tannin and vitamin. Hashemi and Davoodi, 2011, state that the compounds have bioactive substances can increase the metabolism of carbohydrates, proteins and fats in the body.

Further explained that the compound inhibits absorption of cholesterol in the intestine, so causing a decrease in cholesterol concentration of cholesterol in the blood. This matter will lead to increase of cholesterol synthesis in the liver for conversion into bile acid and secreted in to the intestine. This process leads to increase the cholesterol excretion through the faces and therefore contributes to decreasing the cholesterol levels in the blood. Asmarini (2012) state that the bile acid secretion is required cholesterols the primary raw material. That is lead to increase the acid bile secretion and influence on the decrease of cholesterol levels in the blood.

Meat Cholesterol

Broiler fed diet containing goroho banana stem meal fermented in the diet produce meat with low cholesterol content showed in Table 2. The low cholesterol content of meat was obtained with the addition of gohoro banana stem meal fermented replace corn in the diet. The higher level to addition of goroho banana stem meal in the diet, the lower cholesterol content in meat produced. Flavonoid in goroho banana stem meal fermented has an effect to reduce cholesterol in blood serum trough inhibiting micelle formation in small intestine so that decrease intestinal cholesterol absorption (Vermeer et al., 2008). While cholesterol in the body can be eliminated through its conversion by liver into bile acids which is bound to glycine and taurine to form bile salts and secreted to duodenum which is then degraded by microbes in the gut and excreted together with faeces, so that body cholesterol content decrease. Decrease in meat cholesterol levels along with an increase in the level of banana goroho stem meal fermented of 15 % in the ration until 118 mg/100 g. The lower meat cholesterol content in this treatment is due to the increasing use banana goroho stem meal fermented in the ration, so that the content of bioactive compound increases in the diet. Increasing the content of bioactive compounds such as β-carotene, flavonoid and vitamin. Bioactive compounds inhibit the action of the enzyme HMG-CoA reductase (Hydroxymetyl glutary-CoA) which plays a role in the formation is inhibited cholesterol (MCGilvery and Goldstein, 1996; Nuraini, 2006)

CONCLUSIONS

Goroho banana (*Musa acuminafe* sp.) stem meal fermented with *Trichoderma viridae* can be substituted up to 15% corn meal in ration which improved the meat quality.

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REFERENCES

Asmariani, W., Probosan, E. (2012). Pengaruh Pemberian Buah Pepaya (*Carica papaya* L.) Terhadap Kadar Kolesterol LDL dan Kolesterol HDL pada Tikus Sprague Dawilwey dengan Hipokolesterolemia. *J.Nutr.College*, 1(1), 268-264.

Basmaciglu. I., Ergul, A. (2005). Reseach on the factor affecting cholesterol content and some other characteristic of eggs in laying hens. *Turk.J.Vet Anim.Scie.*, 29, 157-164.

Guilaume, R, Sonia, P., Patrick, C., Simone, L., Benoid, M., Charles, C. (2006). Favorable impact of lower caloric cranberry juice, consumption on plasma HDL cholesterol, caloric cranberry juice consumes on plasma HDL cholesterol concentration. *J.Nutr*., 6, 234-245.

Hashemi, S.R., Davoodi, H. (2011). Herbal Plants and their Derivatives as Growth and Health promoters in Animal Nutrition. *Vet Res Commun*., 35, 169-180.

Mongomery, et al. (1993). Biokimia Suatu Pendekatan Biooritasi Kasus Yokyakarta, Universitas Gajah Mada.

Murray, R.K., Bender, D.A., Bothan, K.M., Kennelly, P.J., Weil, P.A, Rodwell, V.W. (2012). *Harper's Illustrated Biochemistry*. USA: The Mc Graw-Hill Companies. Inc. Publishing House.

Najoan, F., Wolayan, B., Bagau, F.N., Sompie, N. (2017). The effect of *Trichoderma viridae* usage of nutritional value on goroho banana stem (*Musa acuminafe* sp). *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development*, 17(1).

Noorrafiqi, M.I., Yasmina A., Hendriyono, F.X. (2013). Efek jus buah karamunting (*Melastoma malabathricum* l.) terhadap kadar trigliserida serum darah tikus putih yang diinduksi propiltiourasil. *Berkala Kedokteran*, 9(2), 219-227

Rumokoy, L.J.M. (2012). Precursor adipocyte development as media of lipid metabolism. *Jurnal Lassalian*, 9(2), 25-31.

Rumokoy, L.J.M., Toar, W.L. (2014). *The content of lipids in intramuscular adipose as a quality determinant of cattle meat production. International Congress: Challenges of Biotechnological Research in Food & Health*, 144-146. http://repo.unsrat.ac.id/976/1/Publikasi_Laurentius-Wisje_di_Surakarta_2015.pdf

Silva, P.R.L., Freitas Neto, O.C., Laurentiz, A.C., Junqueira O.M., Fagliari, J.J. (2007). Blood serum components and serum protein test of hybro-PG broilers of different ages. *Rev. Bras. Cienc. Avic*., 9, 229-232.

Steel, R. G.D., Torrie, J.H. (1980). *Principles and Procedures of Statistics: A Biometric Approach*. New York, USA: McGraw-Hill Book Co. Publishing House.

Situmorang, R., Martha, I.K. (2014). Perbedaan kadar triglyceride setelah pemberian eksrak dan rebusan daun salam (*Eugenia polyantha*) pada tikus Spraque dawley yang diberiinggi lemak. *J. Nutr college*, 3, 26-33.

Vermeer, M.A., Mulder, T.P.J., Molhuizen, H.O.F. (2008). Thea amins from black tea, especially thea flavin-3-gallate, reduce the incorporation of cholesterol into mixed micelles. J. *Agric. Food Chem.*, 56, 12031-12036.

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DEVELOPMENT OF SMALL-SCALE FARMING IN NORTH SULAWESI IN PANDEMIC COVID-19 SITUATION

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Abstract

The Covid-19 pandemic period has spread across almost all countries in the world has resulted a significant negative impact on various socio-economic aspects, including on large-scale and small-scale livestock business activities. This paper aims to present a study of challenges in developing small-scale farms carried out at grassroots level communities affected by the Covid-19 pandemic in North Sulawesi Indonesia. The method used in this study was a meta-analysis pattern approach. The cases of covid-19 infection in this area began to emerge early on March of this year, where data until early May 2020 showed a number of people who were confirmed positive Covid-19 had reached 45 people. The government as an authority holder in North Sulawesi Province does not apply yet a lockdown. This temporary policy gives farmers the opportunity to move and continue developing their livestock activities in order to support the fulfillment of livestock products in local market, while on the other hand, feed row material tended to increase in the market. The recommendation to keep a physical distance did not significantly affect the activities to the small-scale animal farms because in general the workers were family who were the same people in a household.

Key words*: Livestock, small-scale, Covid-19 pandemic.*

INTRODUCTION

No one knows exactly when this pandemic will end. The visible effect is that many companies have reduced their workforce significantly, affecting their purchasing power for their important needs.

After increasing the cases of new corona virus (2019-nCov) various countries or regions applied lockdowns or other similar policy were applied indicating by a high social activity restriction and therefore that many parties in the community in the location have been experienced a difficulty in obtaining certain raw material especially the foodstuffs because they have been absorbed by panic buyers. This phenomenon had appeared in North Sulawesi in the beginning of the pandemic effect raised in this region.

Economic performance which a ratio between income and purchasing power become less balanced. On the other hand, the path of importing animal food is also experiencing obstacles due to restrictions on transportation to and from exporting countries, which can cause an imbalance between food availability and consumer needs.

These factors can drag the volume of food into scarcity which if not addressed properly early will in the future experience a food crisis in the region.

The struggle of farmers until know who run small-scale livestock in North Sulawesi province lead their own business still exist in this situation. This effort is an important matter that can help in sustaining food availability for the community.

Small-scale livestock that are generally raised are livestock genetic diversity (Hoffmann, 2010). Small scale animal husbandry can be sporadically found in various villages scattered in several districts in North Sulawesi.

Small scale livestock business is very vulnerable to losses due to limited capital funds. They are very dependent on price fluctuations in the market. Small-scale animal

husbandry activity is important to support the availability of food, and it is also useful to support the farmer's economy.

MATERIALS AND METHODS

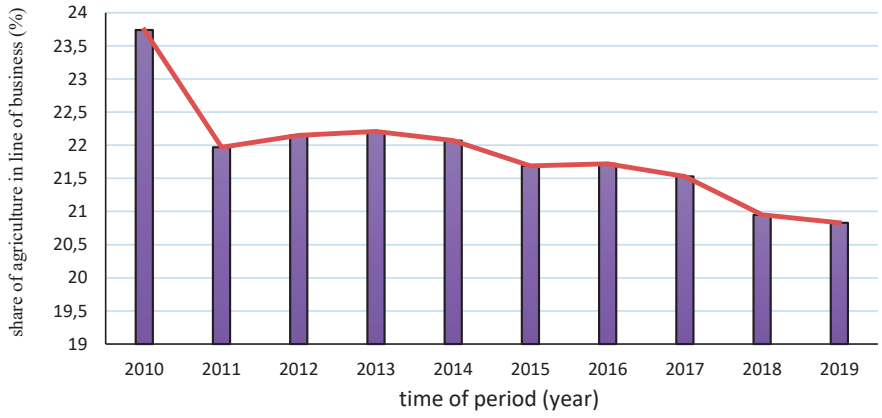
The method used in this study was a meta-analysis pattern approach which several sources information concerning the condition and the policies of authorities in connection with the labor, economic growth, and animal feed alternative application, had been used to support the discussion of this paper. The official discussions, reports, including the scientific paper were used to get the concerned information.

RESULTS AND DISCUSSIONS

Data on the economic structure in the area of North Sulawesi Province in August 2019 showed that among 1.13 million of population workers then 28.52% (the most) had a main employment in agriculture compared to other fields. This situation changed in the early pandemic Covid-19 period in this area where this number decreased to 21.13% in the first quarter (BPS, 2020). Pratomo (2020) reported that there was an increase for livestock commodities in North Sulawesi in 2019 consisting of: beef production reaching 3,552.66 tons, equivalent to 102.92% of the 2019 regional medium-term program target; pork production, as many as 25,661.01 tons in 2019. That amount is equivalent to 130.42% of the target of 19,675.75 tons. Whereas the production of local chicken meat in this region in 2019 produced 2,703.62 tons above the target.

The graph in Figure 1 shows that there are still wide-open opportunities to increase agricultural output leading to an increase in agricultural participation rates including livestock production in 2020. A parameter could be a participation rates of agriculture in line of business in North Sulawesi in spite of the pandemic of Covid-19 moment.

The unprecedented physical distancing inclines practical efforts in working livestock everywhere including. According to Bahri dan Tiesnamurti (2012) at least it is necessary to foster small-scale farm management for communities that rely on the potential of local natural resources to manage the agriculture farming.



Source: BPS *(processed)*

Figure 1. Share of agriculture in line of business in North Sulawesi

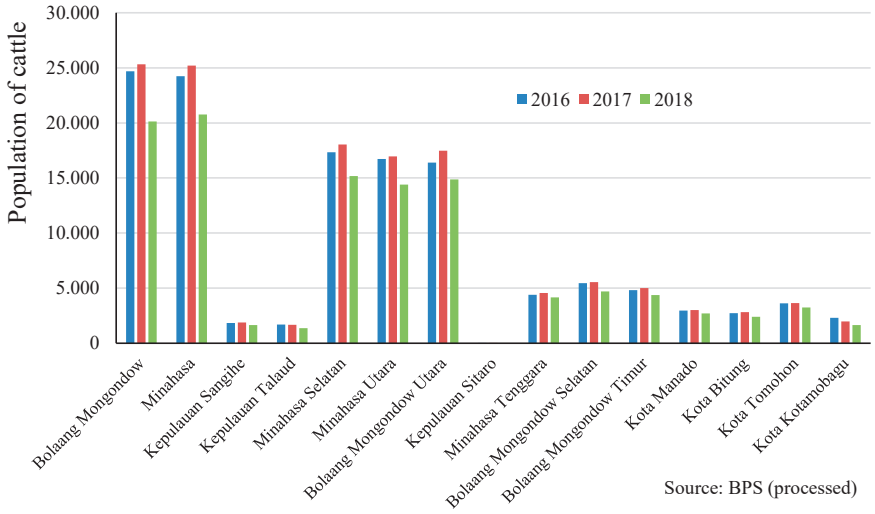


Figure 2. Population of cattle in the areas of North Sulawesi

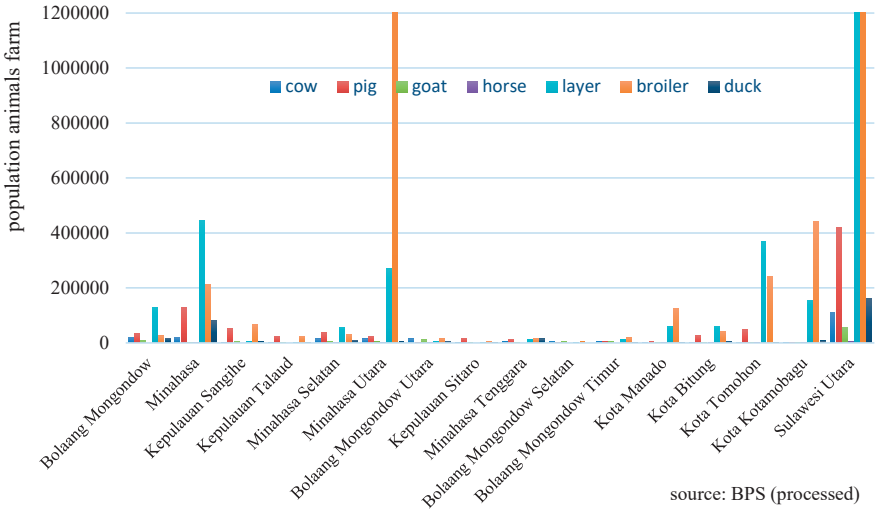


Figure 3. The growth of animal farm population in areas of North Sulawesi in 2018

The Covid-19 pandemic issue has had a major impact on health and the global economy. In Indonesia, as has happened in other countries, this impact has been very visible since March 2020. The number of UMKM (small business, and medium credit) scattered in Indonesia existed by several domain including, agriculture, animal husbandry, forestry, fisheries have been affected by the pandemic. An example that occurred in April 2020 as reported by Detik-Finance (2020) is that some farmers have stated directly that they experienced the 'blow' effect of the Covid-19 pandemic in the form of a drop in the selling price of their livestock production to half of the usual price.

The livestock sector has an influence especially on large scale farms as a consequence of labor

and the transportation path for animal feed ingredients. Besides social restrictions or physical distancing greatly affected the activities and performance of employees in large livestock companies. This was to prevent more cases of new corona virus infection. This condition was a challenge but also an opportunity for small scale farms in areas such as North Sulawesi.

Figure 4. Ongole Crossbreed (PO) cattle foraged under a coconut plantation (*Photo taken by: Laurentius J.M. Rumokoy*)

Figure 5. Piglets in a small-scale farm in North Sulawesi (*Photo taken by: Laurentius J.M. Rumokoy*)

The development of livestock population was directly related to several elements such as livestock production which are supported by the potential of natural resources and the large market demand for consumption of beef cattle products (Otoluwa et al., 2016). Data from BPS concerning cattle farm in North Sulawesi is shown in Figure 2, there were some areas in 2018 which had a population of cattle above 10,000, as well as in Bolaang Mongondow, Minahasa, South Minahasa, North Minahasa, and North Bolaang Mongondow. These figures are accumulative figures derived from small-scale cattle ranches that were scattered in each district areas.

Figure 6. Local breed chicken in an extensive farm (*Photo taken by: Laurentius J.M. Rumokoy*)

The cattle farms in North Sulawesi in general are small-scale (Rumokoy and Toar, 2015). Multiple functions of this farm are: farming labor, transporting agricultural products in some areas, and as livestock producing meat for human consumption. According to Rembang (2017).

The local beef cattle in North Sulawesi plays an important role as a provider of employment, absorbing family labor, livestock labor, transportation equipment, savings, hobbies, determining social status, and as beef cattle, and it could change forage for livestock feed or by-products of agriculture into value-added products. Local natural resources, especially insects that are easily obtained in the environment in various tropical regions, can be used both for feed formulation (Toar et al., 2019), and also as a promotion of livestock immunity (Rumokoy et al., 2017)

The supporting factor for a development of local beef cattle is the increasing market demand for beef, availability of large workforce, and also an existence of government

policies (Kariyasa, 2015). Various natural resources as mentioned by Toar et al. (2018), Toar et al. (2017) and Manangkot et al. (2014) available in the North Sulawesi region, are very supportive in the development of small-scale livestock, because by utilizing them, there is no need to depend on industrial feed which is relatively difficult to find during a pandemic.

Proper health control of livestock will be able to sustain the development of small-scale animal husbandry businesses because of the way they are raised is susceptible to infection by disease agents (Rumokoy and Toar, 2014).

In line with the effort in infrastructure development and farmer corporations is to accelerate the improvement of food production and export as well as improving the welfare of farmers. The potential of economy growth of North Sulawesi in the first quarter of 2020 grew 4.27%. Production side, growth was driven by most of the business fields, with the highest growth achieved by the Information and Communication Business Field which grew by 19.33% (BPS, 2020).

CONCLUSIONS

The development of small-scale farms can be realized well if it is supported by the availability of a well-trained workforce. The sustainable management of local natural resources in the context of developing small-scale farms can be a good example in an effort to improve the people's economy while caring for the environment.

REFERENCES

BPS (2020). Badan Pusat Statistik. https://www.bps.go.id.

Bahri, S., Tiesnamurti, B. (2012). Strategy on Sustainable Livestock Development by Using Local Resources. *J. Litbang Pert.*, 31(4), 142-152.

Detik-finance (2020). *Peternak Ayam 'Babak Belur' Dihantam Dampak Corona*. Detik-fincance. Online media, 16 April 2020. https://finance.detik.com/berita-ekonomi-bisnis/d-4979469/peternak-ayam-babak-belur-dihantam-dampak-corona.

Distanak-Pemprov-Sulut (2018). Fokus RKP pada Tahun 2019: Pemerataan Pembangunan. https://distanak.sulutprov.go.id/home/detailpost?res=24.

Hoffman, J.I.E. (2015). Biostatistics formedical and biomedical practitioners: An Interpretative Guide for Medicine and Biology. 770 pages. Elsevier. https://doi.org/10.1016/C2014-0-02732-3.

Hoffmann, I. (2010). Livestock biodiversity. *Rev. Sci. Tech*., 29(1), 73-86.

Irham, M. (2020). Virus corona: UMKM diterjang pandemi Covid-19 sampai 'kembang kempis'. *BBC News Indonesia.* Accessed on 19 March 2020. https://www.bbc.com/indonesia/indonesia-51946817.

IAARD (Indonesian Agency for Agricultural Research and Development) (2014). Regional Workshop Eco-Health on Poultry Production Cluster. info Aktual 13 Mei 2014. http://www.litbang.pertanian.go.id/info-aktual/1722/

Kariyasa, K. (2005). Sistem integrasi tanaman-ternak dalam perspektif reorientasi kebijakan subsidi pupuk dan peningkatan pendapatan petani. *Analisis Kebijakan Pemerintah Pertanian*, 3(1), 68-80.

Manado-Post (2020a). Pertanian Diminta Genjot Pertumbuhan Ekonomi. ManadoPost. 17 February, 2020. http://manadopost.id/read/2020/02/17/Pertanian-Diminta-Genjot-Pertumbuhan-Ekonomi/65900.

Manado-Post (2020b). Permintaan daging ayam menurun, pengusaha banting harga. https://manado.tribunnews.com/2020/04/24/permintaan-daging-ayam-menurun-pengusaha-banting-harga

Manangkot, H.J., Rondonuwu, S.J., Pinontoan, O.R., Najoan, M., Rumokoy, L.J.M. (2014). Black soldier fly larvae malure degradation as fish meal replacer in native chicken ration. Lucrari stiintifice - *Seria Zootehnie,* 62, 139-142. http://www.uaiasi.ro/zootehnie/Pdf/Pdf_Vol_62/Heidy_Manangkot.pdf

Pratomo, M.N. (2020). Lampaui Target, Ini Pencapaian Produksi Pertanian & Peternakan Sulut 2019. Bisnis.com Sulawesi 06 February 2020. https://sulawesi.bisnis.com/read/20200206/540/1198207/lampaui-target-ini-pencapaian-produksi-pertanian-peternakan-sulut-2019.

Rembang, V.L.H. (2017). *Analisis Efisiensi Pemasaran Ternak Sapi Potong Di Provinsi Sulawesi Utara.* Doctor Thesis. Universitas Brawijaya Malang. http://repository.ub.ac.id/9138/

Rumokoy, L.J.M., Toar, W.L. (2014). The equine colostrums of milk treatment against pathogenic agent. *Scientific Papers Series D. Animal Science*, 52, 174-177.

Rumokoy, L., Adiani, S., Kaunang, C., Toar, W.L., Kiroh, H. (2017). The effect of combination of crude salive gland extract of *Stomoxys calcitrans* (Diptera: Muscidae) with colostrum immunoglobulin on IgG serum level of young horses. *Scientific Papers. Series D. Animal Science*, LX, 253-256.

Rumokoy, L.J.M., Toar, W.L. (2015). The paradox of nutrient fulfilement and immunity challenge on chicken livestock development in tropical humid regions. *Agriculture and Agricultural Science Procedia,* 6, 259-264.

Otoluwa, M.A., Salendu, A.H.S., Rintjap, A.K., Massie, M.T. (2016). Prospek pengembangan usaha ternak sapi potong di Kecamatan Bolaang Timur Kabupaten Bolaang Mongondow Utara. *Jurnal Zootek*., 36(1), 191-197.

Toar, W.L., Rumokoy, L.J.M., Pudjihastuti, E., Manangkot, H., Bagau, B., Untu, I.M. (2019). Effect of Supplementation of Combination of Curcuma and BSF Maggot Meal in Rations on Accumulative Weight of Native Chickens. *IOP Conference Series: Earth and Environmental Science*, 372. 012009. doi:10.1088/1755-1315/372/1/012009

Toar, W.L., Tulung, M., Memah, V., Pudjihastuti, E., Rumokoy, Untu, I.M. (2018). The presence of insect in animal farm L., in North Sulawesi. *Scientific Papers. Series D. Animal Science*, LXI(1), 220-224.

Toar, W.L., Kaunang, C., Untu, I.M., Rumokoy, L., Kiroh, H. (2017). The Empowerment of Crude Extract Antigen-G of Insect on Goats Immunity Enhancement. An Entomology Contribution in Animal Husbandry. *Scientific Papers. Series D. Animal Science*, LX, 271-273.

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A DEVELOPMENT STRATEGY OF SMALL-SCALE GOATS FARM IN PANDEMIC COVID-19

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Abstract

Small-scale goat farm with local breeds which are often referred to 'kambing kacang' in Minahasa area of North Sulawesi Province, which is generally one of the livestock species that are farmed by people in rural areas. Simple maintenance patterns with relatively small maintenance costs become a reason why this type of livestock is selected by the community to maintain this type of livestock. The purpose of this article is to present a scientific study concerning strategies to develop local goat farms that are generally small scale. The method used in this was a field study and combination of quantitative and qualitative approach using various data from scientific references. The use of local feed for local goat production and online marketing is an option that can help to develop this farm during the Covid-19 pandemic.

Key words*: covid-19, goat production, Minahasa region*

INTRODUCTION

Farmers' goats almost characterized by local breeds which locally called 'kambing kacang'. This type of animal in the North Sulawesi areas is generally become one of livestock that are found in rural areas, even can also be found in certain locations in suburbs of the cities. The local goats are farmed privately by households in small quantities.

The livestock of goat in North Sulawesi Province are generally classified as small-scale farms that are handled extensively. Although in terms of numbers are classified as a small quantity, but this type of farm is officially categorized as a livestock. Definition of a livestock in Indonesia was regulated in Act (PP) 2016 No. 4, article 1, section 2. This regulation states that: livestock *('ternak')* are animals which its products are intended to produce food, industrial raw materials, services, and/or its by-products related to agriculture.

Local goat products are mainly for meat products. In order these animals are able to produce meat in good level quantitatively and qualitatively, it need a development strategy in accordance with the natural, socio-economic conditions of the people and the country in the situation of Covid-19 pandemic. These animals are able to "conjure" forage and plants or other material found all regency and cities in North Sulawesi area that are not used by other livestock animals into meat products. This ability is positively relied upon by farmers with limited capital to be a side activity in sustaining their economy. While a part of the farmer did not aware many potential resources naturel could be applied in their farm (Toar et al., 2019)

The existence of local goat livestock and natural resources that are spread in all regencies and cities in North Sulawesi, suggests a development of local goat livestock has great potential to support food security in the region at the current pandemic.

MATERIALS AND METHODS

The combination of quantitative and qualitative approach using various data from scientific references as well as field observation. Survey on extensive goat farms figure was carried out in the field of five regions by evaluating the

condition of goat house and the availability of naturally forages around the farms. Values made from 0 to 100 and divided into 5 grade.

RESULTS AND DISCUSSIONS

We found that the farmer realized an extensive goat livestock activity as a side livelihood to support household source of revenue. The livestock were handled in small quantity by household. This meat product of local goats attracted people as a local culinary destination (Saeroji, 2017), therefore this type of livestock has a great opportunity to be developed into an important business object, or at least as a source of animal protein for general consumption (Figure 1).

Figure 1. A small-scale of extensive livestock of goat *(kambing kacang)* in Minahasa region

The strategy in developing small scale goat farms in the North Sulawesi region during the pandemic period needs to be based on several foundations as follows: In general, this type of animal husbandry needs to be developed because it can help to fulfill animal protein sources for local communities, which synergize with efforts to accomplish national food which is oriented towards a sustainable packaging (FAO, 2014).

Another common problem which is the basis of this development is if the situation of the number of people confirmed to be positive of Covid-19 continues still increase, it will certainly have a negative impact on the productive activities of the people which are directly correlated with the economic situation.

In this point, it is needed to be anticipated to the future times where there will be a decline in food production and in food distribution. Under these conditions small-scale farms carried out by families will help in supporting local food availability, consequently business from household will be able to develop because it automatically helps maintain or improve the economy of farmers. In this situation new farmer could be formed from the people who experienced layoffs, or lost their daily job. In point of view of natural resources, local goat farming is one of the important types of livestock in the area of North Sulawesi because various of this animal products will support the local food availability. Figure 2 shows a comparison of the number of goats compared to cattle and pigs in North Sulawesi areas in the year of 2018. Although the population of goats does not dominate but goat livestock products contributed to consumers in this area.

At that time the number of pigs dominated the three types of mammals, but the figure shows that small scale of local goat livestock existed in all regency and cities in North Sulawesi.

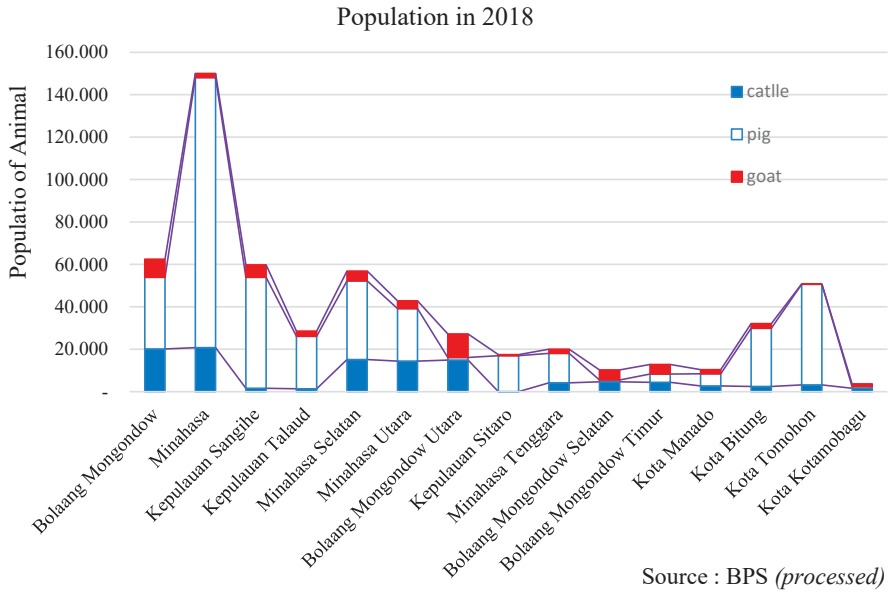

Figure 2. The comparison of population of cattle, pig and goat in North Sulawesi regions in 2018

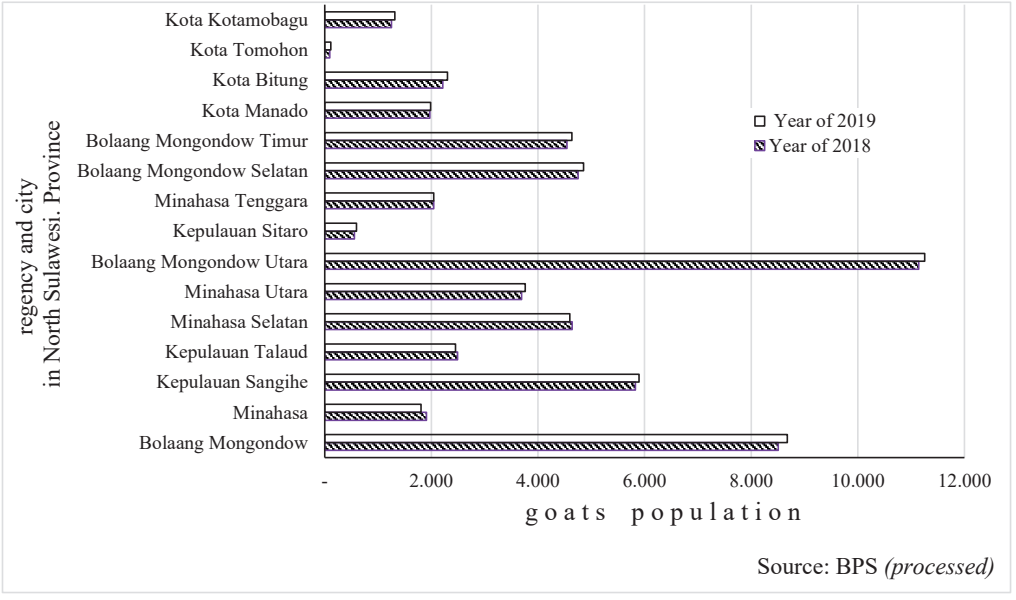

Figure 3. The growth of goat farm population in North Sulawesi in 2018 and 2019

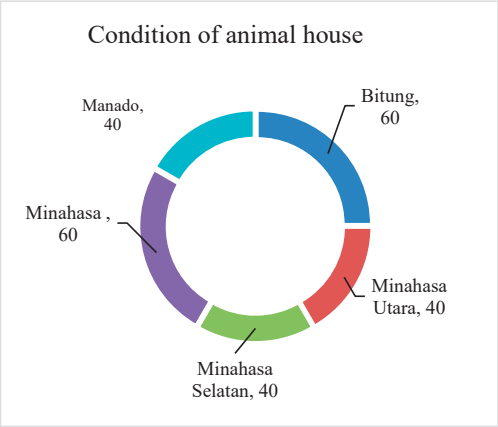

Figure 4. Condition of animal house in extensive livestock of goats observed in some places

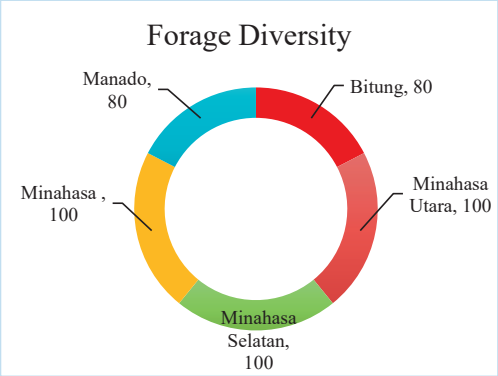

Figure 5. The availability of forage around the location of livestock in some areas

The development of the number of goats in North Sulawesi is shown in Figure 2. The number tended to increase in almost all regions from 2018 to 2019, excepted in the area of South Minahasa, Talaud Islands and Minahasa which experienced a slight decline.

The Figure 3 indicate that the people of North Sulawesi in general already have experience in conducting goat breeding as a local food source, although the amount varies by location.

To anticipate the handling of food availability decreasing as a result of this co-pandemic19, the development of goat livestock needs to be taken into account, moreover this product can be consumed by people with diverse socio-cultural including religious backgrounds.

In reality, goat meat is favored by the majority of the population of Indonesia, especially in North Sulawesi, which in terms of culinary goat meat dishes is considered as a special food that is served at various public thanksgiving events, thus this livestock meat product is easily marketed in this area.

North Sulawesi region has a variety of forages as goats feed. These plants grow easily at various temperatures and humidity in this area (Rumokoy and Toar, 2014). Concentrate feed can be made from agricultural by-products such as bran, cassava, ground corn, table salt and calcium (Distanpangan, 2018). The ability of these animals to convert various food sources to produce meat and milk (Darcan and Silanikove, 2018), by consuming grasses, legumes, and seeds (Rahman et al., 2014)

The ability of local goats to produce meat by consumption various types of plants, even though it is classified as a mediocre quality plant, it is a concern to be developed in difficult situation that happened in this Covid-19 pandemic.

On the other hands this type of goat is generally categorized as an animal that is quite easy to maintain because physiologically it is able to adapt to an environmental condition, including in the dry season (González-Pech et al., 2015ş Raducuta et al., 2015).

The development strategy will be pursued by taking into account important steps to achieve the objectives in the context of developing small scale goat farming during the Covid-19 pandemic in the all regions. If there is a PSSB (large-scale social restrictions) to be applied, it should still provide space for internal household activities in conducting livestock activities so that they can produce food as one of the basic needs of the community in general. On the other hand, social solidarity action needs to be continued for people who experience limited sources of funding to get their basic needs, including for small-scale community farmers who are very affected by this pandemic.

Development activities must refer to government policies and remain oriented to the applicable laws and regulations so that a business undertaken by a community is legal and remains respectful at a dimensions of community life and the public interest. This includes, among others, controlling so that livestock do not interfere with the plant

cultivation around goat farms, even in small numbers.

Formulate health protocol standards set by the government related to carrying out agricultural activities including private livestock activities from households to minimize the risk of transmitting the new Corona virus. A tradition handling in a livestock existed various health problems (Rumokoy and Toar, 2014), keep providing technical assistance and small loans in the context of the integrated farming system development (Reynold, 2002).

Technical guidance is still deemed necessary by goat breeders which can be achieved by strengthening the reproduction of goats (Monintja et al., 2016) simultaneously both online and directly in the field assistance concerning:

CONCLUSIONS

Pandemic Covid-19 demands various efforts to provide sufficient food for humans. To anticipate the decline in food supply and distribution in areas including North Sulawesi, various efforts need to be made, especially those that can support the community's economy. This can be achieved by developing local goat farms.

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REFERENCES

[BPS]-Statistic of Sulawesi Utara Province (2020). Sulawesi Utara Province in Figures 2020. Bada Pusat Statistik Provinsi Sulawesi Utara. Catalog 1102001.71. https://sulut.bps.go.id

Koluman Darcan, N.K., Silanikove, S. (2018). The advantages of goats for future adaptation to Climate Change: A conceptual overview. *Small Ruminant Research*, 163, 34-38.

[Distanpangan] Pemkab Magelang (2018). Cara sederhana pembuatan konsetrat sapi/domba. Dinas Pertanian dan Pangan Kabupaten. Fri. 12. 2018. http://distanpangan.magelangkab.go.id/home/detail/cara-sederhana-pembuatan-konsentrat-sapi-domba/281.

FAO - Food and Agriculture Organisation of United Nations. (2014). Sustainable goat breeding and goat farming in central and eastern Europeans countries. European Regional Conference on Goats. 7 – 3 April 2014. http://www.fao.org/3/a-i5437e.pdf

Monintja, F.J., Hendrik, M.J., Pudjihastuti, E., Ngangi, L.R. (2016). Pengamatan potensi reproduksi kambing betina yang dipelihara secara tradisional di daerah pesisir Kecamatan Tombariri Kabupaten Minahasa. *Jurnal Zootec*., 36(2), 466-475.

González-Pech, P.G., de Jesús Torres-Acosta, J.P., Sandoval-Castro, C.A., Tun-Garrido, J. (2015). Feeding behavior of sheep and goats in a deciduous tropical forest during the dry season: The same menu consumed differently. *Small Ruminant Research*, 133, 128-134.

Răducută, I., Călin, I., Ion (Răducută), E., Nicolae, C.G., Marin, M.P., Priseceanu, H.I. (2015). Research on milk production at goats from Carpathian breed in relation with breeding system. *Agrolife Scientific Journal*, 4(1), 137-140.

Rahman, M.M., Abdullah, R.B., Wan Khadijah, W.E., Nakagawa T., Akashi, R. (2014). Feed intake and growth performance of goats fed with Napier grass and oil palm frond supplemented with soya waste. *Journal of Applied Animal Research*, 43 (3), 256-260.

Reynold, S.G. (2002). Coconuts, cattle and integrated farming systems in Zansibar, Tanzania. *Cocon Journ*., 12.

Rumokoy, L.J.M., Toar, W. L. (2014). The equine colostrums of milk treatment against pathogenic agent. *Scientific Papers. Series D. Animal Science*, LVII, 174-180.

Rumokoy, L.J.M., Toar, W.L. (2014). The forage production of *Brachiaria mutica* under coconut tree canopy. Lucrări Ştiinţifice-Seria Zootehnie, 62, 131-134.

Saeroji, A., Wijaya, D.A. (2017). Pemetaan Wisata Kuliner Khas Kota Surakarta. *Jurnal Pariwisata Terapan*, 1(1), 13-27.

Toar, W.L., Rahasia, C., Sumolang, C., Oroh, F., Untu, I., Najoan, M. (2019). Responses of farmers regarding the role of fly insects against skin defects of local cattle. *Scientific Papers, Series D. Animal Science*, 62(1), 203-207.

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SERUM LIPID PROFILE OF BROILER CONSUMING RATION CONTAINING LAURIC ACID AND FEED FIBER

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Abstract

The aim of this experiment was to evaluate effect of level lauric acid (LA) combined with feed fiber (FF) in ration on serum lipid profile of broiler. A total of 360 unsex 1-d old broiler chicks were obtain from broiler breeding company. They were kept in brooding cage with temperature 23-33°C on a light/dark cycle until 7-d. After 7-d adjustment period, all bird randomly assigned to 12 treatments combination. Each group having three replicate cages with 30 birds was fed diet according experimental design. The experiment was conducted in a completely randomized design with a 3 x 4 factorial arrangement. The first factor was level of LA consisted of 3 levels i.e. 1.30%; 1.95%; and 2.60%, while the second was level of FF i.e. 5%, 6%, 7%, and 8% in the diet. Each treatment was given diet from 21 to 35 day of age during the experimental period. Effect of treatment on serum lipid consists of total cholesterol, triglyceride, HDL-cholesterol, and LDL-cholesterol was determined at the end of experiment (35-d of age). The conclusion of this study indicated that the used of 1.95% LA and 8% FF level on the diet would have an optimize serum lipid profile of broiler.

Key words*: feed fiber, lauric acid, serum lipid profile.*

INTRODUCTION

Functional food is defined as a compound that contains physiologically active compounds (bioactive compounds) and is used for the prevention or cure of a disease or for achieving optimal body health. Increasing the economic status of the community, causing consumption of animal products to increase as well. The essential components in the community's diet are meat and processed meat products (Fernandez-Gines et al., 2005).

According to WHO in 2010, 25% of deaths in developing countries were due to cardiovascular disease. Cardiovascular disease and type-2 diabetes are considered as a result of increased consumption of animal products. Much research has been done to dismiss this assumption.

The results showed that total fat, saturated fatty acids and monounsaturated fatty acids were not associated with deaths from coronary heart disease, and consumption of saturated fatty acids was not related to the risk of coronary heart disease, stroke and cardiovascular disease.

Pure lauric acid (LA) can be used in feed with the same function as coconut oil as an energy source as well as a source of saturated fatty acids. Medium chain fatty acids (MCFA) in LA contain 95.5% lauric acid (Londok et al., 2018). The superiority of MCFA over long chain fatty acids (LCFA) is its metabolic processes in the body. MCFA has a smaller molecular weight so it does not require high energy and only requires a little enzyme to break down the fat into a form that is ready to be absorbed (Gheorghe et al., 2019; Papamandjaris et al., 1998; DebMandal and Mandal, 2015). Crude fiber is one of the important food substances in poultry rations, because it functions to stimulate the digestive tract peristalsis so that the digestion process of feed substances goes well. Poultry has limitations in digesting coarse fibers because the fermenter organ is located at the end of the absorptive organ. High crude fiber causes poultry to feel full, which can reduce consumption because crude fiber is voluminous. The level of energy in the feed will determine the amount of feed consumed. Broilers tend to increase their consumption if the metabolic energy content in feed is low.

Feed formulation by optimizing the use of fiber-rich feed ingredients can reduce meat fat content so that it becomes a safe/healthy meat product for consumers.

Strengthening the supply of food from animal experiences is a dilemma because on one hand meat consumption per capita is still low but on the other hand there is a tendency for certain consumers to limit consumption of livestock meat because of the negative effect of food on health. This issue is certainly a challenge for animal husbandry experts on how to develop businesses that can produce livestock commodities with carcasses that have high edible meat portions as a source of food that is safe and healthy for consumers. Food security which aims to increase the availability of food based on food security and independence, improving the quality of consumption and improving the quality and food safety.

MATERIALS AND METHODS

This study was conducted on 360 day-old unsexed Lohman broilers (MB 202-P) with an average body weight of 45.76 ± 1.73 grams, obtained from the breeding of PT Japfa Comfeed Indonesia Tbk chickens. Poultry Breeding Division Unit 13 Kauditan, Jl. Raya Manado-Bitung, Tumaluntung Village, North Minahasa Regency.

The chicken was placed in a brooder cage at a temperature of 23-33ºC for 7 days. After the random adjustment period the chickens were placed in 12 treatment plots which were repeated 3 times. The study was designed using a completely randomized 3x4 factorial design. As factor A is the level of lauric acid (LA), which is A1 = 1.30%, A2 = 1.95%, and A3 = 2.60%. Factor B is the level of feed fiber (FF), namely B1 = 5%, B2 = 6%, B3 = 7%, and B4 = 8%. Determination of LA level based on the application of coconut oil in feed by 3% which is calculated lauric acid content.

There are 12 treatment combinations. LA used in this study was pure LA (99.5%) FA 1299 rays produced by Sinar Mas. The composition of the feed and its ingredients are presented in Table 1.

Table 1. Composition and ingredient of treatment feed (*as fed*)

Items	A1 B1	A1 B2	A1 B3	A1 B4	A2 B1	A2 B2	A2 B3	A2 B4	A3 B1	A3 B2	A3 B3	A3 B4
Ingredients (%)												
Yellow Corn	31.7	22.7	20.7	8.70	26.05	27.05	20.05	8.05	29.4	23.4	19.4	11.4
SBM	22.0	20.0	23.0	7.0	16.0	19.0	23.0	7.0	21.0	20.0	23.0	7.0
Fish meal	5.0	5.0	6.0	9.0	6.0	6.0	6.0	9.0	5.0	5.0	6.0	8.0
Rice bran	14.0	20.0	28.0	28.0	11.0	21.0	28.0	29.0	14.0	20.0	28.0	29.0
MBM	5.0	4.5	4.0	7.0	5.0	4.0	7.0	6.0	5.0	4.5	4.0	7.0
BR-21F	20.0	25.5	16.0	38.0	19.0	16.0	37.0	32.0	22.0	23.5	16.0	34.0
LA	**1.30**	**1.30**	**1.30**	**1.30**	**1.95**	**1.95**	**1.95**	**1.95**	**2.60**	**2.60**	**2.60**	**2.60**
NaCl	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
DL-methionine (99%)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Premix[1]	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Total	100	100	100	100	100	100	100	100	100	100	100	100
Nutrient content												
ME (Kcal/kg)	3125	3157	3159	3125	3287	3169	3148	3205	3207	3200	3171	3219
CP (%)	20.4	20.3	20.1	20.5	20.5	19.9	20.3	20.3	20.2	19.9	20.4	19.5
EE (%)	5.8	5.9	5.9	6.0	6.7	6.6	6.5	8.0	7.1	7.2	7.2	8.0
CF (%)	**5.1**	**6.3**	**7.2**	**8.1**	**5.1**	**6.2**	**7.2**	**8.2**	**5.1**	**6.2**	**7.2**	**8.1**

SBM, soy bean meal; MBM, meat bone meal; BR-21F, commercial feed; NaCl, natrium chloride; ME, metabolizable energy; CP, crude protein; EE, extract ether; CF, crude fiber. [1]Premix supplied the following per ton of diet: Iron, 40 mg; Copper, 26.16 mg; Zinc, 40 mg; Manganese, 44 mg; Selenium, 0.08 mg; Cobalt, 0.08 mg; Iodine, 0.52 mg; Vit A, 12500 IU; Vit D3, 35000 IU; Vit E, 25 IU; Vit K3, 4 mg; Vit B1, 4 mg; Vit B2, 8 mg; Vit B6, 20 mg; Vit B12, 50 mcg; Pantothenic acid, 15 mg; Niacin, 50 mg; Biotin, 125 mcg; Calcium D-pantothenate, 16.30 mg; Folic acid, 1 mg.

The treatment ration was made at a metabolic energy level between 3100-3200 kcal kg^{-1} with a crude protein level of 20%. The treatment feed and drinking water were applied ad libitum on days 21 to 35 days to the experimental chicken, so that each treatment combination was applied to 30 chickens. An interactions were shown by variables of total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol.

Measurement of serum fat is done through laboratory analysis of total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol. Blood sampling for parameter determination was performed at the end of the study (day 35), in chickens that were fasted for ± 8 hours.

Overall data were analysed using the general linear model in Minitab (version 19). The difference in the mean value of the treatment was tested by Tukey simultaneous test. The difference was evaluated at the P level, 0.05.

RESULTS AND DISCUSSIONS

The average of total cholesterol, triglycerides (TG), HDL-cholesterol, LDL-cholesterol and the ratio of HDL to LDL in chicken serum results of this study were presented in Table 2. Total cholesterol, TG, HDL-cholesterol, LDL-cholesterol and HDL to serum LDL ratio were statistically significant influenced by the level of lauric acid, the level of feed fiber and the interaction of both. The different effect of blood lipids was thought to be caused by differences in fatty acids in the feed, because the transport of MCFA (lauric acid) is not in the form of lipoproteins. In the body, cholesterol is transported through the bloodstream by a type of fat called lipoprotein. There were several types of lipoproteins that were known, namely: very low density lipoprotein (VLDL) and LDL. These types of lipoproteins all contain a lot of TG and cholesterol. VLDL had more TG (60%), whereas LDL and HDL had more cholesterol (44%) (Piliang and Djojosoebagio, 2006). VLDL-cholesterol is usually released into the bloodstream continuously by the liver. This condition ensures a steady supply of TG to other cells of the body as a source of energy for metabolic processes. This increases lipoproteins that are rich in cholesterol particles, in the form of LDL. MCFA does not reduce cholesterol through the transport of LDL cholesterol to the liver but by other mechanisms. Apolipoprotein B (Apo-B) is the main apolipoprotein from chylomicrons and LDL-cholesterol which is responsible for bringing cholesterol to the tissues. Apo-B in MCFA is lower than LCFA (Wang et al. 2015).

The interaction between levelof LA and the level of crude fiber ($P < 0.05$) influences total cholesterol levels, TG, HDL-cholesterol, LDL-cholesterol and the ratio of HDL to LDL serum. The highest total serum cholesterol level was shown by the chicken consumming 1.95% LA and 6% FF (109.67 ± 0.28 $mg.dL^{-1}$), while the lowest concentration was shown by the fed chicken supplemented with 2.6% LA with 7% FF (103.59 ± 0.28 $mg.dL^{-1}$). Saturated fats decrease LDL receptor (LDLr) activity and increase LDL production (Merchant et al., 2008). LDLr is the key to regulating cholesterol homeostasis feedback (Musa et al*.,* 2007). The absorption of LDL cholesterol from peripheral tissues is mediated by LDLr. LDLr is negatively correlated with serum LDL and abdominal fat percentage (Murwani et al., 2011). When LDLr is high, the serum LDL and abdominal fat are low. Research on adding palm oil and coconut oil to broiler feed was carried out by Piliang et al. (1995). It was reported that a decrease in total cholesterol, HDL and serum LDL. Dong et al. (2003), using coconut oil as a source of MCFA in pregnant mice. The use of coconut oil decreases TG, increasing serum HDL-cholesterol at the age of 3 weeks. Fatimah and Rindengan (2011) found the same thing using pure coconut oil in rat feed. Londok et al. (2014) supplemented virgin coconut oil as a source of lauric acid up to 3% in high fiber-based feed in broiler feed, it was able to increase serum HDL, reduce serum LDL and reduce meat cholesterol. Furthermore Londok (2018) supplemented 3% coconut oil with natural antioxidant Areca vestiaria Giseke at a dose of 1250 $mg.kg^{-1}$ showing a marked increase in HDL and a decrease in LDL. Wang et al. (2015) uses coconut oil and soybean oil as a source of MCFA in broiler feed. Linearly, total cholesterol and LDL decreased, but TG increased while HDL did not differ significantly.

Table 2. Average of lipid profile in broiler serum consumed different level of LA and FF (mg dL^{-1})

Parameters	LA Level (%)	FF Level (%)				Average
		5	6	7	8	
Total cholesterol (mg dL^{-1})	1.30	103.91±1.07^{AB}	109.67±0.28^{A}	103.59±0.28^{AB}	101.01±1.16^{BC}	104.54±3.65
	1.95	94.75±1.93C^{DE}	104.96±3.89^{AB}	107.46±3.04^{AB}	103.26±2.27^{AB}	102.61±5.51
	2.60	95.58±2.76^{CD}	91.44±2.49^{DE}	88.12±1.93^{E}	89.08±4.29^{DE}	91.05±3.32
	Average	98.08±5.06	102.02±9.46	99.72±10.23	97.78±7.62	
Triglycerides (mg dL^{-1})	1.30	47.64±2.37^{ABC}	45.87±2.03^{ABCD}	47.04±3.19^{ABC}	46.87±2.37^{ABC}	46.85±0.78.
	1.95	41.59±2.25^{BCD}	36.59±4.28^{D}	43.68±2.14^{ABCD}	40.77±2.31^{BCD}	40.66±2.98
	2.60	48.85±4.23^{AB}	40.39±5.00^{BCD}	38.85±5.00^{CD}	52.23±1.47^{A}	45.08±6.49
	Average	46.03±3.89	40.95±4.67	43.19±4.12	46.62±5.74	
HDL-cholesterol (mg dL^{-1})	1.30	52.28±1.11^{DE}	55.74±1.61^{DE}	59.28±2.43^{BCD}	49.31±3.58^{E}	54.15±4.31
	1.95	66.63±2.82^{AB}	67.79±3.15 ^{AB}	68.22±5.44^{AB}	56.60±3.96^{CDE}	64.81±5.51
	2.60	70.45±1.48^{A}	65.87±2.60^{ABC}	60.19±3.83^{BCD}	56.92±3.15^{CDE}	63.36±6.00
	Average	63.12±9.58	63.13±6.48	62.56±4.92	54.28±4.30	
LDL-cholesterol (mg dL^{-1})	1.30	42.10±1.71^{A}	44.76±1.74^{A}	34.91±3.34^{BC}	42.33±2.93^{A}	41.02±4.25
	1.95	19.80±1.34^{D}	29.85±0.12^{C}	30.50±1.97^{C}	38.50±2.15^{AB}	29.66±7.66
	2.60	15.36±0.43^{D}	20.36±3.05^{D}	20.16±4.76^{D}	21.72±0.85^{D}	19.40±2.78
	Average	25.76±14.33	31.65±12.30	28.52±7.57	34.18±10.96	
HDL: LDL ratio	1.30	1.24±0.08^{D}	1.25±0.08^{D}	1.72±0.23^{CD}	1.17±0.17^{D}	1.35±0.25
	1.95	3.39±0.37^{B}	2.27±0.11^{BCD}	2.26±0.32^{BCD}	1.85±0.66^{CD}	2.44±0.66
	2.60	4.59±0.03^{A}	3.05±0.37^{B}	3.21±0.95^{B}	2.62±0.04^{BC}	3.37±0.85
	Average	3.07±1.69	2.19±0.90	2.40±0.75	1.88±0.72	

The average ratio of HDL to LDL in serum of experimental chickens that consume feed supplemented with different lauric sources and antioxidant concentrations provides a very significant interaction ($P<0.01$) in influencing serum HDL: LDL ratio. The highest average serum HDL: LDL ratio was shown by chicken fed a combination of feed containing 2.6% LA with 5% dietary fiber (4.59 ± 0.03 mg.dL^{-1}), while the lowest HDL: LDL ratio was shown by a combination of 1.3% LA with 8 % FF (1.17 ± 0.17 mg.dL^{-1}). Coronary atherosclerosis is associated with a high plasma cholesterol-HDL: LDL ratio (Mayes et al. 1995). Each 5% increase in energy consumption from saturated fat is proportional to the energy consumption from carbohydrate and causes a 17% increase in the risk of heart disease (Hu et al. 1997). However Kolondam et al. (2008), concluded that administration of virgin coconut oil (VCO) with therapeutic doses (0.95 mL) and two doses in wistar rats (*Rattus norvegicus*) had no effect on blood lipid levels after being consumed for 30 days, increasing the level of VCO did not cause changes in blood lipid levels during the study. Other results also showed that changes in blood lipid levels after administration of virgin coconut oil did not result in changes in the risk of heart disease in test animals, and claims about the dangers of saturated fat consumption in VCO against increased blood lipids were not proven.

CONCLUSIONS

The use of lauric acid 1.95% with 8% feed fiber level provides optimal broiler serum lipid profile.

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REFERENCES

Al-Taleb, S.S. (2003). Effect of an early feed restriction productive performance and carcass quality. *J. Biol. Sci.*, 3, 607-611.

Benevent. (1981). *Quelques Aspects de la Croissance Chez les Animaux Supperieur delevage*. ENSA-Montpellier.

Bortoluzzi, Fernandes, C., Contini, J.I.M., Gurski, J.P., Esser, T.J., Prokoski, A.F.G. (2013). Quantiative feed restriction from 35 to 42 days of age for broiler chicken. *Rev Bras Saude Prod Anim*., 14(4), 778-784.

Broiler Performance and Nutrition Supplement. 2015. (http://www.cobb-vantress.com/docs)

Butzen, F.M., Riberio, A.M.L., Vieira, M.M., Kessler, A.M., Dadalt, J.C., Della, M.P. (2013). Early feed restriction in broiler. I-Performance, body fraction weights, and meat quality. *J. Appl. Poult. Res.*, 22, 251-259.

DebMandal, M., Mandal, S. 2011. Coconut (*Cocos nucifera* L.: Arecaceae): In health promotion and disease prevention. *Asian Pac. J. Trop. Med.,* 4(3), 241-247.

Fernandez-Gines, J.M., Fernandez-Lopez, J., Sayas-Barbera, E., Perez-Alvares, J.A.. 2005. Meat products as functional foods: a review. *J. Food Sci.,* 70(2), 37-43.

Gheorghe, A., Habeanu, M., Tabuc, C., Marin, M. (2019). Effects of dietary pea seeds (*Pisum sativum* L. cv. Tudor) on performance, carcass traits, plasma biochemistry and intestinal microflora in broiler chicks. AgroLife Scientific Journal, 8(1), 99-106.

Jahanpour, H., Seidavi, A., Qotbi, A.A.A., Van Den Hoven, R., Rocha e Silva, S., Laudadio, V., Tuvarelli, V. (2015). Effects of the level and duration of feeding restriction on carcass component of broiler. *Arch. Anim. Breed.,* 58, 99-105.

Leeson, S., Summers, J.D. (2008). *Commercial Poultry Nutrition.* 3rd ed. Nottingham, UK: Nottingham University Pr.

Lohmann Meat. (2007). *Broiler Stock Performance Objectives*. Albama, US: Aviagen.

Londok, J.J.M.R., Manalu, W., Wiryawan, KG., Sumiati. (2017). Growth performance, carcass characteristics and fatty acids profile of broilers supplemented with lauric acid and natural antioxidant from *Areca vestiaria* Giseke. *Pakistan Journal of Nutrition*, 16(9), 719-730.

Londok, J.J.M.R., Tulung, B., Kowel, Y.H.S., Rompis. J.E.G. (2012). Effect of feed restriction on feed efficiency, carcass quality and digestive organs charactheristics of broiler. Proceeding the 2nd International Seminar "Feed Safety for Healthy Food". July 6-7 2011. AINI and Faculty of Animal Husbandry, Univesitas Padjajaran. Jatinangor.

Murtidjo, B.A. (2003). *Pemotongan dan Penanganan Daging Ayam*. Yogyakarta, ID: Kanisius.

Papamandjaris, A.A., McDogall, D.E., Jones, P.J.H. (1998). Medium chain fatty acid metabolism and energy expenditure: obesity treatment implications. Minireview. *Life Sciences,* 62(4), 1203-1215.

Prudhon, M., Benevent, M., Vezinhet, J. P., Dulor A.A.T. (1976). Croissance relative du squalete chez lagneau. Influence du sexe et de la race. *Ann. Biol. Anim. Bioch. Biophys*., 18(1), 5-9.

Robelin, J. (1979). Evalution de la composition corporelle des jeunes bovine males entiers de race limosine entre 9 et 19 moins. *Ann. Zootech*., 26(4), 333-346.

Santoso, U. (2001). Effect early feed restriction on growth, fat accumulation and meat composition in unsexed broiler chicken. *J. Anim. Sci.,* 4, 1585-1591.

[SNI] Standar Nasional Indonesia. 3924-2009. *Mutu Karkas dan Daging Ayam*. Jakarta, ID: Badan Standarisasi Nasional-BSN.

Soeparno (1992). *Ilmu dan Teknologi Daging*. Edisi I. Yogyakarta, ID: Gadjah Mada University Press.

Soeparno (2011). *Ilmu Nutrisi dan Gizi Daging*. Yogyakarta, ID: Gadjah Mada University Press.

Tulung, B. (1999). *Isu, Kontroversi dan upaya Penurunan Kolesterol Produk Hewani*. Pidato Pengukuhan Guru Besar bidang Ilmu Nutrisi & Makanan Ternak Fakultas Peternakan Universitas Sam Ratulangi Manado. Manado, ID: Unsrat Press.

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FATTY ACID COMPOSITION AND ANTIBACTERIAL ACTIVITY OF CLOVE (*Syzygium aromaticum*) AND CARROT (*Daucus carota*) AS CANDIDATE OF ADDITIVE FOR BROILER CHICKENS

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Abstract

This study aimed to evaluate the fatty acids and to determine the antibacterial activity of clove (Syzygium aromaticum) and carrot (Daucus carota) against the pathogen Escherichia coli and Staphylococcus aureus. Clove and carrot were prepared from fresh plants in three concentrations (2.5%, 5% and 10%), the positive control of chloramphenicol and the negative control of DMSO. Disc diffusion method has been used to determine the antimicrobial activities of clove and carrot. The results showed that dominant fatty acids in flower of clove were Linoleic acid, Palmitic acid, Cis-13,16-Docosadienoic acid, Stearic acid, Oleic acid, Linolenic acid, γ-Linolenic acid, and fatty acid total was 9.59% w/w, whereas fat content of clove was 4.19 %w/w. Moreover, in carrot, Linoleic acid was the major component followed by Palmitic acid, Linolenic acid, Oleic acid, Stearic acid, γ-Linolenic acid, and fatty acid total was 37.65% w/w, whereas fat content of carrot was 2.21% w/w. Inhibition zone of clove for E. coli at concentration between 2.5%, 5%, and 10% showed the same response (6 mm),and was lower than that of positive control chloramphenicol (7.69 mm), however, for S. aureus inhibition zone at concentration 10% (8.64 mm) was almost the same with positive control (10.52 mm). Inhibition zone of carrot for E. coli at concentration 2.5% was the same with positive control (9.75 mm), moreover, for S. aureus at concentration 10% was lower than positive control but still proved effective against bacteria. It can be concluded that clove flower was better as natural antimicrobials for E. coli and carrot was for S. aureus. So, clove and carrot can be used as feed additives in broiler diet

Key words*: additive, antibacterial, carrot, clove, fatty acid.*

INTRODUCTION

Recent ban on the use of antibiotic growth promoters (AGP) in poultry feeds has drawn the concerns of researchers towards the presence of natural substances like medicinal herbs as a new class of additives to animal and poultry feeds. There are plenty resources of different kinds of medicinal herbs which can be used as alternative feed additives for poultry (Vinus et al., 2018). Herbs and their mixture can ameliorate the performance of birds by improving digestive tract function by anti-inflammatory, anti-oxidative and anti-microbial effects. Herbs may exert multiple functions in the bird's body system (Hernandez et al., 2004) It has been vivid that the potential of medicinal herbs as the valuable source of therapeutics aids has attained a global significant place in the health system all over the world not only for humans but also for animals as well as birds (Dhama et al., 2015). Antibiotic and antibacterial medications still used in poultry industry in several indications including therapeutic treatment, prevention or as traditional growth promoters (Diarra and Malouin, 2014). Particular attention is drawn to two classes of antimicrobial lipid, namely fatty acids (hydrocarbon chains with a carboxylic acid functional group) and monoglycerides (esterified of a fatty acid and glycerol molecule). Fatty acids are released from lipids, typically by the action of enzymes, to become free fatty acids, which have vast and potent biological activities (Desbois and Smith, 2010). The biological activities of free fatty acids have roles in host defences against potential opportunistic or pathogenic microorganisms. An important aspect of this is growth inhibition or quick destroying of bacteria. Several studies for understanding the mechanism of the anti-bacterial effects of fatty acids from biological sources such as algae, animals and plants have been done by several researchers (Wille and Kydonieus, 2003; Desbois and Smith, 2010). Several researchers investigated the antimi-

crobial activity of fatty acid derivatives. However, they show poor antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* (Shukla et al., 2018; Walvekar et al., 2018).

Many fatty acids, such as lauric, palmitic, linolenic, linoleic, oleic, stearic, and myristic acids are known to have antibacterial and antifungal properties (Seidel and Taylor, 2004). Galbraith et al. (1971) reported that lauric acid is the most potential gram-positive antibacterial agent among the saturated fatty acids, while linoleic acid is the most potential gram-positive antibacterial agent among the unsaturated fatty acids. Yoon et al. (2018) reported that oleic acid shows antibacterial activity against *S. aureus* through damaging the bacterial cell membrane.

The antibacterial activity of long-chain unsaturated fatty acids has been well known for many years. These antibacterial actions of fatty acids are usually attributed to long-chain unsaturated fatty acids including oleic acid, linoleic acid, and linolenic acid, while long-chain saturated fatty acids, including palmitic acid and stearic acid, are less active (Sun et al., 2003). However, their primary molecular target still remains unknown (Zheng et al., 2005).

Fatty acids with antibacterial activity have been isolated from several plants. Cerdeiras et al. (2000) identified 11-O-(6'-O-acetyl-β-D-glucopyranosyl)-stearic acid as the main antibacterial component of aerial parts of *Ibicella lutea*. This fatty acid derivative showed an interesting antibacterial activity, being active against *E. coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *S. aureus* with the Minimal Inhibitory Concentration value of 9 $\mu g ml^{-1}$ against *S. aureus*. Dilika et al. (2000) described the antibacterial activity of linoleic and oleic acids isolated from the leaves of *Helichrysum pedunculatum*. Linoleic and oleic acids inhibited the growth of Gram-positive: *B. subtilis*, *Micrococcus kristinae* and *S. aureus*, and linoleic acid also showed activity against *B. cereus* and *B. pumilis*. Both acids displayed no activity against Gram-negative: *Enterobacter cloacae*, *E. coli*, *Klebsiella pneumoniae*, *P. aeruginosa* and *Serratia marcescens*.

Bacteria *S. aureus* and *E. coli* are the bacteria that cause most infections in the community and nosocomial infections. *S. aureus* is a major pathogen in humans. *S. aureus* is positive coagulase, which distinguishes it from other species. Nearly everyone has experienced a variety of *S. aureus* infections during his lifetime, from severe food poisoning or small skin infections, to infections that cannot be cured (Brooks et al., 2008).

The *E. coli* bacteria are Gram-negative bacteria, while *S. aureus* bacteria are Gram-positive bacteria. The antibacterial agent against the growth of test bacteria has a mechanism of antibacterial activity that is thought to attack various parts of bacterial cells, namely cell walls, cell membranes, cell proteins, or bacterial cell nucleic acids. Besides these antibacterial substances are also able to inhibit bacterial cell metabolism (Gan et al., 1995; Pelczar et al., 2010).

Gram-positive bacteria represent the causative agents of both animal intestinal diseases and potentially lethal foodborne diseases in humans. *Listeria monocytogenes* and *S. aureus* are considered to be the widespread pathogens causing serious illnesses and systematic disorder both in animals and humans (McLauchlin and Rees, 2009). Phytogenic additives, including organic acid, present an alternative as they enhance a number of important processes in the animal body as well as they can be used also in the food industry because of their antibacterial properties (Karaskova et al., 2015).

The primary and secondary habitats of *E. coli* are the intestinal tract of warm-blooded animals and the environment, respectively. In poultry, as in humans, *E. coli* resides in the lower digestive tract, which it colonizes in the first 24 h after hatching or birth (Ballou et al., 2016; Stromberg et al., 2017). Ohta et al. (1995) demonstrated the antibacterial activity of α-linolenic acid against methicillin-resistant *S. aureus* (MRSA). McDonald et al. (1981) reported on the susceptibility of several strains of MRSA to linolenic acid and hydrolysed linseed oil (containing 52% linolenic acid in the ester form). Yoon et al. (2018) reported that all tested Gram + sp. was susceptible to treatment with 0.01 mM arachidonic acid C20:4. Bactericidal effect of arachidonic acid treatment on *S. aureus* depended on treatment time and drug concentration.

Clove (*Syzygium aromaticum*) is the aromatic flower buds of a tree in the family Myrtaceae. They are native to the Maluku Islands (or Moluccas) in Indonesia, and are commonly used as a spice. Clove plants have special characteristics, because all parts starting from the roots, stems, leaves, to the flowers, contain organic acids or essential oils. Cloves has been used as an antimicrobial (Valero and Salmeron, 2003).

Many vegetable plants can be used as medicinal plants, one of which is carrots (*Daucus carota* L.). Carrots are vegetable plants that have many uses for public health services in the world. Besides being rich in nutritional content, especially vitamin A is also efficacious for healing various diseases (Rukmana, 1995). Carrot (*Daucus carota* subsp. *sativus*) is a root vegetable, usually orange in colour, though purple, black, red, white, and yellow cultivars exist. They are a domesticated form of the wild carrot, *Daucus carota*, native to Europe and Southwestern Asia. The plant probably originated in Persia and was originally cultivated for its leaves and seeds. Carrots are widely used in many cuisines, especially in the preparation of salads, and carrot salads are a tradition in many regional cuisines. The roots contain high quantities of alpha- and beta-carotene, and are a good source of vitamin K and vitamin B6.

Fatty acids are widely occurring in natural fats and dietary oils and they are known to have antibacterial and antifungal properties. However, little is known on antibacterial activity of clove and carrot meal, and this study for the first time determines the fatty acid composition and the antibacterial activity potency of fatty acids in clove and carrot.

MATERIALS AND METHODS

The fatty acid analysis was carried out by A.O.A.C. Official Methods 2012.13: 991.33 (fatty acid in oils and fats; preparation methyl ester). And analysis of fat content according to A.O.A.C 2012: 991.36.

The dried clove flower and freshly carrot used in the study was collected from the local market in Manado, North Sulawesi of Indonesia. Carrot sample was shade dried, and then, dried flower clove and carrot were powdered.

Fat and fatty acid were extracted from clove and carrot by hydrolytic method. Fat was extracted into ether, then methylated to fatty acid methylesters (FAMEs). FAMEs then quantitatively measured by gas chromatography. The FAME components were identified using the retention time established using reference standard for FAs and percentage of individual FAME was made in relation to total area of the chromatogram.

Antibacterial tests were carried out by disc diffusion method (Pal et al., 2007). *S. aureus and E. coli* test bacteria were rejuvenated first, then a bacterial suspension was made. The clove and carrot meal were each made with a concentration solution 2.5%, 5%, and 10% using DMSO solvents. As much as 0.3 mL of bacterial suspension was put into a Petri dish then 15 mL of NA media was added to the Petri dish, homogenized and then condensed. The sterile 6 mm filter paper discs (Whatman No. 3) were impregnated with the 2.5%, 5%, and 10%/disc stock solution of clove and carrot, and placed on the inoculated agar. Negative controls were prepared using DMSO. DMSO solvent is used in this study because DMSO can dissolve polar and nonpolar compounds and DMSO will not interfere with observations because it does not provide activity on bacterial and fungal growth. Chloramphenicol (0.1%/disc) was used as positive reference standards to determine the sensitivity of each bacterial species tested. Chloramphenicol is used as a positive control for bacteria because it belongs to a broad spectrum of antibiotics that can inhibit the growth of Gram-positive and Gram-negative (Octaviani et al., 2019).

The inoculated plates were incubated at 37^0C for 24 h. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organisms. All inhibitory tests were performed in triplicate. Growth bacteria were observed, and the zone of inhibition was calculated in millimetres carefully.

RESULTS AND DISCUSSIONS

Fatty acid composition and antibacterial activity of clove (*Syzygium aromaticum*) and carrot (*Daucus carota* L.) as candidate of additive for broiler chickens have shown in Tables 1 and 2. Result showed that dominant

fatty acids in flower of clove were Linoleic acid (C18:2n6c), Palmitic acid (C16:0), Cis-13,16-Docosadienoic acid (C22:2), Stearic acid (C18:0), Oleic acid (C18:1n9c), Linolenic acid (C18:3n3), γ-Linolenic acid (C18:3n6), and fatty acid total was 9.59% w/w, whereas fat content of clove was 4.19% w/w. Moreover, in carrot, Linoleic acid (C18:2n6c) was the major component followed by Palmitic acid (C16:0), Linolenic acid (C18:3n3), Oleic acid (C18:1), Stearic acid (C18:0), γ-Linolenic acid (C18:3n6), and fatty acid total was 37.65% w/w, whereas fat content of carrot was 2.21% w/w. The flower of *Syzygium aromaticum* and root of *Daucus carota* L showed an almost similar fatty acid profile.

Table 1. Fatty acid of clove and carrot

Parameter	Result	
	Clove (% w/w)	Carrot (% w/w)
Fat Content	4.19	2.21
Fatty acid		
Caprilic acid, C8:0	0.06	-
Capric acid, C10:0	0.02	-
Lauric acid, C12:0	0.11	0.04
Tridecanoic acid, C13:0	0.11	-
Myristic acid, C14:0	0.20	0.08
Pentadecanoic acid, C15:0	0.02	0.13
Palmitic acid, C16:0	1.63	4.62
Palmitoleic acid, C16:1	0.06	0.15
Heptadecanoic acid, C17:0	0.06	0.16
Cis-10-Heptadecanoic acid, C17:1	0.04	0.14
Stearic acid, C18:0	0.65	0.87
Elaidic acid, C18:1n9t	-	0.07
Oleic acid, C18:1n9c	0.62	1.49
Linoleic acid, C18:2n6c	2.72	25.41
Arachidic acid, C20:0	0.31	0.36
γ-Linolenic acid, C18:3n6	0.39	0.75
Cis-11-Eicosenoic acid, C20:1	0.07	0.10
Linolenic acid, C18:3n3	0.52	2.74
Heneicosanoic acid, C21:0	0.02	0.06
Cis-11,14-Eicosedienoic acid, C20:2	-	0.03
Behenic acid, C22:0	0.21	0.23
Arachidonic acid, C20:4n6	-	0.03
Cis-13,16-Docosadienoic acid, C22:2	1.28	-
Tricosanoic acid C23:0	-	0.09
Lignoceric acid, C24:0	0.51	0.13
Fatty acid – total	9.59	37.65

Inhibition zone of clove for *E. coli* at concentration between 2.5%, 5%, and 10% showed the same response (6 mm), and was lower than that of positive control chloramphenicol (7.69 mm), however, for *S. aureus* inhibition zone at concentration 10% (8.64 mm) was almost the same with positive control (10.52 mm). Inhibition zone of carrot for *E. coli* at concentration 2.5% was the same with positive control (9.75 mm), moreover, for *S. aureus* at concentration 10% was lower than positive control but still proof effective against Gram-positive bacteria.

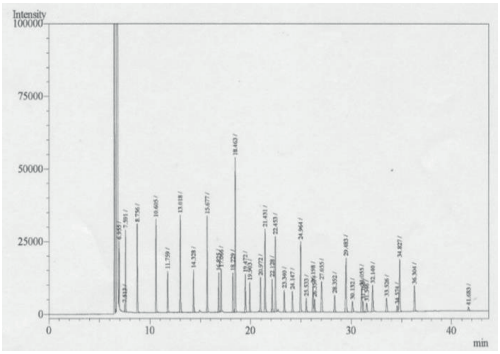

Figure 1. Graphic of Standard of FAME

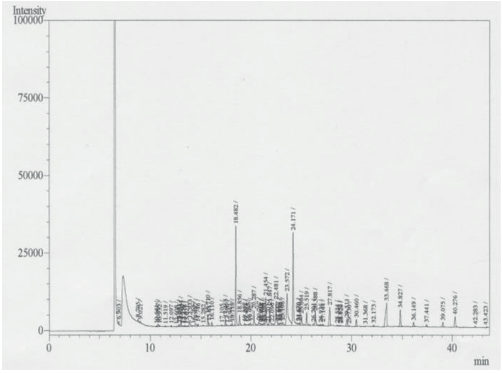

Figure 2. Graphic of Clove Fatty Acids

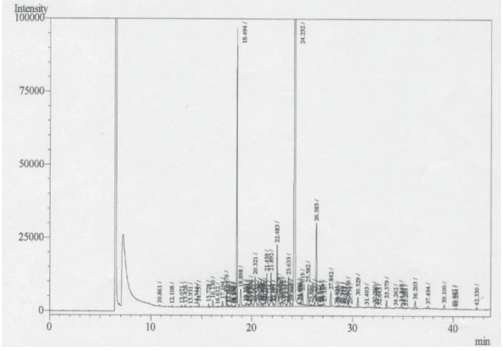

Figure 3. Graphic of Carrot Fatty Acids

Clove demonstrated the highest zone of inhibition at 10% concentration on *S. aureus* 8.64 mm and the lowest inhibition on *E. coli* with a diameter 6 mm. The clove generally proved effective against Gram- positive bacteria used in this study even at the highest concentration while other Gram-negative organisms showed resistance at the lowest concentration. On the other hand, carrot demonstrated the highest zone of inhibition at lowest concentration (2.5%) on *E. coli* but showed highest zone of inhibition at highest concentration (10%) on *S. aureus*. This study recommended the use of this clove in combating some of *E. coli* that are resistance.

Overall it can be seen for clove inhibition on *S. aureus* that the higher the concentration of the compound given, the greater the diameter of the inhibitory region formed. This is consistent with the theory put forward by Pelczar and Chan (1988), that the greater the concentration of the antimicrobial compounds tested, the greater the antimicrobial activity of the compound.

Table 2. Diameter zone of inhibition of clove and carrot for bacterial

Sample	Concentration (%)	Zone of inhibition (mm)	
		E. coli	*S. aureus*
Clove	2.5	6.00	6.00
	5	6.00	6.00
	10	6.00	8.64
Chloramphenicol (C+)	0.1	7.69	10.52
DMSO (C-)		0	0
Carrot	2.5	9.75	6.00
	5	6.00	6.65
	10	7.2	7.96
Chloramphenicol (C+)	0.1	9.75	12.38
DMSO (C-)		0	0

Compared to Huda et al. (2018) reported that the clove flower extract of 10% to 100% concentration was able to inhibit the growth of *S. aureus* bacteria. The lowest concentration of clove flower extract which can inhibit the concentration of 10% with average of 15.87 mm. and highest concentration of clove flower extract 100% concentration obtained average 21.40. While the clove flower extract at 70% concentration with mean of 19.16 mm has effectively inhibited bacterial growth when compared with positive control of Amikacin antibiotics with mean of 18.8 mm. The essential oils in clove flower showed the largest antibacterial activity which was about 25.85-26.75 mm, while in the flower stalks and clove leaves showed an activity with inhibition zone of 20.60-21.20 mm and 18.04-18.58 mm, respectively (Lova et al., 2018). In our study, we used clove meal for against the bacteria, so, maybe we have to add the level of concentration of clove to increase the inhibition zone.

Compared to Sirait et al. (2016) reported that extract ethanol 96% of carrot (*Daucus carota* L.) root in 5%, 10%, 20%, 40%, and 80% concentrations have to provide activities that inhibits the growth of test bacteria. Any increase in concentrations of 5% (3.50 mm), 10% (3.67 mm), 20% (4.83 mm), 40% (5.16 mm) and 80% (6.67 mm) for a review of *E. coli* and a concentration of 5% (3.17 mm), 10% (3.83 mm), 20% (4.00 mm), 40% (4.17 mm) and 80% (4.33 mm) to review the bacterium *S. aureus*. In this study, any increase in concentration of carrot meal maybe will increasing the inhibition zone.

Gonelimali et al. (2018) reported that plant extracts significantly affected the cell membrane of Gram-positive and Gram-negative bacteria. Plant extracts: roselle (*Hibiscus sabdariffa*), rosemary (*Rosmarinus officinalis*), clove (*Syzygium aromaticum*), and thyme (*Thymus vulgaris*) are of great value as natural antimicrobials and can use safely as food preservatives.

The antimicrobial properties of fatty acids are well known and there is a close relationship between the structure of fatty acids and their ability to function as antimicrobial agents. Saturated fatty acids are effective against microorganisms at lower chain lengths, while monounsaturated and polyunsaturated fatty acids with longer chain lengths are more effective. The position of double bonds is significant for long chain fatty acids (McGaw et al., 2002). Anzaku et al. (2017) reported that free fatty acids (FFA) of various chain lengths (C8-C18) have antibacterial activity against a range of Gram-positive bacteria, but not against a number of Gram-negative bacteria. In this study, carrot have high Linoleic acid, so it is effective against *E. coli* compared to clove.

Clavijo and Flo´rez (2018) reported that the extraction of energy and nutrients from food

requires interaction between the biochemical functions of the chicken and the microbiota present in the gastrointestinal tract. Thus, the selection of beneficial microbiota plays an important role in the production, health, protection from pathogens, detoxification, and modulation of the immune system. The presence of pathogenic bacteria in the broiler chicken microbiota is important to animal and human health alike. Among the taxa that can cause illness in humans and that have been reported in the chicken microbiota are *Campylobacter* (principally *Campylobacter jejuni* and *Campylobacter coli*), *Salmonella enterica*, *Escherichia coli*, and *Clostridium perfringens* (Oakley et al., 2014).

In poultry, *E. coli* infections include egg peritonitis, omphalitis, coligranuloma, swollen head syndrome, cellulitis, and colisepticaemia. Colisepticaemia is a severe systemic form of infection. Omphalitis is a major factor responsible for early chick mortality during the first few days after hatching (EL-Sawah et al., 2018). In this study, clove and carrot were not unconventional feed for poultry, moreover, it relating to its part of feed supplement.

CONCLUSIONS

From the results, that these plants contains high percentage of linoleic and significant amount of palmitic acids. Plants showed the power of antibacterial activity. It can be concluded that clove flower was better as natural antimicrobials for *E. coli* and carrot was for *S. aureus*. However, further work needs to be carried on characterization of fatty acid compounds and test that fatty acids potential for antibacterial activity.

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REFERENCES

Anzaku, A. A., Akyala, J. I., Juliet, A., Obianuju, E. C. (2017). Antibacterial activity of lauric acid on some selected clinical isolates. *Ann. of Clinic. and Lab. Res.,* 5(2), 170–174.

Ballou, A. L., Ali, R. A., Mendoza, M. A., Ellis, J. C., Hassan, H. M., Croom, W. J. (2016). Development of the chick microbiome: how early exposure influences future microbial diversity. *Front Vet*. Sci., 3, 2.

Cerdeiras, M. P., Fernández, J., Soubes, M., Vero, S., Ferreira, F., Moyna, P., Olano, I., Vázquez, A. (2000). A new antibacterial compound from *Ibicella lutea*. *J. of Ethnopharm*. 73, 521–525.

Clavijo. V., Flo´rez, M. J. V. (2018). The gastrointestinal microbiome and its association with the control of pathogens in broiler chicken production: A review. *Poult. Sci*., 97, 1006–1021.

Dilika, F., Bremner, P. D., Meyer, J. J. M. (2000). Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*: a plant used during circumcision rites. *Fitoterapia*, 71, 450–452.

Desbois, A. P., Smith, V. J. (2010). Antibacterial free fatty acids: Activities, mechanisms of action and biotechnological potential. *Appl. Microbiol. Biotechnol*., 85, 1629–1635.

Diarra, M. S., Malouin, F. (2014). Antibiotics in Canadian poultry productions and anticipated alternatives. *Front Microb*., 5, 282. doi:10.3389/fmicb.2014.00282.

Dhama, K., Latheef, S. K., Mani, S., Samad, H. A., Karthik, K., Tiwari, R., Khan, R. U., Alagawany, M., Farag, M. R., Aiam, G. M., V. Laudadio, V., Tufarelli, V. (2015). Multiple beneficial applications and modes of action of herbs in poultry health and production-A Review. *Int. J. of Pharm*., 11(3), 152-176.

EL-Sawah, A. A., Dahshan, A. H. M., El-Nahass, El-S., El-Mawgoud, A. I. A. (2018). Pathogenicity of Escherichia coli O157 in commercial broiler chickens. Beni-Suef Univ. *J. of Basic and Appl. Sci*., 7, 620–625.

Galbraith, H., Miller, T., Paton, A., Thompson, J. (1971). Antibacterial activity of long chain fatty acids and the reversal with calcium, magnesium, ergocalciferol and cholesterol. *J. Appl. Microbiol*., 34, 803-813.

Gibbons, S. (2004). Anti-staphylococcal plant natural products. *Natural Product Reports*, 2, 263-277.

Gonelimali, F. D., Lin, J., Miao, W., Xuan, J., Charles, F., Chen, M. S. R. (2018). antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Frontiers in Microb*., 9, 1-9.

Hernandez, F., Madrid, J., Garcia, V., Orengo, J., Megias, M. D. (2004). Influence of tow plant extracts on broiler performance, digestibility, and digestive organ size. *Poult. Sci*., 83, 169-174.

Huda, M., Rodhiansyah, Ningsih, D. S. (2018). Efektivitas ekstrak bunga cengkeh (*Eugenia aromatic*) terhadap pertumbuhan bakteri *Staphylococcus aureus*. *Jurusan Analis Kesehatan,* 7(1), 710-716.

Kammon, A., Almaeyoufi,A., Asheg, A. (2019). In vitro antimicrobial activity of clove oil *in vitro* against gram negative bacteria isolated from chickens.

Approches in Poult., Dairy and Vet. Sci., 6(2), 542-456.

Karaskova, K., Suchy, P., Strakova E. (2015). Current use of phytogenic feed additives in animal nutrition: A review. *Czech J. of Anim. Sci.,* 60, 521-530.

Lova, I. P. S. T., Wijaya, W. A., Paramita, N. L. P. V., Putra, A. A. R. Y. (2018). Perbandingan uji aktivitas antibakteri minyak atsiri daun, tangkai bunga dan bunga cengkeh bali (*Syzygium aromaticum* L.) terhadap bakteri *Propionibacterium acne* dengan metode difusi disk. *J. Kimia*, 12(1), 30-35.

McDonald, M. I., Graham, I., Harvey, K. J., Sinclair, A. (1981). Antibacterial activity of hydrolysed linseed oil and linolenic acid against methicillin-resistant *Staphylococcus aureus*. *The Lancet*., 2, 1056.

McGaw, L. J., Jäger, A. K., Van Staden, J. (2002). Isolation of antibacterial fatty acids from *Schotia brachypetala*. *Fitoterapia*, 73, 431-433.

McGaw, L. J., Jäger, A. K., van Staden, J. (2002). Antibacterial effects of fatty acids and related compounds from plants. *South African J. of Botany*, 68, 417-423.

McLauchlin J., Rees, C. E. (2009). *Genus Listeria*. In: Whitman W.B. (Ed.): Bergey's Manual of Systematic Bacteriology, Vol. 3. New York, USA: Springer-Verlag Publishing House, 244-257.

Oakley, B. B., Lillehoj, H. S., Kogut, M. H., Kim, W. K., Maurer, J. J., Pedroso, A., Lee, M. D., Collett, S. R., Johnson, T. J., Cox, N. A. (2014). The chicken gastrointestinal microbiome. *FEMS Microbiol. Lett*., 360, 100–112.

Ohta, S., Y. Shiomi, A. Kawashima, O. Aozasa, T. Nakao, T. Nagate, K. Kitamura, H. Miyata (1995). Antibiotic effect of linolenic acid from Chlorococcum strain HS-101 and *Dunaliella primolecta* on methicillin-resistant *Staphylococcus aureus*. *J. of Appl. Phycology*, 7, 121-127.

Pal, S., Kyung, T. Y., Myong, S. J. (2007). Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. *Appl. Environ. Microbiol*., 73(6), 1712-1720.

Rukmana, R., Sugandi, S. 1995. *Hama Tanaman dan Teknik Pengendalian*. Bumi Aksara. Jakarta.

Seidel, V., Taylor, T. P. W. (2004). In vitro activity of extracts and constituents of *Pelagonium* against rapidly growing mycobacteria. *Int. J. Antimicrob. Agents*, 23, 613-619.

Shukla, S., Hegde, S., Kumar, A., Chaudhary, G., Tewari, S. K., Upreti, D. K., Pal, M. (2018). Fatty acid composition and antibacterial potential of Cassia tora (leaves and stem) collected from different geographic areas of India. *J. of Food and Drug Anal*., 26, 107-111.

Sirait, A. Y., Pelealu, N. C., Yamlean, P. V. Y. (2016). Uji daya antibakteri ekstrak etanol umbi wortel (*Daucus carota* L.) terhadap *Staphylococcus aureus* dan *Escherichia coli* secara *in vitro*. *Pharmacon J. Ilmiah Farmasi*, 5(4), 145-154.

Sun, C. Q., O'Connor, C. J., and Roberton A. M. (2003). Antibacterial actions of fatty acids and monoglycerides against *Helicobacter pylori*. *FEMS Immunol. Med. Microbiol*., 36, 9-17.

Valero, M., Salmeron, M. C. (2003). Antibacterial activity of 11 essential oils against *Bacillus cereus* in tyndallized carrot broth. *Int. J. Food Microbiol*., 85, 73-81.

Wille, J. J., Kydonieus, A. (2003). Palmitoleic acid isomer (C16:16) in human skin sebum is effective against Gram-positive bacteria. *Skin Pharmacol Appl. Skin Physiol*., 16, 76-87.

Yoon, B. K., Jackman, J. A., Valle-González, E. R., Cho, N. J. (2018). Antibacterial free fatty acids and monoglycerides: biological activities, experimental testing, and therapeutic applications. *Int. J. Mol. Sci*., 19(1114), 1-40.

Zheng, C. J., Yoo, J. S., Lee, T. G., Cho, H. Y., Kim, Y. H., Kim, W. G. (2005). Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. *FEBS Letters*, 579, 5157–5162.

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EFFECT OF ROSEMARY, SEA BUCKTHORN AND GINGER AS FEED ADDITIVE ON HEMATOLOGICAL PROFILE AND SOME BIOCHEMICAL PARAMETERS OF *Oreochromis niloticus* SPECIES

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Abstract

In the present study, three phytobiotics were added to the basal diet of Oreochromis niloticus reared in a recirculating aquaculture system. Therefore, was investigated the influence of phytobiotics on hematological profile, some blood biochemical indices and leukocyte reaction. The experiment was carried out during 12 weeks. The experimental variants were: V1 - control; V2 - 1% Rosmarinus officinalis/kg feed; V3 - 1% Hippophae rhamnoides/kg feed and V4 - 1% Zingiber officinale/kg feed. The results of some parameter of hematological profile showed that supplemented diet with sea buckthorn and ginger exhibited significantly ($p<0.05$) lowest values of red blood cells count, white blood cells count, absolute number of lymphocytes and neutrophils, relative number of neutrophils. Regarding to blood biochemical analysis was observed a significant ($P<0.05$) reduction of plasma cortisol concentration and a slight decrease ($P>0.05$) of glucose concentration in V3 variant. Also, only the sea buckthorn (V3) and ginger (V4) showed an immunomodulatory effect during the experiment because they determined the intensifying the lysozyme activity. In conclusion, dietary supplementation with sea buckthorn and ginger reduced the technological stress and improved the immunity of Nile tilapia.

Key words*: hematological profile, lysozyme activity, Nile tilapia, phytobiotics, recirculating aquaculture system.*

INTRODUCTION

The use of dietary additives in fish farms is one of the methods commonly used to improve weight gain, feed efficiency, and/or disease resistance in cultured fish (Akrami et al., 2015). Recently, the immunostimulants, due to increased resistance to infectious diseases by enhancing both specific and nonspecific defence mechanisms of fish and animals (Harikrishnan et al., 2010), are used for fish disease control because they offer an alternative and a cost-prohibitive or even limited efficacy to currently existing drugs, chemicals and antibiotics (Sakai, 1999). They are improving the immune status of the fish by enhancing the lysozyme, leukocyte reaction and the values of haematological profile parameters.

The latest studies showed that a large number of medicinal chinese herbs were used as immunostimulants such as *Viscum album*, *Urtica dioica* and *Zingiber officinale* (Dugenci et al., 2003), *Radix astragalin* and *R. angelicae* (Jian and Wu, 2004), *Astragalus radix* and *Scutellaria radix* (Yin et al., 2006), *Achyranthes aspera* (Rao et al., 2006), *Eclipta alba* (Christybaptita et al., 2007), *Rosmarinus officinale* (Xie et al., 2008), *A. radix* and *Ganoderma lucidum* (Yin et al., 2009) who reported an improvement in the innate immunity of fishes.

The most widely used medicinal herbs worldwide is a rosemary (*Rosmarinus*

officinalis), due to its good antioxidant activity (Caillet et al., 2007). The most significant feature of the antioxidant activity of rosemary is the association between diterpenes and radical scavenging activity (Nieto et al., 2018). The biological activities of this plant are mainly related to the phenolic and the volatile constituents (Arranz et al., 2015) such as carnosol, carnosic acid and rosmarinic acid present in the extract of rosemary and a-pinene, bornyl acetate, camphor and eucalyptol present in the essential oil of this plant (Arranz et al., 2015). Minor components may have a potential influence on the biological activity due to the possibility of synergistic effect among their components (Hussain et al., 2010).

Rosemary extract has been tested as a feed additive in mammals, and has been demonstrated to have strong antidiabetic (Al-Jamal and Alqadi, 2011), hepatoprotective (Cui et al., 2012), choleretic (Romo Vaquero et al., 2012) and antiadipogenic (Gaya et al., 2013) effects. However, to our knowledge, only one study revealed some general effects on the physiological condition of the fish as reduced hepato-somatic index and increased spleen-somatic index and bile-somatic index (Yilmaz et al., 2013).

Several studies confirmed that the oral administration of rosemary leaf powder could enhance growth production, improve antioxidant status and immunological parameters, and alleviate the adverse effects of high stocking density stress on common carp fingerlings (Yousefi et al., 2019). Additionally, fish fed a diet supplemented with rosemary (*Rosmarinus officinalis*) showed significantly improving growth rates and feed efficiency compared with those in the control group at Nile tilapia, *Oreochromis niloticus* (L.) (Hassan et al., 2018). Moreover, dietary supplementation with 0.5% rosemary significantly enhanced innate immunity and antioxidant status of *O. niloticus* fed aflatoxin B1 contaminated diet (Naiel et al., 2019). Also, Jiang et al. (2011) found that rosemary extract revealed great antibacterial activity against Gram-positive and Gram-negative stained bacteria. Besides these positive effects, rosemary-supplemented sea bass (*Dicentrarchus labrax*) diet did not affect kidney function indicators and liver enzymes (Yılmaz et al., 2013).

At the same time, sea buckthorn (*Hippophae rhamnoides* L.) is a versatile food and nutraceutical crop with various applications, from con-trolling soil erosion to being a source of horse fodder, nutritious foods, drugs, and skin-care products (Kumar et al., 2011). It should be noted that all parts of sea-buckthorn are a rich source of bioactive components, their highest concentration being found in fruit (vitamins A, C, E, K, carotenoids, organic acids, minerals etc.) (Yang & Kallio, 2002). Moreover, the berries are rich in carotenoids, such as zeaxanthin, beta β-carotene, β-cryptoxanthin, litein, lycopene and γ-carotene (Anderson et al., 2009). However, all parts of rΓthis wonder plant are considered to be a good source of a large number of bioactive compounds, including carotenoids, tocopherols, sterols, flavonoids, lipids, vitamins, tannins, minerals, etc. which contribute to its wide usage as a natural anti-oxidant (Kumar et al., 2011).

Sea-buckthorn was used as an immune-modulator agent in case of animal breeding and veterinary medicine, the outcomes being spectacular, fact that led to the administration of sea buckthorn in animal feeds in order to prevent some health problems (Morar, 2003).

In fish diets, *Hippophae rhamnoides* it is used successfully for improving disease resistance and growth performance (Todoran, 2015).

Ginger (*Zingiber officinale*) is widely used around the world in food as a spice. Ginger is generally considered as a safe herbal medicine; contains alkaloids, flavonoids, polyphenols, saponin, steroids, tannin, fiber, carbohydrate, vitamins, carotenoids and minerals; natural antioxidants as gingerols, shogaols and zingerone; essential oils which has potent anti-inflammatory effects and oleoresin (Jahanjoo et al., 2018). Previously, studies have indicated that ginger is effective for the controling of a range of bacterial, fungal and parasitic conditions (Chrubasik et al., 2005). In different fish, it has been demonstrated that ginger administration significantly increased growth performance, different immune responses, and resistance to against different pathogenic bacteria (Ahmadifar et al., 2019).

Thus, in order to evaluate the welfare status of the fish it is taken into account the determination of the haematological parameters, some biochemical blood parameters and immunological tests.
Therefore, the present study aims to investigate the efficiency of some herbal as feed additive on haematological changes and leukocyte reaction in case of *Oreochromis niloticus* reared in a recirculating aquaculture system.

MATERIALS AND METHODS

Experimental design
This experiment was carried out in the research laboratory of the Department of Food Science, Food Engineering, Biotechnology and Aquaculture, from "Dunarea de Jos" University, Galati. The recirculating system was described in our precedent paper (Antache et al., 2013). The design of this system consists in four rearing units, with a volume of $1m^3$ each, and a series of water quality conditioning units (Cristea et al., 2002). The experiment lasted 12 weeks. In this research the biological material consisted in a total number of 168 individuals of Nile tilapia, with an initial average weight of 359.57 ± 61.44 g/fish that were randomly distributed in four rearing units. The experimental variants were organized as follows: V1 - control, V2 - 1% rosemary (*Rosmarinus officinalis*)/kg feed, V3 - 1% sea buckthorn (*Hippophae rhamnoides*)/kg feed and V4 - 1% ginger (*Zingiber officinale*)/kg feed. These phytobiotics were purchased from a Plafar market, like dried plants, after which they were grounded and used as powder.
The additivation of fish feed with phytobiotics was achieved using an aqueous solution of gelatine with 2% concentration. The feed was sprayed, mixed and then dried at 25ºC. Fish were fed with SOPROFISH pelleted feed, with 38% crude protein and 7% crude fat. The feed biochemical composition was related by Antache et al. (2013). Fish were fed four times per day with a daily ration of 2% from fish body weight. At the end of the experiment the individual average weight was 597.18 ± 113.48 g/fish in V1, 589.33 ± 90.42 g/fish in V2, 620.20 ± 84.40 g/fish in V3 and 616.93 ± 103.64 g/fish in V4.
Blood sampling and analysis
Blood sampling has been carried out at the beginning (V0) and at the ending of experimental period. Before to start the sampling method, fish were anesthetized with 2-phenoxyethanol (8 mL/40 L of water for 5 minutes) in order to reduce handling stress. Was sampling 4 mL of blood at 7 fish, by caudal venous puncture using heparin as anticoagulant, from each growth unit. For each sample were used two Eppendorf tubes. So, for haematology analyses was added anticoagulant in Eppendorf tubes and for biochemical analyses was not added anticoagulant. Blood analysis was performed by method used in fish haematology described by Blaxhall and Daisley (1973). This analysis consisted in determination of red blood cells count (RBCc, x 10^6 cells/mm^3), hemoglobin (Hb, g/dl) and hematocrit (PVC, %).
The erythrocyte number was determined by counting the erythrocytes from 5 small squares of Neubauer hemocytometer. The hematocrit was performed by duplicate using capillary tubes and a micro hematocrit centrifuge. The hemoglobin concentrations were measured spectrofotometrically with SPECORD 210 Analytikjena at λ-540 nm, using Drabkin reagent. Then, using standard formulas described by Ghergariu et al. (1985) and Svobodova (2001) were calculated the erythrocyte constants: mean corpuscular volume (MCV, μm^3), mean corpuscular hemoglobin (MCH, pg), and mean corpuscular hemoglobin concentration (MCHC, g/dl).
Regarding to blood biochemical parameters have been analysed the glucose concentration (GLU - mg/dL), cortisol concentration (ng/mL), total protein (TP - g/dL and lysozyme activity (LYS Units/mL). To obtain blood serum, the blood without anticoagulant was centrifuged 10 minutes, at 3500 rotation/min. Determination of glucose, total protein and lysozyme activity from serum was performed spectrophotometric using the spectrophotometer SPECORD 210 Analytikjena. Dosage of glucose was made by colorimetric method with o-toluidine, readings were made at 635 nm wavelength. Total protein from serum were determined by Biuret method, the readings was done at a 546 nm wavelength. Lysozyme activity was measured, from serum, based on the turbiometric assay, Enzymatic

Activity of Lysozyme Protocol (Sigma, EC 3.2.1.17). For this test was prepared a substrate, in 66 mM Potassium Phosphate Buffer, with 6.24 pH at 25°C, a volume of 0.01% (w/v) suspension of *Micrococcus lysodeikticus* (Sigma, M3770). Lyophilised powder of chicken egg white lysozyme (Sigma, L6876) was used as standard. One unit of lysozyme activity was defined as a reduction in absorbancy of 0.001/min, at a 450 nm wavelength. Serum cortisol determination was performed using the kit: NovaTec Cortisol-DNOV001 based on competitive immunoenzymatic colorimetric method for quantitative determination of cortisol in human serum or plasma. Absorption was read at 450 nm using an ELISA microwell plate reader.

The leukocytar reaction was obtained by microscopic examination of 200 leukocytes on blood smears (in dublicate for each fish), using Zeizz Axio Imager microscop and immersion objective (10 oc. x 100 ob.). Blood smears were immediately dried, fixed with methanol and then colored with May-Grünwald Giemsa panoptic method (MGG). The type of leukocytes were determined based on identification characters listed by Svobodova et al. (1991). Absolute number of circulating blood leukocytes and thrombocytes was determined in comparison with 1000 erythrocytes counted on haemocytometer, per blood volume unit.

Statistical analysis

The results, of haematological and biochemical parameters, of the experimental groups were statistically analysed using descriptive statistics and ANOVA test. Programs used were Microsoft Excel 2010 and SPSS Statistics 17.0. The results were presented as mean ± standard deviation.

RESULTS AND DISCUSSIONS

The determination of the haematological profile represent an important analysis in the detection of the nutritional deficiencies which can cause anaemia, appeared as a result of the significant reduction of hematocrit values and hemoglobin concentrations. From this reason, in fish, the determination of the hematological parameters represent a useful tool that can be used to monitor the changes at the physiological and pathological level (Kori-Siakpere et al., 2005).

The values of hematological parameters obtained at the beginning (V0) and at the end of the experiment (V1 - control, V2 - 1% rosemary/kg feed, V3 - 1% sea buckthorn/kg feed and V4 - 1% ginger/kg feed), are presented in the Figures 1-6.

Thus, the changes that appeared during the experimental research, following the administration of rosemary, sea buckthorn and ginger, at the level of the haematological picture are presented below.

At the end of the experiment the ***red blood cells count (RBCc)*** registered a significant reduction ($p < 0.05$) in case of V3 variant in which was administered sea buckthorn as phytobiotic (Figure 1). However, compared to V1 variant, there was a reduction in the number of erythrocytes in all variants in which phytobiotics were administered (by 31.56% in V3 variant; 11.65% in V4 variant; respectively by 0.99% in V2 variant). It should be noted that the values obtained in the case of our research were registered in the range of 1.08-2.44 x 10^6 erythr./µL blood, these values being within the optimal limits for the *Oreochromis niloticus* species, respectively between 0.7 and 2.8 x 10^6 erythr./ µL blood (Bittencourt et al., 2003).

The ***hematocrit*** registered a slowly increase ($P>005$) at the end of the experiment compared to the initial moment (Figure 2), but between the experimental variants there were no significant differences ($P>0.05$). Thus, the PVC values increased with 4.93% in V1 variant, 3.52% in V4 variant; 2.82% in V2 variant and 0.70% in V3 variant. During the experiment PVC (%) values ranged from 23 to 33%. The average values obtained are recorded in the reference ranges reported in the literature for the *Oreochromis niloticus* species (23-41%, Tavares Dias and Faustino, 1998; 15-45%, Bittencourt et al., 2003).

At the end of the experiment, from the four experimental variants, the lowest ***haemoglobin concentratio***n was also obtained in the variant in which sea buckthorn was administered (V3 variant - 9.67 ± 1.21 g/dL) (Figure 3). The increase in haemoglobin concentrations obtained at the end of the experiment was significant ($P<0.05$) compared to the value of haemoglobin concentration obtained at the

initial moment (V0). The decrease of the haemoglobin concentration in V3variant shows that the administration of sea buckthorn had a positive effect on the physiological state of the biological material throughout the experimental period. However, compared to the control variant (V1), the physiological state of the biological material from the variants in which sea buckthorn was administered, but also from the other two variants (V2 and V4) is an optimal one. During the experiment, the minimum value of the haemoglobin concentration was 5.5 g/dL, and the maximum value was 11.81 g/dL, these values delimiting the interval in which all the values of the haemoglobin concentration were included. The values obtained are found in the optimal range for Nile tilapia: 5.4-12.7 g/dL (Tavares Dias and Faustino, 1998) and 6.58-15.98 g/dL (Bittencourt et al., 2003).

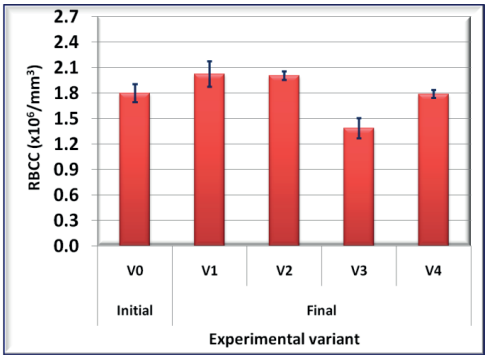

Figure 1. Changes in erythrocytes number (RBCc) of different experimental groups

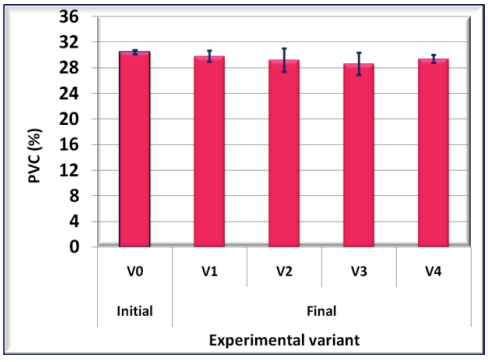

Figure 2. Changes in hematocrit (PVC) of different experimental variants during the experiment

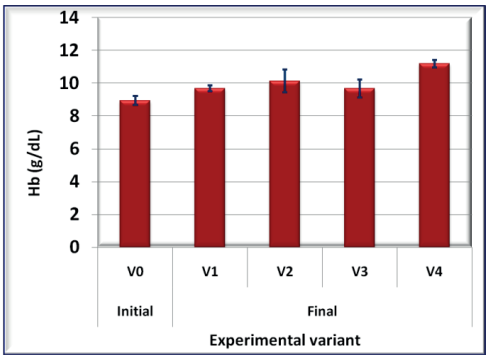

Figure 3. Changes in hemoglobine concentration (Hb) of different experimental groups

Regarding to the erythrocytar constants the ***mean corpuscular volume (MCV)***, in the variant in which sea buckthorn was administered (V3), the MCV concentration increased significantly ($P<0.05$) compared to the control variant (V1), also compared to the value obtained at the beginning of the experiment (V0) (Figure 4). The MCV concentration increased in V3 variant with 43.76% compared to V2 variant; with 40.46% compared to V1 variant; respectively with 29.26% compared to V4 variant.

Also in case of the mean of MCV concentration, the same trend was observed in the occurrence of significant differences as in the case of the number of erythrocytes (RBCc), only that the values recorded in the case of MCV concentration are indirectly proportional. Hamid et al. (2013) showed that the average of MCV can reach a maximum value, but at the same time an optimal value, of 214 μm^3 (Hamid et al., 2013).

Concerning to ***mean corpuscular hemoglobin (MCH)***, at the end of the experiment, there was a significant increase ($P<0.05$) in the variants in which were administered phytobiotics compared to the control variant (V1) and compared to the initial value (V0). Compared to the control (V1), the MCH concentration increased with 3.02% in V2 variant, 27.24% in V4 variant and 48.01% in V3 variant (Figure 5). This is correlated with the hemoglobin concentration, because even in the case of hemoglobin the highest values were recorded at the end of the experiment.

The results obtained during the experiment are in the range of 41.11-109.20 pg. In the literature, at the Nile tilapia, a mean range

between 5 pg and 80.4 pg has been reported for MCH concentration (Hamid et al., 2013), but average values between 50.2 pg and 65.4 pg have also been reported (Goda, 2008).

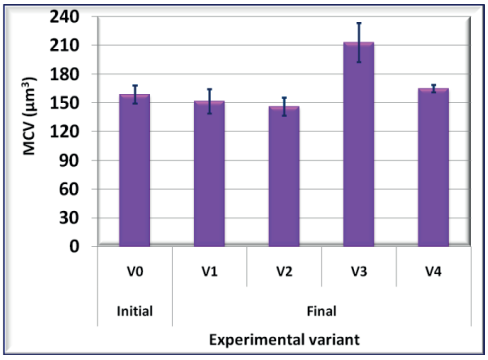

Figure 4. Changes in MCV concentration of different experimental variants during the experiment

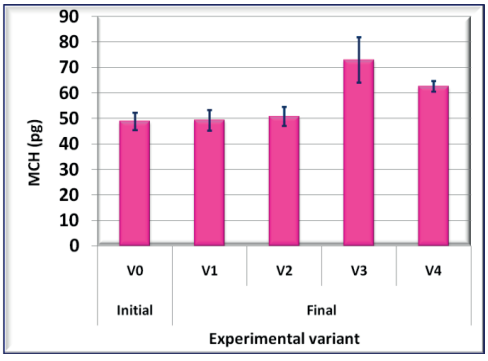

Figure 5. Changes in MCH concentration of different experimental variants during the experiment

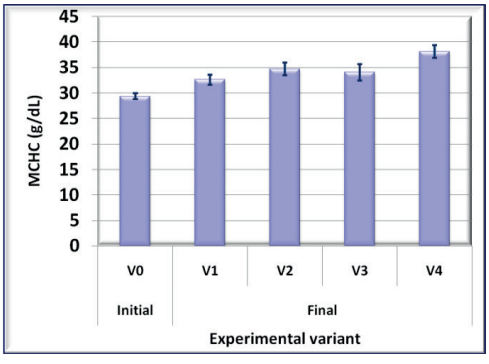

Figure 6. Changes in MCHC concentration of different experimental variants during the experiment

Due to the increase in hemoglobin concentration, as in the case of MCH concentration, there was a significant increase ($p < 0.05$) in ***mean corpuscular hemoglobin concentration (MCHC)*** at the initial momement (V0). However, there were no significant differences between the experimental variants ($P > 0.05$). The highest mean of the MCHC concentration was recorded in the variant in which was administered ginger (V4 - 38.16 ± 2.78 g/dL) (Figure 6). Regarding to the mean of the MCHC concentration, the values ranged from 25.18 to 42.50 g/dL. The values obtained fall within the reference range for the *Oreochromis niloticus* species, respectively 19.84 g/dL and 87.73 g/dL (Bittencourt et al., 2003).

At the end of the experiment, it was observed that with increasing of hemoglobin concentration, the average of MCH concentration and the average of MCHC concentration also increased in the variants in which rosemary (V2) and ginger (V4) were administered. The MCH concentration recorded a high value in the variant in which sea buckthorn was administered due to the small number of red blood cells in this variant.

In order to evaluate the fish welfare status, besides the analysis of the hematological profile, the determination of some biochemical indicators of the blood is also used.

Jawad et al. (2004), showed that the biochemical parameters of blood plasma can vary from species to species, being influenced by both biotic and abiotic factors, such as water quality, temperature, season, age, sex and last but not least by the food composition. However, the biochemical parameters of the blood can be used as biomarkers, due to their sensitivity and the fact that they are less variable (glucose, cortisol, total proteins) (Owolabi, 2011).

Cortisol and glucose are the most common stress indicators in fish, which elevate during stress and increase energy expenditure (Abdel-Tawwab, 2012; Ghelichpour et al., 2018).

In terms of ***glucose concentration***, at the end of the experiment, there were no significant differences between the experimental variants ($P > 0.05$). The results obtained in the variants in which the diet was supplemented with phytobiotics were close to the value obtained in the control variant (V1) (Figure 7). However, the values recorded at the end of the experiment were significantly lower ($P < 0.05$)

than the value obtained at the initial of the experiment (V0). In this case, it can be seen that the administration of sea buckthorn led to a lower concentration of glucose compared to the variants in which rosemary and ginger were administered. The average of the glucose concentration are registered within the normal limits for the *Oreochromis niloticus* species, respectively 22.7-107.0 mg/dL (Bittencourt et al., 2003). Though, Hamid Ahmed et al. (2013) extended the glucose range from 33.3 mg/dL to 250 mg/dL in Nile tilapia.

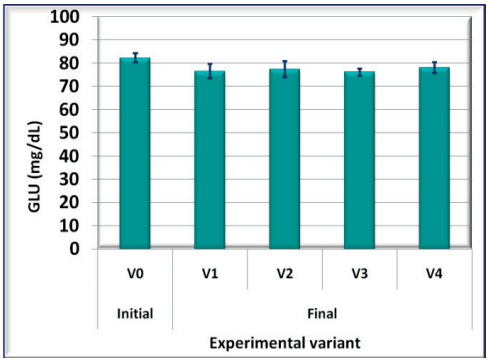

Figure 7. Changes in glucose concentration (GLU) of different experimental variants during the experiment

Regarding to ***cortisol concentration***, although the mean values varied, there were no significant differences (P>0.05) between the experimental variants (Figure 8). The lowest cortisol concentrations were recorded in the variant in which sea buckthorn was administered (V3 - 366.26 ± 94.29 ng/mL). The low value of cortisol concentration obtained in variant V3 can be associated with the large amount of antioxidants present in sea buckthorn. Apines-Amar et al. (2013) showed that ginger administration significantly reduced plasma cortisol levels in *Epinephelus fuscoguttatus*. Also, demonstrated that ginger is a strong anti-stress agent, the ginger-treated fish had significantly lower cortisol levels compared to the ascorbic acid-treated ones, a well-known anti-stress agent in fish (Jalali et al., 2010; Barros et al., 2014). Apines-Amar et al. (2013) found lower plasma cortisol levels in the ginger-treated *E. fuscoguttatus*, which was accompanied by higher growth performance. Similar results were also obtained in *O. mykiss* fed with lycopene or cineole supplemented diets and reared under high stocking density (Taheri Mirghaed et al., 2018). This explains in our case the reduction of cortisol in the variant in which sea buckthorn was administered (V3).

In case of the ***total proteins***, a reduction with 8.07 percent was observed in the variant in which the diet was supplemented with 1% *Rosmarinus officinalis*, and an increase by 3.64% and 6.65% in the variants in which the fish diet was supplemented with 1% *Hippophae rhamnoides*, respectively 1% *Zingiber officinale*, compared to the variant in which were not administered phytobiotics (V1) (Figure 9). However, no significant differences were obtained between the experimental variants (P>0.05) (Figure 9).

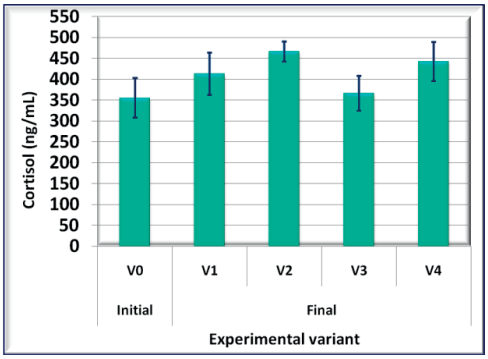

Figure 8. Changes in cortisol concentration of different experimental variants during the experiment

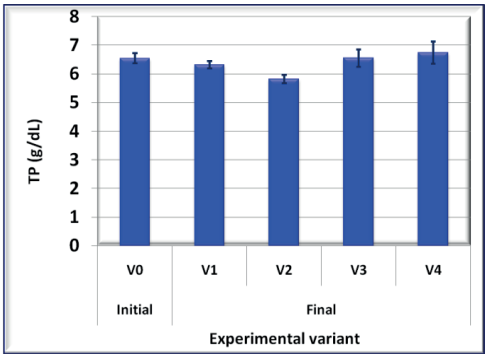

Figure 9. Changes in total protein concentration (TP) of different experimental variants during the experiment

At the end of the experiment, an intensification of ***lysozyme activity*** was observed in variant V3 (10.37 ± 1.39 U/mL) (Figure 10).

Thus, there was an increase in lysozyme activity by 10.08% and 11.46% in V3 variant, respectively in V4 variant, and a decrease by

4.25% in lysozyme activity in V2 variant compared to lysozyme activity recorded in the control variant (V1), but the differences were statistically insignificant (P>0.05).

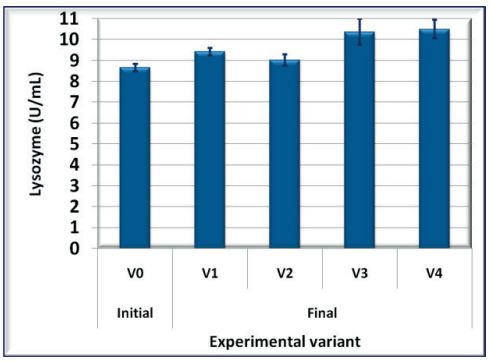

Figure 10. Changes in lysozyme activity (Lys) of different experimental variants during the experiment

From the analysis of lysozyme activity, an improvement of immunity can be observed in the variant in which sea buckthorn and ginger were administered, fact for which we can say that these phytobiotics have an immunomodulatory action.

The analysis of blood smears from a quantitative and qualitative point of view can give us the necessary information in order to study innate/native immunity. Leukocytes are the body's first line of immune defence (Cristea et al., 2012), so they play a very important role in defending the body if it is attacked by various pathogens.

The relative number of the leukocytes are presented in Table 1.

Table 1. Variation in the relative number of leukocytes at Nile tilapia during the experiment

Experimental variant		Relative number of leukocytes (%)			
		Lymphocytes		Monocytes	Neutrophilic granulocytes
		small	large		
V0 (initial)		96.42±1.92	1.23±0.86	0.58±0.18	1.78±0.89
Final	V1	95.53±0.78	0.79±0.22	1.09±0.34	2.58±0.54
	V2	96.44±0.93	1.03±0.41	1.12±0.20	1.40±0.46
	V3	96.81±0.30	0.58±0.19	1.07±0.20	1.54±0.39
	V4	95.71±0.61	0.80±0.22	1.69±0.46	1.79±0.22

Small lymphocytes (%). At the end of the experiment, the highest value of the relative number of small lymphocytes was recorded in the variant in which sea buckthorn was administered (V3 - 96.81 ± 0.30%), but which was insignificantly higher than in the other experimental variants (P>0.05). However, the initial relative number was significantly higher (P<0.05) than the relative number obtained in the control variant at the end of the experiment (Table 1).

Large lymphocytes (%). It was found that after 12 weeks the administration of phytobiotics did not influence the relative number of large lymphocytes. Thus, the highest value was recorded in variant V2 (1.03 ± 0.41%) and the lowest in V3 (0.58 ± 0.19%), the differences being insignificant (P>0.05). Compared to the initial value, was obtained a significant difference (p<0.05)

Monocytes (%). An insignificant increase (P>0.05) of the mean monocytes value was obtained in variant V4 (1.69 ± 0.46%) compared to the other experimental variants. The lowest average value was obtained in the variant in which sea buckthorn was administered (V3 - 1.07 ± 0.20%). The mean values increased significantly (p<0.05) compared to the value obtained at the initial moment (V0) (Table 1).

Neutrophil granulocytes (%). At the end of the experiment, a significant increase (P<0.05) of the relative number of neutrophils was observed compared to the average number obtained in the variants in which phytobiotics were administered. Therefore, the average values decreased by 45.74% in V2 variant, by 40.31% in V3 variant; respectively by 30.62% in the V4 variant.

The aspects indicated by the absolute number of leukocytes are presented in Table 2.

The absolute number of leukocytes. In the experiment, significant differences were observed between the experimental variants

(P<0.05). The average value obtained in variant V2 is significantly (P<0.05) higher than the initial value. This aspect can be observed in Table 2. During the experiment, the values obtained were in the range 43.93-132.71 x 1000 leukocytes/mm^3. The values obtained are included in the reference range described in the literature for tilapia, respectively 21.559-154.690 x 1000 leukocytes/mm^3 (Hrubec et al., 2000).

The absolute number of small lymphocytes. Because from all the leukocytes, the small lymphocytes are dominated by the changes that occurred during the experiment, they had approximately the same tendency as in the case of the absolute number of leukocytes. The absolute number of small lymphocytes recorded the highest average value also in the variant in which rosemary was administered, being preceded by the average value from variant V1, V3 and V4. Thus, significant differences were noticed between the experimental variants (P<0.05). If we report the results obtained to the initial mean value, we find a significant increase (P<0.05) of the mean values at the end of the experiment (Table 2).

The absolute number of small lymphocytes showed during the experimental research a minimum value of 45.42 x 1000 small lymphocytes/mm^3 and a maximum value of 128.90 x 1000 small lymphocytes/mm^3, this falling within the gap described in the literature (Hrubec et al., 2000).

Table 2. Variation in the absolute number of leukocytes and platelets at Nile tilapia during the experiment

Experimental variant		Absolut number (x 1000 cel./mm^3)					
		Leukocytes	Lymphocytes		Monocytes	Neutrophilic granulocytes	Platelets
			small	large			
V0 (Initial)		62.16±3.86	59.96±3.41	0.71±2.22	0.45±0.17	1.04±0.83	22.51±9.21
Final	V1	86.83±19.60	83.06±19.26	0.83±0.27	0.90±0.25	2.17±0.35	32.00±6.65
	V2	108.97±14.16	105.20±14.29	1.20±0.22	1.20±0.12	1.48±0.34	28.78±8.91
	V3	74.32±18.38	72.00±18.00	0.53±0.23	0.77±0.15	1.14±0.32	25.51±6.20
	V4	63.61±9.42	60.88±9.04	0.67±0.21	1.08±0.32	1.12±0.07	23.57±7.44

The absolute number of large lymphocytes registered a significant increase (p<0.05) also in the variant in which rosemary was administered (V2 - 1.20 ± 0.22 x 1000 cell/mm^3). In our experiment, the absolute number of large lymphocytes ranged from 0.275 to 1.632 x 1000 cell/mm^3. The obtained results are not found in the limits described for tilapia, respectively 2.852-30,833 x 1000 large lymphocytes/mm^3 (Hrubec et al., 2000).

The absolute number of monocytes. There were no significant differences between the experimental variants (P>0.05). The mean values obtained at the end of the experiment were significantly higher (P<0.05) than the value obtained at the beginning of the experiment (V0). The lowest average value of the absolute number of monocytes was obtained in the variant in which sea buckthorn was administered (V3 - 0.77 ± 0.15 x 1000 monocytes/mm^3). The results recorded by us are found in the reference range for the absolute number of monocytes for tilapia ranging between 0.511 and 1.550 x 1000 monocytes/mm^3 (Hrubec et al., 2000). The variation of the absolute number of monocytes is presented in Table 2.

The absolute number of neutrophils. At the end of the experiment, a significant reduction (P<0.05) of the number of neutrophils was observed in the variants in which phytobiotics were administered compared to the control variant (V1). The lowest values were recorded in the variant in which sea buckthorn (V3) and ginger (V4) were administered. The mean values of the absolute number of neutrophils, obtained during the experiment, fall within the reference range for tilapia, 0.557-9.873 x 1000 neutrophils/mm^3 (Hrubec et al., 2000).

Absolute number of platelets. At the end of the experiment, was observed a reduction tendency, but insignificantly (P>0.05), of the average values in the variants in which phytobiotics were administered compared to

the control variant. Therefore, the average values decreased by 26.34% in V4 variant, by 20.28% in V3 variant; respectively by 10.06% in the V2 variant compared to control variant (Table 2). Recent studies have shown that platelets are involved in homeostasis process and play a defending role in organism, it is produced, in Teleostean fish in the spleen and kidneys (Tavares-Dias and Oliveira, 2009).

The increase in the number of leukocytes at the end of the experiment, especially in the variant in which rosemary was administered, can be corroborated with the increase in antibody production, which led to a good state of comfort, so tilapia specimens are less affected by stressor or being less susceptible to disease. Tawwab et al. (2010) obtained similar results when administered green tea (*Camelia sinensis*) to *Oreochromis niloticus*.

In the variant in which sea buckthorn was administered, it was observed that at the end of the experiment the number of monocytes was lower than in the other experimental variants, this aspect shows the absence of foreign substances in the blood circulation (Bocioc, 2011).

CONCLUSIONS

Following the analysis of the haematological parameters, we can notice an improvement in the variants in which phytobiotics were administered, their positive action was observed especially in the variants in which sea buckthorn and ginger were administered.

Following the biochemical analysis of the blood we can say that the supplementation of the Nile tilapia diet with 1% *Hippophae rhamnoides*, respectively 1% *Zingiber officinale* led to the end of the experimental period, after the obtained results, to the reduction of technological stress and to the improvement of immunity. Although the administration of phytobiotics did not contribute to significant changes in cortisol and total protein throughout the experimental period, the administration of sea buckthorn led to a lower concentration of glucose compared to variants in which rosemary and ginger were administered. At the same time, only sea buckthorn and ginger showed an immunomodulatory effect during the experiment due to the intensification of lysozyme activity.

After the analysis of the relative number of leukocytes (%) we can conclude that at the end of the experiment the administration of phytobiotics contributed to obtaining significant changes ($P<0.05$) compared to the control variant at the level of neutrophil granulocytes, and compared to the initial mean value at the level of the relative number of small lymphocytes and monocytes.

In conclusion, dietary supplementation with sea buckthorn and ginger has reduced the technological stress and improved the immunity of *Oreochromis niloticus* species.

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REFERENCES

Abdel-Tawwab, M. (2012). Effects of dietary protein levels and rearing density on growth performance and stress response of Nile tilapia, *Oreochromis niloticus* (L.). *Int. AquaticRes*. 4, 3.

Ahmadifar, E., Sheikhzadeh, N., Roshanaei, K., Dargahi, N., Faggio, C. (2019). Can dietary ginger (*Zingiber ofcinale*) alter biochemical and immunological parameters and gene expression related to growth, immunity and antioxidant system in zebrafish (Danio rerio)? *Aquaculture*, 507, 341–348.

Akrami, R., Gharaei, A., Razeghi Mansour, M., Galeshi, A. (2015). Effects of dietary onion (*Allium cepa*) powder on growth, innate immune response and hemato e biochemical parameters of beluga (*Huso huso* Linnaeus, 1754) juvenile. *Fish & Shellfish Immunology*, 45, 828–834.

Al-Jamal, A.R., Alqadi, T. (2011). Effects of rosemary (*Rosmarinus officinalis*) on lipid profile of diabetic rats. *Jordan J Biol Sci,* 4, 199–204.

Anderson, S.C., Olsson, M.E., Johansson, E., Rumpunen, K. (2009). Carotenoids in Sea buckthorn (*Hippophae rhamnoides* L.) berries during ripening and use of pheophytin A as a maturity marker. *J. Agric. Food Chem*., 57, 250–258.

Antache, A., Cristea, V., Dediu, L., Grecu, I., Docan, A., Vasilean, I., Mocanu (Creţu), M., Petrea, Şt. M. (2013). The influence of some phytobiotics on growth performance at *Oreochromis niloticus* reared in an

intensive recirculating aquaculture system. *Lucrări Ştiinţifice, Seria Zootehnie*, 204-208.

Apines-Amar, M.J.S., Amar, E.C., Faisan Jr.J.P. (2013). Growth, plasma cortisol, liver and kidney histology, and resistance to vibriosis in brown-marbled grouper, *Epinephelus fuscoguttatus* fed onion and ginger. *Aquac. Aquar. Conserv. Legislation-Int. J. Bioflux Soc. (AACL Bioflux)*, 6, 530–538.

Arranz, E., Mes, J., Wichers, H. J., Jaime, L., Mendiola, J. A., Reglero, G. (2015). Anti-inflammatory activity of the basolateral fraction of Caco-2 cells exposed to a rosemary supercritical extract. *Journal of Functional Foods*, 13, 384–390.

Barros, M.M., Falcon, D.R., de Oliveira Orsi, R., Pezzato, L.E., Fernandes Jr., A.C., Guimarães, I.G., Fernandes Jr., A., Padovani, C.R., Sartori, M.M.P. (2014). Non-specific immune parameters and physiological response of Nile tilapia fed β-glucan and vitamin C for different periods and submitted to stress and bacterial challenge. *Fish Shellfish Immunol*., 39, 188–195.

Bittencourt, N.L.R., Molinari, L.M., Scoaris, D.O., Pedroso, R.B., Nakamura, C.V., Nakamura, T.U., Abreu Filho, B.A., Dias Filho, B.P. (2003). Haematological and biochemical values for Nile tilapia *Oreochromis niloticus* cultured in semi-intensive system. *Acta Scientiarum. Biological Sciences Maringá*, 25(2), 385–389.

Blaxhall, P. C., Daisley, K. W. (1973). Routine Haematological Methods for Use Fish Blood, *Journal of Fish Biology*, 5(6), 771–781.

Bocioc, E. (2011). Doctoral thesis: Research on the use of probiotics in industrial aquaculture in recirculating systems, "Dunărea de Jos" University, Galaţi.

Caillet, S., Yu, H., Lessard, S., Lamoureux, G., Ajdukovic, D., Lacroix, M. (2007). Fenton reaction applied for screening natural antioxidants. *Food Chem.,* 100, 542–552.

Cui, L, Kim, M.O., Seo, J.H., Kim, I.S., Kim, N.Y., Lee, S.H., Parkb, J., Kim, J., Lee, H.S. (2012). Abietane diterpenoids of *Rosmarinus officinalis* and their diacylglycerol acyltransferase- inhibitory activity. *Food Chem*, 132, 1775–1780.

Chrubasik, S., Pittler, M.H., Roufogalis, B.D. (2005). Zingiberisrhizoma: a comprehensive review on the ginger effect and efficacy profiles. *Phytomedicine*, 12, 684–701.

Christybapita, D., Divyagnaneswari, M., Michael, R.D. (2007). Oral administration of *Eclipta alba* leaf aqueous extract enhances the non-specific immune responses and disease resistance of *Oreochromis mossambicus*. *Fish Shellfish Immunol,* 23,840–52.

Cristea, V., Grecu, I., Ceapa, C. (2002). *Recirculating aquaculture systems engineering*, Bucharest Didactic and Pedagogic Publishing House.

Cristea, V., Mocanu (Creţu), M., Antache, A., Docan, A., Dediu, L., Ion (Placinta), S., Coadă, M.T. (2012). Effect of Stocking Density on Leuckocyte Reaction of *Oncorhynchus mykiss* (Walbaum, 1792). *Animal Science and Biotechnologies*, 45(2), 31–36.

Dugenci, S.K., Arda, N., Candan, A. (2003). Some medicinal plants as immunostimulant for fish. *J Ethnopharmacol*, 88, 99–106.

Gaya, M., Repetto, V., Toneatto, J., Anesini, C., Piwien-Pilipuk, G., Moreno, S. (2013). Antiadipogenic effect of carnosic acid, a natural compound present in *Rosmarinus officinalis*, is exerted through the C/EBPs and PPARc pathways at the onset of the differentiation program. *BBA Gen Subj,* 1830, 3796–3806.

Ghelichpour, M., Mirghaed, A.T., Mirzargar, S.S., Joshaghani, H., Mousavi, H.E. (2018). Modification of saltwater stress response in *Cyprinus carpio* (Linnaeus, 1758) preexposed to pesticide indoxacarb. *Ecotoxicol. Environ. Saf.,* 147, 139–143.

Ghergariu, S., Pop, A., Kadar, L., (1985). *Veterinary Clinical Laboratory Guide/Ghid de Laborator Clinic Veterinar*. Bucharest, RO: Ceres Publishing House.

Goda, A. M. A.S. (2008). Effect of dietary ginseng herb (Ginsana G115) supplementation on growth, feed utilization, and hematological indices of Nile tilapia, *Oreochromis niloticus* (L.), fingerlings. *Journal of the World Aquaculture Society*, 39, 205–214.

Hamid, A.S.H., Ahmed, M.F.A., Mohammed, A.M.I., Ali, M.S.I. (2013). Physical and chemical caracteristics of blood of two fish species (*Oreochromis niloticus* and *Clarias lazera*). *Worlds Vet. J.*, 3(1), 17–20.

Harikrishnan, R., Balasundaramb, C., Heo, M.S. (2010). Herbal supplementation diets on hematology and innate immunity in goldfish against *Aeromonas hydrophila*. *Fish & Shellfish Immunology,* 28, 354e361. doi:10.1016/j.fsi.2009.11.013

Hassan, A.A.M., Yacout, M.H., Khalel, M.S., Hafsa, S.H.A., Ibrahim, M.A.R., Mocuta, D.N., Rahoveanu, A.T., Dediu, L. (2018). Effects of Some Herbal Plant Supplements on Growth Performance and the Immune Response in Nile Tilapia (*Oreochromis niloticus*). *Agricul. Life Life Agric. Conf. Proc.*, 1, 134–141.

Hrubec, T.C., Cardinale, J.L., Smith, S.A. (2000). Hematology and Plasma Chemistry Reference Intervals for Cultured Tilapia (*Oreochromis* Hybrid), *Veterinary Clinical Pathology*, 29(1), 7–12.

Hussain, A. I., Anwar, F., Chatha, S.A.S., Jabbar, A., Mahboob, S., Nigam, P.S. (2010). Essential oil: antiproliferative, antioxidant and antibacterial activities. *Brazilian Journal of Microbiology*, 41, 1070–1107.

Jahanjoo, V., Yahyavi, M., Akrami, R., Bahri, A. H. (2018). Influence of Adding Garlic (*Allium sativum*), Ginger (*Zingiber officinale*), Thyme (*Thymus vulgaris*) and Their Combination on the Growth Performance, HaematoImmunological Parameters and Disease Resistance to Photobacterium damselae in Sobaity Sea Bream (*Sparidentex hasta*) Fry. Turkish *Journal of Fisheries and Aquatic Sciences,* 18, 633–645.

Jalali, M.A., Hosseini, S.A., Imanpour, M.R. (2010). Physiological characteristics and stress resistance of great sturgeon (*Huso huso*) juveniles fed with vitamins C, E, and HUFAenriched *Artemia urmiana* nauplii. *Fish Physiol. Biochem*., 36, 555–564.

Jawad, L.A., Al-Mukhtar, M.A., Ahmed, H.K. (2004). The relationship between haematocrit and some biological parameters of the Indian shad, *Tenualosa*

ilisha (Family Clupidae). *Anim Biodivers Conserv*, 27, 478–483.

Jian, J., Wu, Z. (2004). Influence of traditional Chinese medicine on non-specific immunity of Jian Carp (*Cyprinus carpio* var. Jian). *Fish Shellfish Immunol,* 16, 185–191.

Jiang, Y., Wu, N., Fu, Y.-J., Wang, W., Luo, M., Zhao, C.J., Zu, Y.G., Liu, X.L. (2011). Chemical composition and antimicrobial activity of the essential oil of Rosemary. *Environ. Toxicol. Pharmacol*., 32, 63–68.

Kori-Siakpere, O., Alje, J.E.G., Idoge, E. (2005). Haematological characteristcs of the African snakehead, *Parachacnna obscura*, *Afr J Biotechnology*, 4(6), 527–530.

Kumar, M.S.Y., Dutta, R., Prasad, D., Misra, K. (2011). Subcritical water extraction of antioxidant compounds from sea buckthorn (*Hippophae rhamnoides*) leaves for the comparative evaluation of antioxidant activity. *Food Chem*., 127, 1309-1316.

Morar, R. (2003). *Eridiarom, Erisol, Polivitarom or Herbal Alternatives*. Ed. Todesco, Cluj-Napoca.

Naiel, M.A., Ismael, N.E., Shehata, S.A. (2019). Ameliorative effect of diets supplemented with rosemary (*Rosmarinus officinalis*) on aflatoxin B1 toxicity in terms of the performance, liver histopathology, immunity and antioxidant activity of Nile Tilapia (*Oreochromis niloticus*). Aquaculture. 511, 734264.

Nieto, G., Ros, G., Castillo, J. (2018). Antioxidant and antimicrobial properties of rosemary (*Rosmarinus officinalis*, L.): a review. *Medicines*. 5, 98.

Owolabi, O.D., (2011). Haematological and serum biochemical profile of the upside-down catfish, Synodontis membranacea Geoffroy Saint Hilaire from Jebba Lake, Nigeria. *Comp Clin Pathol*., 20, 163–172,

Rao, Y.V., Das, B.K., Jyotyrmayee, P., Chakrabarti, R. (2006). Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. *Fish Shellfish Immunol*, 20, 263–73.

Romo Vaquero, M., Yanez-Gascon, M.J., Garcıa Villalba, R., Larrosa, M., Fromentin, E., Ibarra, A., Soller, M., Tomas-Barbera, F., Espın de Gea, J.C., Garcıa-Conesa, M.T. (2012). Inhibition of gastric lipase as a mechanism for body weight and plasma lipids reduction in Zucker rats fed a rosemary extract rich in carnosic acid. *PLoS One*, 7:39773.

Sakai, M. (1999). Current research status of fish immunostimulants. *Aquaculture*, 172, 63–92.

Svobodova, Z., Fravda, D., Palakova, J., (1991). *Unified methods of haematological examination of fi*sh. Research Institute of fish Culture and Hydrobiology, VURH Vodnany, Edice Metodik, Czechoslovakia.

Svobodova, Z. (2001). Stress in Fishes (A Review), *Bull. Vurh Vodnany*, 4, 169–191.

Taheri Mirghaed, A., Hoseini, S.M., Ghelichpour, M. (2018). Effects of dietary 1, 8-cineole supplementation on physiological, immunological and antioxidant responses to crowding stress in rainbow trout (*Oncorhynchus mykiss*). *Fish Shellfish Immunol*., 81, 182–188.

Tavares-Dias, M., Faustino, C.D. (1998). Hematological parameters of Nile tilapia *Oreochromis niloticus* (Cichlidae) in extensive culture, *Ars Vet.,* 14, 254–263.

Tavares-Dias, M., Oliveira, S. R. (2009). A review of the blood coagulation system of fish, *Brazilian Journal of Biosciences, Porto Alegre*, 7(2), 205–224.

Tawwab, A.M., Amad, M.H., Seden, M.E.A., Sakr, S.F.M. (2010). Use of green tea, *Camelia sinensis* L., in practical diet for growth and protection on Nile tilapia, *Oreochromis niloticus*, against *Aeromonas hydrophila* infection, *Journal of the world aquaculture society*, 41(2), 203–213.

Todoran, L. (2015). Researches on assessing and improving quality indicators in salmonid exploitation. Ph.D. Thesis, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania.

Xie, J., Liu, B., Zhou, Q., Su, Y., He, Y., Pan, L. (2008). Effects of anthraquinone extract from rhubarb *Rheum officinale* Bail on the crowding stress response and growth of common carp *Cyprinus carpio* var. *Jian Aquacult*, 281, 5-11.

Yang, B., Kallio, H. (2002). Composition and physiological effects of sea buckthorn (*Hippophaë*) lipids, Trends *in Food Science and Technology*, 13(5), 160–167.

Yılmaz, S., Ergun, S., Celik, E.S. (2013). Effect of dietary herbal supplements on some physiological conditions of sea bass *Dicentrarchus labrax*. *J Aquat Anim Health*, 25, 98–103.

Yin, G., Jeney, G., Racz, T., Xu, P., Jun, X., Jeney, Z. (2006). Effect of two Chinese herbs (*Astragalus radix* and *Scutellaria radix*) on non specific immune response of tilapia, *Oreochromis niloticus*. *Aquaculture*, 253, 39–47.

Yin, G., Ardo, L., Thompson, K.D., Adams, A., Jeney, Z., Jeney, G. (2009). Chinese herbs (*Astragalus radix* and *Ganoderma lucidum*) enhance immune response of carp, *Cyprinus carpio*, and protection against *Aeromonas hydrophila*. *Fish Shellfish Immunol*, 26, 140–145.

Yousefi, M., Hoseini, S.M., Vatnikov, Y.A., Kulikov, E.V., Drukovsky, S.G. (2019). Rosemary leaf powder improved growth performance, immune and antioxidant parameters, and crowding stress responses in common carp (*Cyprinus carpio*) fingerlings. *Aquaculture*, 505, 473–480

Enzymatic Activity of Lysozyme Protocol - Sigma Aldrich. Home page address: http://www.sigmaaldrich.com/technicaldocuments/ protocols/biology/enzymatic-assay-oflysozyme. html

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PHOSPHOLIPID SPECTRUM AT CRYOTECHNOLOGICAL STAGES OF GAMETES PROCESSING OF FARM ANIMALS

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Abstract

Due to the fact that the bulk of the lipid bilayer of biological membranes is phospholipids, the purpose of the researches, the results of which are presented in this paper, was to study the phospholipid composition and its cryogenic changes during preservation of bulls, rams and boars sperm. Ram gametes are characterized by a high content of phosphatidylcholine and phosphatidylethanolamine. The minor components of boar gametes are phosphatidylserine; ram - sphingomyelin and bull - phosphatidylserine and cardiolipin. By thin-layer chromatography it was found that phospholipids of gametes are represented by the following fractions: 1. Phosphatidylserine; 2. Phosphatidylcholine; 3. Sphingomyelin; 4. Phosphatidylethanolamine; 5. Cardiolipin. Of these, the largest amount is contained in the fractions of phosphatidylcholine and phosphatidylethanolamine, the content of the remaining fractions is slightly lower, namely phosphatidylserine, sphingomyelin and cardiolipin. With the passage of the technological stages from dilution to thawing of biological material, the phospholipids content gradually decreases.

Key words*: phospholipids, cryopreservation, gametes.*

INTRODUCTION

Phospholipids belong to the class of highly specific lipids, are components of cell membranes and organelles contribute to the metabolic process. Currently, new properties of phospholipids have been discovered, one of which contributes to an increase in the intensity of reproduction of animals involved in activation and acrosomal reactions, as if they were specially created by nature to stimulate the reproductive system of humans and animals (Skatkov, 2002). Diet fortified with phospholipids along with other biotechnological methods (Narijnii et al., 2001) such as hydrotherapy, the introduction of placental preparations, herbal preparations based on *Rhodiola rosea*, *Silybum marianum* and others (Vodeannikov et al., 1999; Djamaldinov, 2006), improves the activity of the reproductive system. For example, in the studies of (Narijnii et al., 2014) as an addition to the ration of boars were used the drug Moslecitin which contains essential phospholipids, which acted very effectively on sperm indicators and sows fertility when fed for 45 days and 90 days.

MATERIALS AND METHODS

The main experimental studies were carried out in the laboratory "Physiology and Reproductive Health" of the Institute of Physiology and Sanocreatology.

The object of the study was the semen material of bulls of the Black-Motley; rams of the Karakul, Tsigay and East Friesian; boars of Large White breeds. The experimental animals were kept under conditions corresponding to zoo-veterinary requirements.

The optimal composition and concentration of the components of cryoprotective mediums during freezing of the animal genome was determined by mixing isotonic solutions of the studied substances in an arithmetic or geometric series (Milovanov, 1962).

Seed was frozen in the form of granules with a volume of 0.1-0.2 ml on a fluoroplastic plate at a temperature of –110…–120°C. The material was thawed and carried out in a water bath or

using a constructed aerodynamic device using a dry and wet method.

As the base medium for cryopreservation of the bull, ram and boar sperm, respectively, was used the medium of (Kononov et al., 1975; Nagase et al., 1964; Watanabe et al., 1976; Kopeika, 1986). In the study of biochemical parameters from the composition of the medium the yolk is excluded.

Quantitative determination of phospholipids by phosphorus is based on the ability of ammonium molybdenum acid to form a phosphorus-molybdenum complex in an acidic medium with inorganic phosphate, which, after reduction, gives products colored blue in proportion to the phosphorus content.

Samples were evaporated on a rotary evaporator. The precipitate was washed with acetone and washed with chloroform. A mixture of lipids was applied to chromatographic plates. The separation of phospholipids was carried out in a system of chloroform: methanol: acetic acid: distilled water (65: 43: 1: 4). Staining was performed on crystalline iodine. Chromatograms were photographed and processed using an NF-4 densitometer (GDR).

RESULTS AND DISCUSSIONS

As we mentioned above, in the conditions of the laboratory of "Physiology and Reproductive Health", using the method of thin layer chromatography, the amount of cholesterol and five phospholipid fractions were identified and determined, of which: the phosphatidylcholine fraction. Phosphatidylcholine (PC) is one of the two most massive phospholipids of the brain membranes. The nitrogen-containing base, which carries a positive charge - choline - is connected by an ether bond with the radical of phosphoric acid, which ensures the balance of electric charges. In most cases, saturated fatty acids predominate in the 1st position of glycerol, and in the 2nd unsaturated. The hydrophobic apolar tails of phosphatidylcholine are the same as those of phosphatidic acid.

Phosphatidylethanolamine (PE) - in this phospholipid, the remainder of phosphoric acid is etherified with amino alcohol ethanolamine. At pH 7, it carries a small negative charge. Phosphatidylethanolamine fatty acids are usually less saturated than phosphatidylcholine. Phosphatidylethphnolamine is also one of the most massive brain phospholipids.

By analogy with phosphatidylcholine, mammalian phosphatidylethanolamine contains relatively high amounts of arachidonic and docosahexaenoic acids. However, the PE has a smaller “polar head” than the PC, and, in contrast to it, is able to form additional hydrogen bonds using the terminal amino group.

Phosphatidylserine (PS) is a diacyl glycerophospholipid containing the amino acid serine in an ether bond, through the OH- group of serine, with phosphoric acid. Although PS is well distributed in nature in animals, plants, and microorganisms, it usually accounts for less than 10% of the total amount of phospholipids. The highest content of PS is found in brain tissues, but in some cases in the plasma membrane and endoplasmic reticulum (ER) of the cells its amount can reach 20% of all phospholipids. By its chemical structure, PS is an anionic phospholipid with three ionized groups: phosphate, amino, and carboxyl. Like other acidic lipids, it exists in nature in salt form. He is the third most abundant phospholipid in the brain. This phospholipid carries a negative charge and is among the phospholipids. Its plasmalogenous form is found in the brain, but in very small quantities.

Cardiolipin (CL) - diphosphatidylglycerol (sometimes poly) consists of a phosphatidylglycerol molecule in which the 3rd OH- group of the second glycerol residue is esterified with the phosphate group of the phosphatidic acid molecule. Thus, cardiolipin consists of three glycerol molecules connected by two diester bridges. The two OH- groups of both outer glycerin molecules are esterified with fatty acids. The inner mitochondrial membranes are rich in cardiolipin. It was first isolated from the muscles of the heart, where mitochondria are very numerous. The content of cardiolipin in the brain is small, significantly lower than in the heart, but it is constantly detected.

In the brain and in other animal tissues, sphingophospholipids are represented by sphingomyelin (SM), which contains a choline group and 4-sphingenin (sphingosine). Since the sphingosine base with a fatty acid is called

ceramide, sphingomyelin can be referred to as ceramide-1-phosphorylcholine. Sphingomyelin is contained in fairly significant amounts (5-10% of total phospholipids) in the white matter of the brain, especially in myelin, but also in gray matter and other tissues. This is a common saturated lipid free of polyenoic acids. About 2/3 of the sphingomyelin fatty acids from the gray matter of the brain are represented by stearic acid, while in sphingomyelin from the white matter of the brain, the main fatty acids are lignoceric and nervonic. Due to the phosphorylcholine group, sphingomyelin is a neutral lipid (Kreps, 1981; Ipatova, 2005).

In the conditions of our laboratory, the following phospholipid fractions were identified from the bull, boar and ram spermatozoa using standard solutions of lecithin and cholesterol, specific staining reactions and original photographs of chromatograms: phosphatidylserine (PS), sphingomyelin (SM), phosphatidylcholine (PC), phosphatidylethanoamine (PE), cardiolipin (CL), as well as cholesterol (C). The PC fraction included cholin plasmalogen (CP) and ethanolamine plasmolagen (EP), the PS - phosphatidylinositol (PI) fraction, and the CL fraction - phosphatidic acid (Nauk, 1991).

In accordance with modern ideas about the mechanisms of cryodamage of biological objects, an important place is given to changes in the state and content of lipids in them. Therefore, subsequent studies were devoted to the study of these particular chemical compounds.

As a result of the performed experiments, it was found that in the gametes of all species of animals under study contain the greatest amount of phospholipid fractions, such as phosphatidylcholine, phosphatidylethanolamine and cholesterol (Table 1).

Table 1. The content of phospholipids at the technological processing of animals gametes, mg/100 g

Name of lipids	Elements of technological processing		
	Dilution	Refrigeration	Thawing
Bull			
Phosphatidylserine	221.9±9.9	189.4±8.3	159.9±5.4*
Sphingomyelin	238.1 ± 24.4	189.4 ± 20.2	140.7±17.9*
Phosphatidylcholine	1649.4± 40.6	1253.3± 64.0	979.4±78.5*
Phosphatidylethanolamine	607.1±11.3	541.1±16.7*	389.6±39.0*
Cardiolipin	216.5±17.9	173.2±16.6	140.7±18.0*
Cholesterol	415.6±10.9	379.9±10.6	342.0±10.6*
Ram			
Phosphatidylserine	199.7±23.5	151.5±13.8	119.9±11.6*
Sphingomyelin	132.6±12.9	107.3±11.6	88.4±8.0*
Phosphatidylcholine	1848.5±55.1	1685.6±27.6*	1602.3±24.2*
Phosphatidylethanolamine	630.1±22.8	559.3±11.6*	512.6±15.2*
Cardiolipin	246.2±16.2	202.1±12.7	157.8±15.2*
Cholesterol	426.0±8.0	409.1±12.8	364.9±12.9*
Boar			
Phosphatidylserine	94.7±13.6	66.3±9.5	52.1±6.9*
Sphingomyelin	356.1±16.3	307.8±11.2	274.6±9.5*
Phosphatidylcholine	1468.8±39.1	1344.8±24.4*	1226.3±24.1*
Phosphatidylethanolamine	639.2±14.5	562.5±11.9*	18.0±12.2*
Cardiolipin	217.8±13.9	151.5±10.1	113.6±10.1*
Cholesterol	482.0±4.0	456.4±10.4	424.2±11.4*

Note: *Statistically authentic differences compared with diluted semen.

In the quantitative content of individual phospholipids and cholesterol are observed specific features. Thus, boar gametes contain less phosphatidylserine and phosphatidylcholine with a higher content of sphingomyelin, phosphatidylethanolamine and cholesterol. Ram gametes are characterized by a high content of phosphatidylcholine and

phosphatidylethanolamine. The minor components of boar gametes are phosphatidylserine, bull - phosphatidylserine and cardiolipin, and ram - sphingomyelin.
In addition, as a result of the studies, it was shown that no significant species differences were found in the total phospholipid content of freshly diluted gametes of bulls, rams and boars.
Refrigeration and maintenance of diluted bull, ram and boar semen at 4°C leads to a significant decrease in the total number of gamete phospholipid fractions. These changes take place due to such fractions as phosphatidylcholine and phosphatidylethanolamine. This, in our opinion, indicates a greater vulnerability of these fractions, in the process of cryopreservation - deconservation, in comparison with other phospholipid fractions.
With further technological processing, a decrease is observed in the remaining fractions of phospholipids.
Therefore, during freezing and thawing of the bull, ram and boar sperm, changes in the content of phospholipid fractions, unlike protein fractions, are unidirectional character - their content decreases. At technological processing of farm animals sperm, the content of various fractions of phospholipids in gametes, regardless of the type of animal, decreases, which indicates the non-specific nature of the change in their content during cryopreservation.
Phospholipids are the main lipid component of cell membranes, they accompany fats in food and serve as a source of phosphoric acid necessary for human life. Phospholipids belong to the class of highly specific lipids and are components of cell membranes and organelles (mitochondria). They improve metabolic processes (Grishenko et al., 1994).
At the same time, new properties of phospholipids are currently discovered, one of which is that essential phospholipids contribute to the improvement and intensification of spermatogenesis (Grishenko et al., 1992). Of the essential phospholipids, the main one is phosphatidylcholine (lecithin). This phospholipid is involved in fertilization, namely in the activation of acrosomal reaction. In experiments with boars, the diet of which was enriched in phospholipids, it was demonstrated that phospholipids are a building material for cell membranes, cellular and subcellular structures. Phosphatidylcholine is involved in many biochemical and physiological processes, and has a direct effect on the functioning of cells, both somatic and reproductive (Gotsalka, 1969).
Skatkov (2002) found that poultry eggs and soybeans are the main sources of phospholipids. These sources of phospholipids, respectively, represent the components of the reproductive organs of animals and plants.

CONCLUSIONS

As a result of cryopreservation of farm animals sperm, the amount of phospholipid spectrum is gradually reduced, which obviously also occurs with other methods of preserving biological objects.
Fresh foods should contain more phospholipids since over time they can be subjected to peroxidation with the formation and accumulation of malondialdehyde, which has genotoxic, mutagenic, and carcinogenic effects.
The loss of phospholipids can be made up for by including them in the diet of humans and animals.
Animal feeding diets enriched with phospholipids affect the reproductive systems of both females and males.

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REFERENCES

Djamaldinov, A.C. (2006). *Intensification of the reproductive function of boars using biotechnological methods.* 03.00.13 – physiology: doctoral thesis in biological sciences. Dubrovitsy (In Russian).
Gotsalka, A. (1969). *Glycoproteins.* Moscow: Peace (In Russian).
Grishenko, V.I., Kalughin, I.V., Lucko, N.A. (1994). Some aspects of embryo and sperm cryopreservation. *Problems of Cryobiology,* 2, 29-37 (In Russian).

Grishenko, V.I., Ostashko, F.I., Isacenko, V.V., et al. (1992). Vitrification, ultrafast freezing of mouse, rat and cow embryos. *Problems of Cryobiology,* 1, 35-39 (In Russian).

Ipatova, O.M. (2005). *Phosphogliv: mechanism of action and use in the clinic.* Moscow (In Russian).

Kononov, V.P., Golisev, N.A., Haciapuridze, E.L. (1975). Sperm freezing mediums. *Swine breeding,* 11, 26-27 (In Russian).

Kopeika, E.F. (1986). *Instructions for the low-temperature conservation of carp sperm.* Moscow (In Russian).

Kreps, E.M. (1981). *Cell membrane lipids.* Sankt Petersburg: Science. (In Russian)

Milovanov, V.K. (1962). *Reproduction biology and artificial insemination of animals.* Moscow. (In Russian)

Nagase, H., Niwa, T. (1964). Deep-freezing bull semen in concentrated pellet form. *Proceedings of V Intern. Congr. Anim. Reprod,* 503-506.

Narijnii, A.G., Vodeannikov, V.I., Pomorova, E.G., Breslavet, V.M., Pohodnea, G.S. (2001). *Boar productivity improvement.* Belgorod (In Russian).

Narijnii, A.G., Djamaldinov, A.C., et al. (2014). Study of the effect of feeding phospholipids on the qualitative indicators of boar semen. *Swine breeding,* 65, 203-208 (In Russian).

Nauk, V.A. (1991). *The structure and function of sperm of farm animals during cryopreservation.* Chișinău: Science (In Russian).

Skatkov, S.A. (2002). The effect of phospholipids on fertility. *Reproduction problems*, 3, 57-60 (In Russian).

Vodeannikov, V.I., Djamaldinov, A.C. (1999). Correction of reproductive function of boars with low potency. *Proceedings of the All-Russian research Institute of Breeding*, 56-60 (In Russian).

Watanabe M., Terada T. (1976). A new diluent for deep freezing preservation of fowl spermatozoa. *Proceedings of VIII Intern. Congr. Anim. Reprod. and Al. Krakow*, 4, 1096-1099.

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ANTIBIOTIC RESISTANCE ANALYSIS OF *Staphylococcus aureus* AS A MAIN CAUSE OF MASTITIS IN THE SOUTHERN REGION OF BANDUNG REGENCY

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Abstract

Pangalengan in Southern Bandung Regency, was known as a center for dairy farming. Mastitis caused by Staphylococcus aureus is a disease that often arises and difficult in treatment, because of lack of knowledge that causes arise resistance to antibiotics. Therefore, observation of milk samples, udder swabs, milking utensils, milking buckets and stable floors, from the Warnasari, Babakan Kiara and Tarumajaya small holder ranches, were tested using antibiotic discs. Results observations showed that Ampicillin, Oxytetracycline and Sulfametoxazole - Trimethoprim, ciprofloxacine and Chloramphenicol are five groups of antibiotics that are often used for the treatment of mastitis, and are now experiencing resistance. The highest percentage of resistance was to Ampicilin which reached 100% and the lowest was Sulfametoxazole - Trimethoprim, Ciprofloxacine and Chloramphenicol which reached 3.12%. Generally, the percentage of resistance of these five antibiotic group, shows the highest number in Babakan Kiara and the lowest in the Tarumajaya region. The factors that support increased antibiotic resistance in the treatment of mastitis are the use of antibiotics that is continuous and uncontrolled, poor farm management and the cage very close to farmer houses.

Key words: *antibiotics, dairy cows, milk, resistance, Staphylococcus aureus.*

INTRODUCTION

The rules for setting up farms, animal hygiene, shelters, milking and milk hygiene are important concerns of dairy farming. Due to different kind of microorganisms, the number of germs in milk may increase by exceeding the norms allowed for milk consumptions. The average total number of bacteria in milk (Total Plate Count) in the working area of the Southern Bandung Dairy Cattle Cooperative (KPBS) in Bandung Regency is 2.59 million Colony Forming Units (CFU) (KPBS, 2019). The low quality of milk produced due to high total bacteria, is inseparable from the production patterns of small holder farms, namely the number of livestock, the small size of land and the very limited skills of farmers (Mauludin et al., 2017). To avoid this situations, antibiotic are sometimes used excessively and uncontrollably, which causes the animals to develop resistance to their action. In addition, poor maintenance management affects up to 70% of milk production (Suprayogi et al., 2019). Distance of the cage adjacent to the farmer's house, as well as dairy cattle dung is usually collected in the cage, which often causes pollution of equipment used for milking, is one of the causes of infection of the udder/mastitis (Kero et al., 2002).

For this reason, research is needed on the level of antibiotic resistance and the use of antibiotic for the treatment of mastitis infections in dairy cattle farms in the Bandung Region, by analyzing the resistance level of *Staphylococcus aureus* bacteria and the factors that influence mastitis. *Staphylococcus aureus* is a major bacterium that causes mastitis in West Java, especially in the Bandung regency. The prevalence of subclinical mastitis in Bandung Regency reaches 53.1% (Supar and Ariyanti, 2008; Afnita, 2016). Bacteria *Staphylococcus aureus*, the cause of mastitis has become resistant to antibiotics Penicillin G, Ampicillin, Vancomycin, Basitrasin and, Ciprofloxacin (Campion et al., 2004), but are still very sensitive to the antibiotics Gentamicin

and Oxacillin (Meng et al, 2018). Poor livestock environmental factors can also increase bacterial resistance to antibiotics (Collignon and Scott, 2019). For this reason, research on how antibiotic resistance levels are used for the treatment of mastitis infections in dairy farms in Bandung Regency, by analyzing the resistance level of *Staphylococcus aureus* bacteria and the factors that influencing of mastitis.

MATERIALS AND METHODS

Sample preparation and isolation of Staphylococcus aureus

This study used 192 subclinical mastitis milk samples, 48 udder swabs, 18 milking utensils swabs, 18 dairy bucket swabs and 18 stable floor swabs, which were isolated from stable farms in Warnasari and Babakan Kiara areas in Pengalengan District and Tarumajaya in Kertasari areas, Bandung district. Determination of subclinical mastitis is done according to the Californian Mastitis Test (CMT). Isolation and identification of *Staphylococcus aureus* was done through bacterial growth screening on Mannitol Salt Agar (MSA), Gram staining, catalase test, coagulase, Voges-Proskauer test and growth properties on 5% sheep blood agar plates (PAD) conducted at the Processing Technology Laboratory, Faculty of Animal Husbandry, University of Padjadjaran. Isolate *Staphylococcus aureus* positive 23S rRNA with code American Type Culture Cell (ATCC) 25923 (Mbrio).

Phenotypic Test of Antibiotic Resistance Properties

This test was determined using a standard inhibition zone test on Müller Hinton agar (Himedia) using Oxoid disk, consisting of: Ampicillin (AMP 10 mcg), Gentamicin (CN 10 mcg), oxytretracycline (OT 30 mcg), Chloramphenicol (C 30 mcg), Sulphamethoxazole - trimethroprim (S x T 25 mcg) and Ciprofloxacine (CIP 5 mcg). *Staphylococcus aureus* ATCC 25923 (Mbrio) was used as a reference strain. Furthermore, isolates are categorized as vulnerable and resistant based on interpretive criteria developed by The Clinical and Laboratory Standards Institute (CLSI) 2019 (Table 1).

Table 1. Standardization of the MIC Zone Antibiotics CLSI 2019

Antibiotic	Diameter (mm)		
	Sensitive	Intermediate	Resistance
Peniciline	≥ 29	–	≤ 28
Gentamicine	≥ 15	13-14	≤ 12
Ciprofloxacine	≥ 21	16-20	≤ 15
Sulfa dan Thrimeth	≥ 16	11–15	≤ 10
Chloramphenicol	≥ 18	11–17	≤ 12
Oxytetrasikline	≥ 19	11–18	≤ 14
Ampiciline	≥ 26	–	≤ 25

Source: CLSI 2019

RESULTS AND DISCUSSION

The results showed that all of 192 milk samples studied, 48 udder swabs, 18 milking utensils swabs, 18 dairy bucket swabs and 18 stable floor swabs, positively contained *Staphylococcus aureus*. This was confirmed phenotypically based on identification results stating that the bacteria were Gram-positive, able to ferment mannitol on MSA media, compact white colonies on Blood Agar Plate media by hemolysis of sheep blood, showing positive reactions on catalase test, positive coagulase test. This is in accordance with the references of Quinn et al. (2002) and Todar (2005), which stated that *Staphylococcus aureus* is Gram-positive, capable of fermenting mannitol, a compact white colony with variations in hemolysis properties, positive on the catalase, coagulase test (Figures 1, 2 and 3).

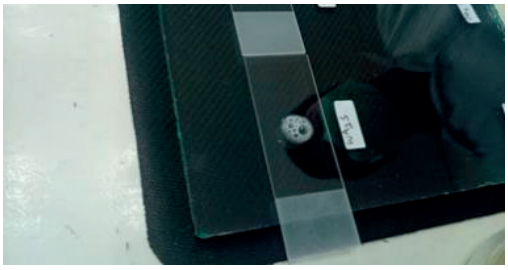

Figure 1. Catalase Test on *Staphylococcus aureus*

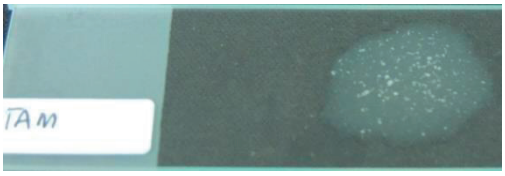

Figure 2. Coagulase Test on *Staphylococcus aureus*

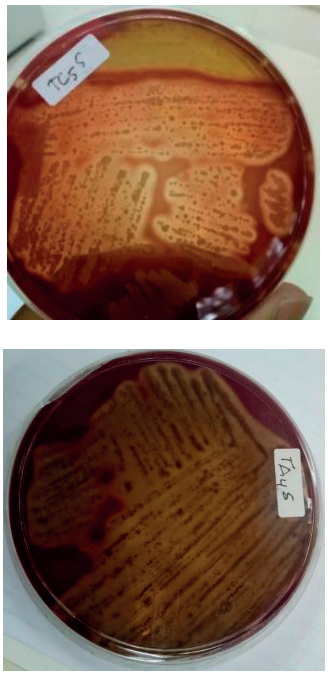

Figure 3. Growth of *Staphylococcus aureus* bacteria on Sheep Blood Agar Media

Based on the nature of bacterial hemolysis isolation in sheep blood agar media, Staphylococcus aureus can lyse sheep blood. Thus these bacteria have virulence factors including alpha hemolysis (hlα) and beta hemolysis (hlβ). Hassuny (2014) mentions that in general the virulence factor of exotoxin haemolisin works by lysis of red blood cells and cause damage to mammalian epithelial cells.

In Gram staining, the bacterium *Staphylococcus aureus*, shows a violet color and is shaped like grapes (Figure 4), is in accordance with the statement of Carter and Cole (2019), that *Staphylococcus aureus* bacteria are Gram-positive, violet-colored bacteria and are shaped like clustered grapes.

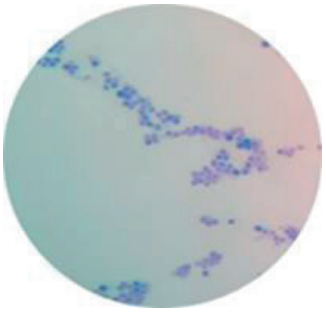

Figure 4. Gram staining of *Staphylococcus aureus*

Staphylococcus aureus, is a major bacterium that causes mastitis in West Java, especially in the Bandung Regency (Sugiri and Anri, 2010). This bacterium often causes mastitis infections both clinically and subclinically.

In dairy farming, *Staphylococcus aureus*, is a threat in increasing the quality and quantity of cow's milk. This threat is due to the difficulty in handling and preventing mastitis infection.

In the observation of this study, it showed that Ampicillin, Oxytetracycline, Sulfametoxazole-Trimethoprim, Ciprofloxacine and Chloramphenicol are five groups of antibiotics for the treatment of mastitis which have become resistant.

The lowest percentage of resistance level occurred in Sulfametoxazole - Trimethoprim, Ciprofloxacine and Chlorampenicol antibiotics (3.12%) and the highest was Ampicilin (100%) (Figure 5, Table 2).

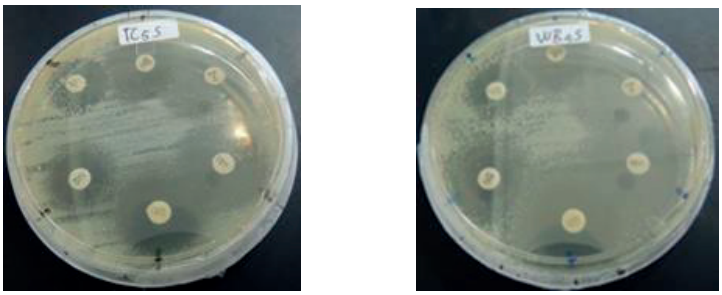

Figure 5. Observation Results of Growth Inhibition Zones (MIC), *Staphylococcus aureus* bacteria in S x T Antibiotic Discs, Cip 5, C30, OT30, CN 50 and AMP 10.

Notes: S x T: Sulfametoxazole - Trimethroprim; Cip 5: Ciprofloxacine; C30: Chloramphenicol; OT30: Oxitetracycline; CN50: Gentamicin; AMP 10: Ampicillin

Table 2. Percentage Data (%) of Antibiotic Resistance Levels in Milk, Udder, Milking utensils, Milking buckets and Stable floor in Small holder Dairy Farming in the KPBS Pangalengan Area

No	Area	Milk		Udder Swabs		Milking utensils		Milking Buckets		Stable Floors	
		Sample	%	Sample	%	Sample	%	Sample	%	Sample	%
1	**Warnasari**										
	S x T	16/64	25	2/16	12,5	3/6	50	0/6	0	2/6	33,33
	C30	0/64	0	0/16	0	0/6	0	0/6	0	0/6	0
	OT30	4/64	6,25	5/16	31,25	3/6	50	3/6	50	2/6	0
	CN 50	0/64	0	0/16	0	0/6	0	0/6	0	0/6	0
	AMP 10	64/64	100	16/16	100	6/6	100	6/6	100	6/6	100
	Cip 5	0/64	0	2/16	12,5	0/6	0	0/6	0	0/6	0
	Total	64		16		6		6		6	100
2	**Babakan Kiara**										
	S x T	23/63	36,51	2/16	12,5	3/6	50	3/6	50	4/6	66,67
	C30	3/63	4,77	0/16	0	0/6	0	0/6	0	0/6	0
	OT30	0/63	0	8/16	50	5/6	83,3	4/6	66,67	4/6	66,67
	CN 50	0/63	0	0/16	0	0/6	0	0/6	0	0/6	0
	AMP 10	63/63	100	16/16	100	6/6	100	6/6	100	6/6	100
	Cip 5	0/63	0	1/16	6,25	0/6	0	0/6	0	0/6	0
	Total	63		16		6		6		6	
3	**Tarumajaya**										
	S x T	2/64	3,12	1/16	6,25	0/6	0	0/6	0	0/6	0
	C30	2/64	3,12	1/16	6,25	0/6	0	1/6	16,67	0/6	0
	OT30	0/64	0	4/16	25	1/6	16,67	0/6	0	1/6	16,67
	CN 50	0/64	0	0/16	0	0/6	0	0/6	0	0/6	0
	AMP 10	64/64	100	16/16	100	6/6	100	6/6	100	6/6	100
	Cip 5	2/64	3,12	3/16	18,75	3/6	50	1/6	16,67	1/6	16,67
	Total	64		16		6		6		6	

Notes: S x T: Sulfametoxazole – Trimethroprim; Cip 5: Ciprofloxacine; C30: Chloramphenicol; OT30: Oxitetracycline; CN50: Gentamicin; AMP 10: Ampicillin

The observations results of antibiotic resistance levels against *Staphylococcus aureus* bacteria (Table 2) show that in general the lowest antibiotic resistance occurs in milk samples, from the stable groups in the Tarumajaya Region against the Cloramphenicol (C30) antibiotic group, the Ciprofloxacine (CIP 5) group, and the Sulfametoxazole - Trimethroprim antibiotic group (Cimetroprimic) S x T) were 3.12% respectively, and the highest was on the antibiotic Ampicillin (AMP 10) of 100% in milk, udder, milking devices, milk bucket and stable floor samples.

The low resistance of *Staphylococcus aureus* to Sulfametoxazole - Trimethoprim (S x T), Ciprofloxacine (CIP 5) and Cloramphenicol (C30) antibiotics (3.12%) occurred in stables in the Tarumajaya region, Kertasari District. Stables in the Tarumajaya area are groups/collective enclosures, with locations far from settlements, bordered by protected forests and with a clean housing system, separated between lactation stables and heifer stables, the feed warehouse is separated from the location of livestock waste disposal. This is consistent with the statement conveyed by Rahayu (2015), that the high level of mastitis infection is highly correlated with cleanliness of the stables, and tends to reduce the use of antibiotics that also to reduce the antibiotic resistance. However, the highest percentage of resistance of *Staphylococcus aureus* to antibiotics is Ampicillin (100%), occurring in all three study areas and in all isolate samples. Ampicillin (AMP 10) is the most studied antibiotic group and has become resistant. This is in accordance with the data submitted by CIVAS (2016) that the highest resistance and most found on animal husbandry is the antibiotic group of Sulphamethoxazole - Trimethoprim, Ampicillin and Tetracycline. This occurs due to several factors that support the inappropriate use of antibiotics and too often using of monotherapy, the very long use and uncontrolled of antibiotics and irrational usages (Utami, 2011).

Dairy stables in the Babakan Kiara and Warnasari area are integrated with farmers homes or only a few meters away, tend to be more humid and with very densely populated

settlements. Cow dung is usually stacked in one corner of the stable and then discharged directly into the water channel. Even so, although the Tarumajaya enclosure is relatively cleaner, in fact the ampicillin (AMP 10) has become resistant. Such conditions, if uncontrolled will result in the spread of antibiotic resistance and residues into the surrounding environment, including water sources.

Based on the isolation, identification and detection of bacterial resistance of *Staphylococcus aureus,* milk isolates from the working area of the KPBS Pangalengan, which is positive for mastitis, indicate a real threat to dairy farms in Bandung Regency, due to the presence of *Staphylococcus aureus*, not only causing clinical and subclinical mastitis but also the potential for resistance to antibiotics that are commonly and uncontrolled used (the Ampicillin, Tetracycline, Quinolone, Aminoglycoside and Chloramphenicol groups). *Staphylococcus aureus* infections treatments, that cause mastitis is known to be very difficult because these bacteria have virulence factors (hlα and hlβ) and genetic changes, especially in the encoding gene for penicillin binding protein, in the use of beta-lactam class antibiotics (Hartman and Alexander, 1984; Olsen et al., 2006) and the presence of the dfrG gene which decreases the affinity of antibiotics, so that it quickly leads to resistance in the aminoglycoside group (Lowy, 2003). *Staphylococcus aureus* infection is also known to be contagious, so that it spreads quickly between the nipples and even between cows. The spread of bacterial infections that cause mastitis in the field, mainly due to poor milking management, location of farms that is close to settlements and poor environment, so the spread of infection between individuals in a farm is increasing.

The observational data on the percentage of antibiotic resistance in the three regions, showed that in small holder dairy farming in Babakan Kiara Region, in general had the highest level of antibiotic resistance compared to the other two regions. The percentage of antibiotic resistance that occurs between 4.77% in Chloramphenicol (C30) antibiotics to 100% in Ampicillin (Amp 10) antibiotics. The lowest resistance was found in the milk sample, then increased in the udder, milking bucket, milking devices, and the highest in the stable floor samples. However, all samples were resistant to Ampicillin antibiotics (AMP 10) (Table 2). The Babakan Kiara region, is known as a dairy farming area located in a densely populated area. Even the stables are very close to their homes and the sewage is directly into the Cileunca lake as water sources, which is less than 100 meters away, so that the spread of bacteria that are resistant to antibiotics in the environment will increase.

This is consistent with the statement of Frieri et al. (2017) that the spread of antibacterial resistant bacteria in the environment is currently increasing and bacteria become resistant due to horizontal gene transfer (HGT). HGT between bacterial species that can cause the spread of Antibiotic Resistance Genes (ARGs) is becoming easier and through various media in the environment, such as in water, soil, air, food, and living things. Facts have been found that the resistance to antibiotics is very large in the environment, due to poor environmental management (Hadi et al., 2018). This bacterial resistance can then spread to animals, humans and the surrounding environment. Bacterial resistance in animals can spread to other animals that are genetically diverse. Whereas in humans, the spread of resistant bacteria can spread directly between patients in the hospital or indirect transmission through health workers. This case, greatly influences the epidemic pattern and transmission of *Staphylococcus aureus* (FOPH, Switzerland, 2019).

CONCLUSIONS

Based on the observations of the resistance of *Staphylococcus aureus* bacteria from milk, udder, milking utensils, milking buckets and stable floors, in the KPBS working area of Bandung Regency, it was concluded that antibiotics (Ampicillin, Oxytetracycline, Sulfamethoxazole - Trimethoprim and Chloramphenicol) that are become resistant, while the Gentamicin are still sensitive.

Therefore, to prevent the increasing resistance of *Staphylococcus aureus* bacteria to antibiotics used for the treatment of mastitis, it is necessary to take steps among others, the use of

wise and well-controlled antibiotics, implementing good farm management by paying attention to environmental health aspects.

Collective system dairy farms stables must far from human settlements; to be one of the livestock systems that can be chosen to minimize the increase the mastitis and also in antibiotic resistance. Antibiotics that can still be recommended for use in dairy farms in the KPBS region, Bandung Regency are antibiotics of the Gentamicin group (CN 50).

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REFERENCES

Afnita, N. N. (2016). Implementation of the Prevention and Treatment of Mastitis in the West Java Pangalengan Area. (*Implementasi Pencegahan dan Pengobatan Penyakit Mastitis pada Peternakan Rakyat Daerah Pengalengan Jawa Barat)*. Animal Husbandry p. 4. Paper Innovation Animal Science Competition, Padjadjaran University.

Campion, J. J., McNamara, P. J., Evans, M. E. (2004). Evolution of ciprofloxacin-resistant Staphylococcus aureus in vitro pharmacokinetic environments. *Journal of Antimicrobial agents and chemotherapy*, 48(12), 4733–4744.

Carter, G. R., John, R., Cole, Jr. (2019). *Diagnostic Procedures in Veterinary Bacteriology and Mircobiology*, 5th Edition. San Diego, California, USA: Elsevier Publishing House.

CIVAS (2016). Antibiotic resistance in livestock: A global problem and its relevance for Indonesia. (*Resistensi antibiotik pada ternak: Suatu masalah global dan relevansinya untuk Indonesia)* Poultry Farmers' Workshop, Antibiotics Awareness Week. (*Saresehan Peternak Unggas, Pekan Kesadaran Antibiotika*) (Indonesian, Malang, 18th Nov 2018).

CLSI (2019). *Performance Standard of Antimicrobial Susceptible Testing*, 29th Edition. Clinical and Laboratory Standard Institute.

Collignon, J. P., Scott, A. M. (2019). One Health-Its Importance in Helping to Better Control Antimicrobial Resistance. *Trop Med Infect Dis*., 4(1), 22.

FOPH (2019). *Strategic on Antibiotic Resistance. Federal Office of Public Health*, Switzerland.

Frieri, M., Kumar, K., Boutin, A. (2017). Antibiotic resistance. *Journal of Infection and Public Health*, 10, 369-378.

Hadi, M. P., Fadlillah, L. N., Widasmara, M. Y., Muziasari, W. I., Subaryono (2018). Potential sources of antibiotic resistant bacteria based on water quality and land use conditions in the Code River, Yogyakarta: A methodological review. *(Potensi sumber bakteri resisten antibiotik berdasarkan kondisi kualitas air dan penggunaan lahan di Sungai Code, Yogyakarta: suatu tinjauan metodologis). Jurnal Pengelolaan Lingkungan Berkelanjutan (JPLB),* 2(1), 88-100 (in Indonesian).

Hartman, B. J., Alexander, T. (1984). Low-Affinity Penicillin-Binding Protein Associated with Beta Lactam Resistance in *Staphylococcus aureus. Journal of Bacteriology*, 158(2), 513-516.

Hassuny, A. A. (2014). Detection of Virulence Factor *hlα* dan *hlβ* of *Staphylococcus aureus* using PCR technic. *Medical Journal of Babylon*, 11(4).

Kero, D., Van Dijk, O., Nederbragt J. E. (2002). Factors involved in The Early Pathogenenis of Bovine *Staphylococcus aureus* Mastitis with Emphasis on Bacterial Adhesion and Invasion, A Review. *Veterinary Quartery*, 155-160.

KPBS (2019). Data on Milk Quality of Southern Bandung Dairy Farm Cooperative (KPBS) from 2017 to 2019 (*Data Kualitas Susu Koperasi Peternakan Sapi Perah Bandung Selatan (KPBS) tahun 2017 sampai dengan 2019*). Annual report (Laporan Tahuna Kesehatan Ternak KPBS, 2017-2019) (In Indonesian).

Lowy, F. D. (2003). Antimicrobial Resistance The Example of Staphylococcus aureus. *Journal of Clinical Investigation*, 111(9). 1265-1273.

Mauludin, M. A., Syahirul A., Viani, P. S (2017). Development and Dynamics of Production Modes of Dairy Cattle Business in Pangalengan, West Java *(*Pengembangan dan Dinamika Moda Produksi Usaha Peternakan Sapi Perah di Pangalengan Jawa Barat). *Sosiohumaniora,* 19(1), 37-44 (In Indonesian).

Meng, D., Wu, Y., Meng, Q., Qiau, J., Zhang, X., Ma, S., Cai, K., Zhang, J., Cheng, Z., Zhang, Z., Cai, X. (2018). Antimicrobial Resistance, Virulance gen Profile and moleculer typing of *Staphylococus aureus* isolate from dairy cows in Xinjiang Province. *Journal of Global Antimicrobial Resistance*, 16, 98-104.

Olsen, J. E., Christensen, H., Aarestrup F. M. (2006). Diversity and Evolution of *blaZ* from *Staphylococcus aureus* and Coagulase-Negative *Staphylococci. J Antimicrob Chemother. Maret*., 57(3), 450-460.

Quinn, P. J., Markey, B. K., Carter, M. E., Donnelly, W. J., Leonard, F. C. (2002) *Veterinary Microbiology and Microbial Disease*. Iowa, West Sussex, UK: Blackwell Science Ltd.Chichester Publishing House, 840-845.

Rahayu, S. (2015). Detection of Streptococcus agalactiae Causes Subclinical Mastitis in Dairy Cows in Cendana District Enrekang Regency. (*Deteksi Streptococcus agalactiae Penyebab Mastitis Subklinis pada Sapi Perah di Kecamatan Cendana Kabupaten Enrekang*) (in Indonesian). Thesis, Faculty of Medicine, Hassanudin University.

Sugiri, Y. D., Anri, A. (2010). Prevalence of pathogens that cause subclinical mastitis (*Staphylococcus aureus* and *Streptococcus agalactiae*) and other

subclinical pathogens that cause mastitis in small and medium scale farmers in several centers of dairy farming in Java (*Prevalensi Patogen Penyebab Mastitis Subklinis (Staphylococcus aureus dan Streptococcus agalactiae) dan Patogen Penyebab Mastitis Subklinis lainnya pada Peternak Skala Kecil dan Menengah di Beberapa Sentra Peternakan Sapi Perah di Pulau Jawa*). *JICA* (In Indonesian). Research report.

Supar, A., Ariyanti, T. (2008). Subclinical Mastitis Control Study in Dairy Cattle. Proceeding: Prospect of the Dairy Cattle Industry Towards Free Trade 2020 (Kajian *Pengendalian Mastitis*).

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PITUITARY - LOBULATION AND SEASONAL CHANGES OF THE BASOPHIL PITUITARY IN CYPRINIDS

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Abstract

The pituitary gland is noted through distinct particularities to those of the other vertebrates. Within this class there are morphological variations in form, the size and lobulation of the pituitary gland. Thus, in some species, adenohypophysis is not anatomically divided but it is consisting of a single lobe. In many species of Teleostei adenohypophysis it consists of two lobes, one anterior or distally subdivided into two zones and the other intermediate. The present paper aims to highlight the structure of the pituitary gland and cell types present in cyprinids lobes. The studies were performed on microscopic preparations containing sagittal sections through carp pituitary. The sagittal histological sections made of 5µm reveal the presence of two distinct parts: neurohypophyses of nervous origin, and the adenohypophysis of epithelial origin. Considering the scientific importance and the practice of pituitary hormones in stimulating the maturation of gonads in fish also followed the changes of the pituitary structure, especially of the middle gonadotropic lobe. The histological study of the pituitary gland in the H. molitrix species at different times of the year has given us the possibility to track the seasonal variations of the basophil gonadotropic lobe structure. In the present study demonstrated that during the annual cycle, GTH cells go through different stages of activity that are not synchronous in all cells GTH. We were able to draw conclusions regarding the best period for harvesting pituitary glands used for pituitary injections under artificial reproduction conditions.

Key words*: adenohypophysis, basophil gonadotropic, basophilic cells GTH, lobe, neurohypophysis, pituitary.*

INTRODUCTION

Given the problematic complexity and difficulties of approaching a fish endocrine mechanism, we need a claim on this topic. We are just trying to highlight the structure of the pituitary gland, aspects of hormonal regulation, related to a form of reproduction of a certain cyprinid.

The pituitary gland is located at the base of the brain, which is in close connection with the hypothalamus (Grasse, 1970). It is a small, soft, whitish body whose size and shape vary with species (Shanthanagouda et al., 2018).

The pituitary, considered a master gland of the endocrine system, consists of two lobes, the anterior pituitary or adenohypophysis, the glandular part of the pituitary consisting of cells which secrete pituitary hormones, and the posterior pituitary or neurohypophysis, containing nerve bundles originating from the hypothalamus as well as other parts of the brain (Ooi et al., 2004; Zohar et al., 2010).

Anterior pituitary is derived from the embryonic pouch, Rathke's pouch arising from the roof of the buccal cavity as an outward evagination and the posterior pituitary which originates from the downward evagination from floor to third ventricle (Yadav, 2009; Ball et al., 1969).

The adenohypophysis is subdivided into three parts, histologically, and in the nomenclature of the adenohypophysis fish is presented differently by the authors:

- after Pickfort and Atz, in the year 1957:

Adenohypophysis: pro-adenohypophysis, meso-adenohypophysis, meta-adenohypophysis;
Neurohypophysis;

- after Gorbman, in the year 1965:

Adenohypophysis: rostral pars distalis, pars distalis proximal, pars intermedia;
Neurohypophysis.

Most current authors have adopted Gorbman's nomenclature (Ekici et al., 2013; Evans, 2003).

MATERIALS AND METHODS

For histological research, pituitary samples were taken from adult females of *H. molitrix*, aged 4-5 years, with an average weight of 5-6 kg, from fish farm Cârja 1 - Vaslui county.

Sampling periods coincided with:

- vitellogenetic growth period (November 2015-April 2016);
- maturation period, after the end of vitellogenesis (April-May 2016);
- ovulation period, which took place in June 2016.

The biological material taken for the histological examination was processed by classical methods. Fixation was done in Bouin and Formalin, and after inclusion in paraffin, the pieces were cut at 5 μm by microtome Slee. Sections were stained with hemalaun-eosin (HE).

RESULTS AND DISCUSSIONS

Distribution of cells in the lobes of the adenohypophysis and neurohypophysis

In each of these parts the cells can be identified both by the arrangement and morphology and by the characteristic staining.

The types of cells contained in the adenohypophysis are: chromophobic, which does not participate directly in the secretion process and chromophilic, which in turn are of two types: acidophilic and basophilic.

The anterior lobe (rostral pars distalis) consists of fine-grained acidophilic cells and chromophobic cells mixed with small basophilic cells: prolactin cells, ACTH cells, TSH cells (Figure 1).

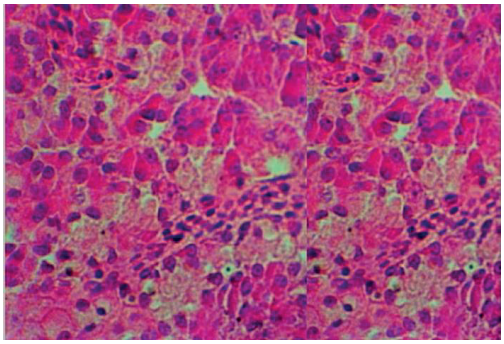

Figure 1. Anterior lobe of the pituitary gland. Acidophilic cells that produce prolactin predominate, HE, 40x

The middle lobe (proximal pars distalis) contains two types of cells, the cells pass successively from the basophilic type to the chromophobic type, then to the acidophilic type: STH cells, GTH cells. STH cells secrete pituitary somatotropic hormone that stimulates growth, and GTH cells secrete gonadotropic hormone that intervenes in the maturation of the gonads (Figure 2).

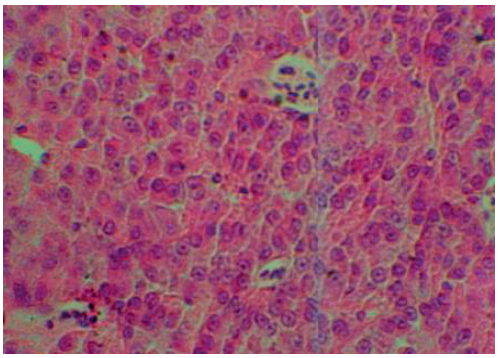

Figure 2. Gonadotropic middle lobe. Basophilic cells (GTH) are well represented. There are also sinusoidal capillaries that take over the secretion product, HE, 20x

The intermediate lobe (pars intermedia) is made up of a single type of cell that secretes a stimulating melanophore hormone, MSH (Takaski, 1982).

In the histological sections made by us, the cellular elements characteristic of the adenohypophysis lobes is highlighted (Figure 3).

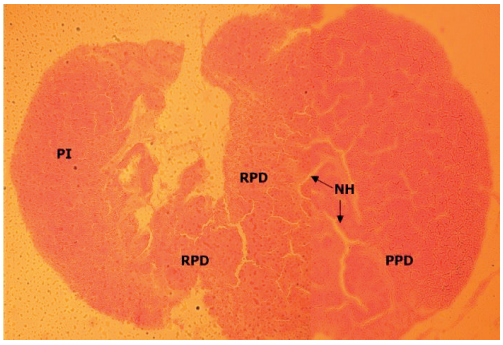

Figure 3. The pituitary gland, sagittal section, shows the adenohypophysis with the three lobes (RPD - rostral pars distalis, PPD - proximal pars distalis and PI - pars intermedia) and the neurohypophysis (NH), HE, 5x (original photo)

The neurohypophysis, like all teleosthenes, branches deeply to the middle of the adenohypophysis in a very particular multiple arborization (Figure 4).

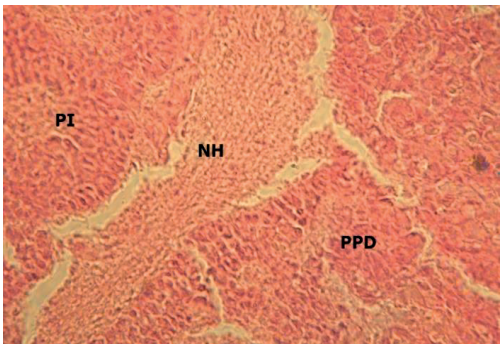

Figure 4. Arborizations of the neurohypophysis inside the adenohypophysis (PPD - proximal pars distalis and PI - pars intermedia), HE, 6x (original photo)

The neurohypophysis has a fibrous consistency being made up of numerous neuroglial fibers, but especially the neurosecretors that originate mostly in the preoptic nucleus of the hypothalamus and glial cells called pituitary.
These cells have an irregular shape and have short branched extensions.
According to some authors, through the hypothalamic - pituitary fibers mentioned above, a functional connection is established between the gonadotropic lobe in the adenohypophysis and the neurohypophysis (Figure 5).

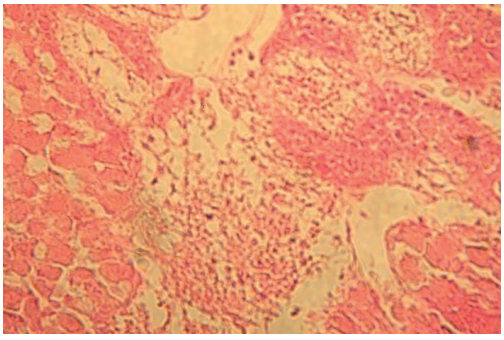

Figure 5. Neurohypophysis, amyelin nerve fibers and pituitary cells are observed, HE, 40x (original photo)

These anatomical connections also demonstrate the existence of a hypothalamic neurosecretory control over pituitary function that occurs in teleost fish as well as in mammals.
The two types of octopeptide hormones produced by the neurohypophysis, arginine vasotocin and isotocin are produced by neurosecretory cells of the preoptic nucleus transported along the axon and released into the capillaries of the neurohypophysis (Figure 6).

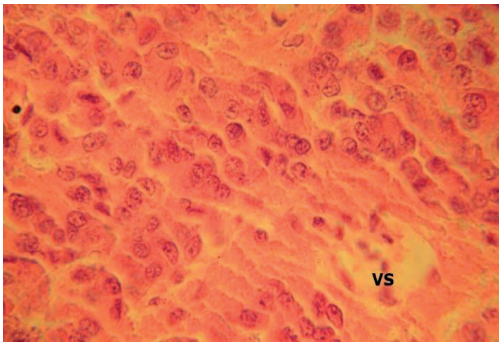

Figure 6. Neurohypophysis and PI - pars intermedia covering blood vessels (VS) for secretion, HE, 90x (original photo)

Seasonal changes in the basophilic pituitary gland in *Hypophthalmichthys molitrix*

The histological study of the pituitary gland in *H. molitrix* species at different times of the year, gave us the possibility to follow the seasonal variations of the gonadotropic lobe structure.

Vitellogenetic period

During this period on the histological sections performed on pituitary glands taken from mature females of *H. molitrix* from November to April in pars distalis proximalis (PPD), the middle lobe or meso-adenohypophysis predominates glycoprotein basophilic gonadotropic cells.
They have an eccentric nucleus, and the cytoplasm is loaded with intensely colored granules, which suggests a hormonal storage (Figures 7, 8, 9 and 10). There is a gradual loading of cells with granules of hormone secretion during this winter, which suggests a high secretory activity of the pituitary gland.
In pars distalis proximalis (meso-adenohypophysis) acidophilic somatotropic cells (STH) can also be observed which are smaller and with fine granulations.

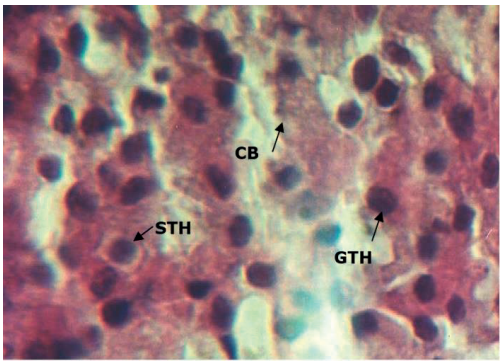

Figure 7. Proximal pars distalis - PPD: among corded GTH cells, rare STH cells and chromophobic (CB) cells, HE, 90x (original photo)

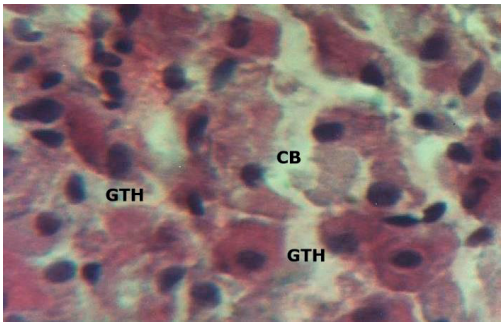

Figure 8. Gonadotropic lobe (PPD): GTH cells elongated with an eccentric or round nucleus, including chromophobic (CB) cells whose cytoplasm does not stain, HE, 90x (original photo)

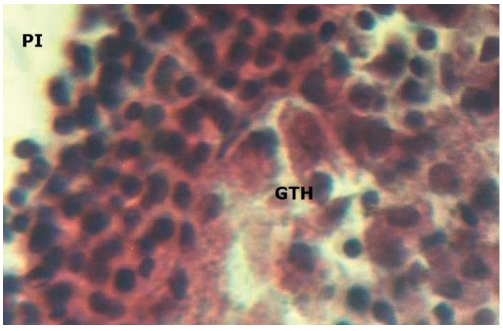

Figure 9. GTH cells at the limit of pars intermedia - PI, HE, 90x (original photo)

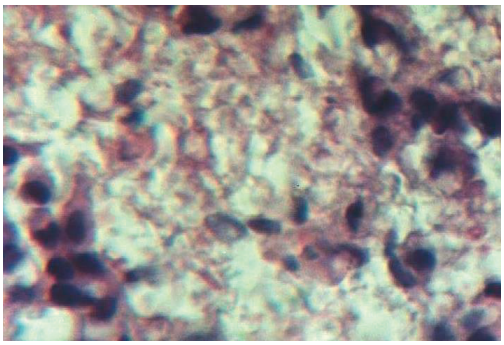

Figure 10. GTH cells ending on nerve fibers in this area their number is smaller, HE, 90x (original photo)

Maturation period

At maturity, the arrangement of highly granulated gonadotropic cells is observed near the blood capillaries where it secretes its secretion product (Figure 11).

The gonadotropic area is distinguished by an increase in the number and size of cells.

After the eggs are laid, the cells lose their polarity to the blood vessels.

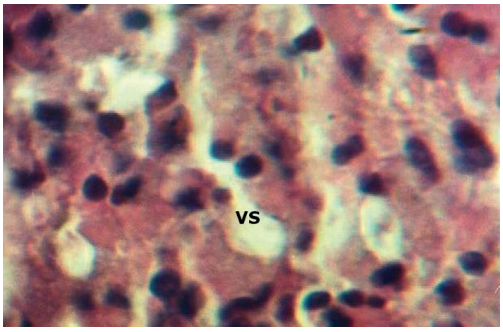

Figure 11. Proximal pars distalis - PPD: GTH cells arranged in cords, facing the blood vessels, HE, 90x (original photo)

Ovulation period

During the spawning season, the gonadotropic area changes considerably. Progressive degranulation and internal vacuolation are observed that occur in most cells. In some areas the cells appear hypertrophied, degranulated and vacuolated almost completely (Figures 12, 13 and 14).

Some gonadotropic cells are located in the dorsal part of the PPD among the branches of the neurohypophysis and are considered to produce GtH-I (FSH), and others located ventrally release the hormone GtH-II (LH) (Evans, 2003).
Gonadotropic cells therefore have a cycle of secretory activity correlated with the evolution of oocytes, having a role in vitellogenesis, maturation and ovulation.

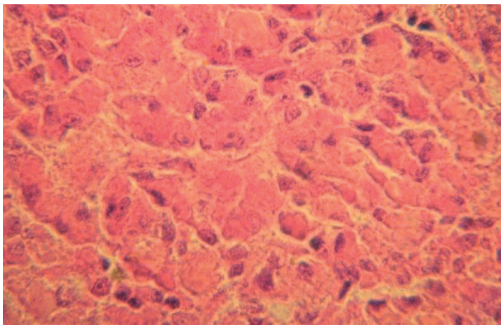

Figure 12. Partially degranulated GTH cells, pycnotic nuclei, HE, 40x (original photo)

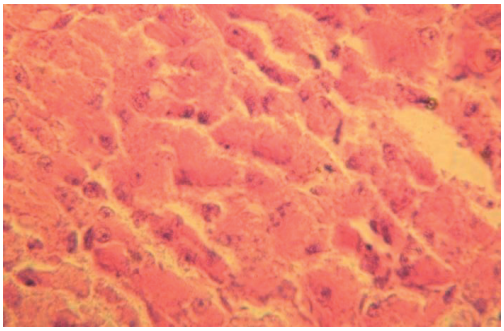

Figure 13. GTH cells, partially degranulated, pycnotic nuclei, HE, ob. 40x (original photo)

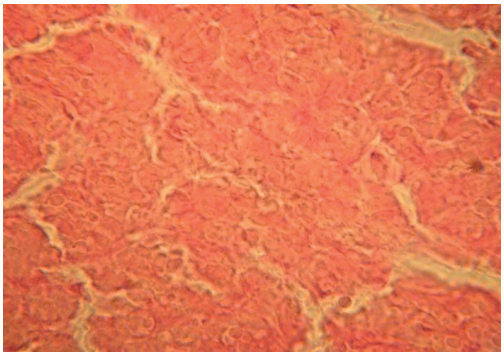

Figure 14. Hypertrophied and vacuolated GTH cells during egg laying, HE, 40x (original photo)

CONCLUSIONS

- From a structural point of view, the adenohypophysis of cyprinids has a glandular structure.
- From a histological point of view, the carp adenohypophysis consists of three distinct lobes: the anterior lobe, the middle lobe and the intermediate lobe, with all the variations of the pituitary gland from one species to another. The technique used highlighted the types of chromophobic cells that do not participate directly in the secretion process and chromophilic cells that participate directly in the hormone secretion process.
- This leads to the important conclusion for the practice of hormone induction in aquaculture, to take pituitary glands from sexually mature females, for pituitary injections, in the season preceding reproduction (December-April). This being the period in which the largest amount of gonadotropic hormones accumulates in the pituitary gland, which it releases in the spring during the maturation period of the oocytes.

ACKNOWLEDGEMENTS

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REFERENCES

Ball, J.N., Baker I. Bridget (1969). The Pituitary Gland: Anatomy and Histophysiology, *Fish Physiology*, 2, 1–10.

Ekici, A., Timur, M. (2013). An anatomical and histochemical examination of the pituitary gland of carp (*Cyprinus carpio*). *Turkish Journal of Veterinary and Animal Sciences*, 37, 399–403.

Evans, D. H. (2003). *The physiology of fishes*, second edition, CRC Press LLC.

Grasse, P. P. (1970). *Traite de Zoologie*, vol. XIII, Paris, F: Masson Publishing House.

Yadav, M. (2008). *Fish Endocrinology*. New Delhi, India: Discovery Publishing House, 296.

Ooi, G.T., Tawadros. N., Escalona, R.M. (2004). Pituitary cell lines and their endocrine applications. *Mol. Cell Endocrinol.*, 228, 1–21.

Shanthanagouda, A.H., Sachin O. Khairnar (2018). Breeding and spawning of fishes: Role of endocrine

gland, *International Journal of Fisheries and Aquatic Studies*, 6(4), 472–478.

Takaski, H. (1982). *Fish Histology*, Stuttgart, GE: Gustav Fischer Verlag. Publishing House.

Zohar, Y., Muñoz-Cueto, J.A., Elizur, A., Kah, O. (2010). Neuroendocrinology of reproduction in teleost fish. *Gen. Comp. Endocrinol.,* 165, 438–455.

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THE EFFECT OF ^{22}Na UPTAKE UPON IONIC COMPARTMENT IN AGING RAT SKELETAL AND CARDIAC MUSCLE

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Abstract

Aim of study is to point out the role of charges distribution at the level of contractile proteins in Contraction and Relaxation state of skeletal and cardiac muscle cells from rats of different ages ,by means of ^{22}Na uptake method in order to bring new arguments in the favor of swelling theory (lateral expansion of filaments network) of muscle contraction in skeletal and cardiac muscle during aging process .Material and method: The study was done on 16 male Wistar rats divided into two groups of 8 rats each: I) 6 months old and II) 37 months old. The animals were anesthetized and then sacrificed and samples from sartorius skeletal muscle and from papillary muscle from left ventricle were excised and placed in glycerol solution in order to remove cell membranes and to expose the contractile apparatus. Our Na22 uptake studies pointed out another structural aspect of functional implication of contractile proteins with modified features from 37 months old skeletal and cardiac muscle and the data concerning the charges distribution at the level of contractile proteins in Contraction and Relaxation which bring new arguments in the favor of swelling theory(lateral expansion of filaments network) of muscle contraction in skeletal and cardiac muscle during aging process.

Key words: *^{22}Na radioisotope, myosin, actin, swelling mechanism, hexagonal lattice, contraction*

INTRODUCTION

Molecular mechanisms of heart muscle contraction have been described by numerous scientists in order to find out drugs able to improve any intermediary phases of the disease affected by the pathological process (Popa et al., 2017).

At each contraction, Ca^{2+} enters in cardiac cells, inducing liberation of a high quantity of Ca^{2+} from the sarcoplasmic reticulum in order to activate contraction and to prevent overloading the myocyte with Ca^{2+}, the quantity of Ca^{2+} which has induced contraction must be reexported in extra cellular space (Rüegg, 1987). Because Ca^{2+} from myocyte cytosol is of micromoles order or even less, while in extra cellular space it is found in millimoles concentrations, its re exporting requires an active transport. There are two systems in charge with this function: an ionic exchanger Na^{+}/Ca^{2+} and a specific ATP-ase; these are working in parallel, but have different kinetics properties, being qualified for full filing different roles (Marin and Goga, 2018a).

The ATP-ase has a great affinity for Ca^{2+}, but a decrease rate of pumping, and can be in such a way considered fine regulator of calcium of cell calcium; it functions in the same manner in all cells where is present (Marin et al., 2017).

The ionic exchanger Na^{+}/Ca^{2+} has a weak affinity for Ca^{2+}, but a great rate of transport, which determine its activation when it is necessary to be ejected high quantity of Ca^{2+}.

There are studies (Curtin et al., 1988; Marin et al., 2018b) which pointed out a dependency of force developed by muscle contraction on intracellular pH - i.e. the muscle force enters into decline with the change into pH - towards acid values. This finding put forward for analysis two possibilities: (1) there are less cycles of cross bridges binding, each producing the same integral force-time, and (2) each cycle produces a less integral force-time.

The second possibility is sustained by the fact that the reduction of pH-ul results in reduction of maximum speed of shortening of muscle fiber, that points out that the kinetics of cycles of binding cross bridges is altered, not only their number.

Almost ¼ from the total consumed ATP- in muscle contraction is used by the calcium pump of the sarcoplasmic reticulum (Curtin and Woledge, 1981).

This ATP-ase activity is diminished by the increased of acidity *in vitro* (MacLennan, 1970), and *in vivo*; it is possible that this effect to be responsible for the obvious slowing down of relaxation produced by the increase in CO_2.

The conclusion of their study was that the mechanical impulse generated by the splitting of a ATP molecule increasing with the value of intracellular pH.

The action potential of heart muscle usually has a duration of more hundreds of milliseconds. In majority of cases the contraction time is approximately equal with duration of action potential. For this reason, is adequate to consider the excedent of action potential as a trigger of contraction, and rapid repolarisation as a shutting down of it. Each contraction is lengthening enough in order to be maximal; in such a way, that the tension corresponds to a titanic contraction in skeletal muscle.

An interesting aspect of cardio myocyte is that the duration of action potential depends on cardiac frequency: as high the frequency is, the duration is less. The duration of potential action is approximately ½ from the interval between two beats.

The action potential of cardiac muscle differs of that of a nerve or of skeletal muscle, in the sense that has a greater duration and a great variability of duration in rapport with frequency.

The rhythm dependency and the exceeding of action potential on external concentration of natrium (Na) demonstrates that the excess of action potential is determined by a great increase in membrane permeability for Na.

But, the nature of permeability modifications which accounts for a much longer depolarization platau phase is not known.

The total resistance of membrane is increases in platau phase, that means that in this phase the permeability to (Na) is increased, and permeability to potassium (K) and/or chloride (Cl) is reduced.

It can be given simple explanation for long duration of action potential and its dependency on rhythm, if we suppose that exists two types of Na conductance.

The first type of conductance responsible for platau phase of action potential is initially high but, is rapidly inactivated during milliseconds after depolarisation and as far as rapid activated after repolarisation.

The second type of conductance responsible for platau phase is in comparison much smaller, but it is slowly inactivated (during seconds) and as slow as activity after repolarisation.

After platau phase of action potential and inactivation of rapid conductance to (Na), the slow inactivated conductance, even reduced of Na persists.

This keep membrane potential towards zero, because conductance of K and Chloride (Cl) have decreased, because it is supposed that these depends on the membrane action potential.

As far as the inactivated conductance of slow Na decreases, the potential decreases slowly, up to a potential at which one or more conductance start to change rapidly in accord with membrane voltage. The (Na) conductance is “stopped”, and the conductance of K and Cl are “triggered on”, in such a way that repolarisation is performing faster and faster. After repolarisation, excitability returns once with activation of fast conductance of (Na), but the action potential will be short because the lent conductance of Na is slowly activated. The platau will appear at a smaller voltage in such a way that the potential at which is produced rapid repolarisation will be reached faster.

Muscle cell is a polyelectrolyte system made up of proteins with many ionizable groups in the same molecule. Basic groups are in charge with positive charges and those acidic with negative charges.

Usually the acidic groups are much more numerous and the net charge at the surface of filaments is negative. The lateral side chains of amino acids from contractile proteins make up a network by weak H bonds with H_20 molecules at the proteins surface leading to an increase in electric load at the level of filaments.

Heterogeneity of protein surface suggests that numerous H bonds fix water at the protein surface some being stronger than others. Such a network of lateral side chains electrically charged exist between 2 myosin tails or

between two polypeptides chains from a single tail.

Concerning the swelling mechanism of muscle contraction the pioneer research of Elliot et al. (1968) has led to the conclusion that the estimation of dimensions of hexagonal lattice of actin and myosin depends on the relation between electrostatic repulsive and attraction forces between the filaments.

Those the last ones seem to be mainly caused by the transverse structural restrictions such as attached cross bridges presented in Rigor, line Z and line M.

An expansion of filament lattice such as is produced at high salt concentrations may be achieved if the repulsive forces are increased or if the forces which determines structural restrictions diminish or due to a combination of those two.

Interactions at the level of contractile proteins are either pure electrostatic, or interactions due to ionic polarizing or they may be caused by the dipoles contribution.

Paper aim was to study the role of charges distribution at the level of contractile proteins in Contraction and relaxation state in contractile apparatus from skeletal and cardiac muscle cells from rats of different ages, using the ^{22}Na uptake method in order to bring new arguments in the favor of swelling theory (lateral expansion of filaments network) of muscle contraction in skeletal and cardiac muscle during aging process

MATERIALS AND METHODS

The study was done on 16 male Wistar rats divided into two groups of 8 rats each: I) 6 months old and II) 37 months old.

The animals were sacrificed and sartorius skeletal muscle and papillary muscle from left ventricle were removed and placed in glycerol solution in order to remove the membranes according to the published method (Revnic et al., 2013).

Glycerinated muscle samples 50-100 mg each were washed in bidistiled water for 15 minutes in 3 successive baths for 5 minutes each. Then the biological samples were placed in test tubes with 1 ml of:

1) Contraction medium with the following composition: KCl 150 mM, CaCl 2.2 mM, $MgCl_2$ 2 mM, TRIS buffer 10 mM, pH 7.2, ATP 2 mM.

2) Relaxation solution with the same composition as contraction solution, excepting CaCl2 which has been replaced with EDTA 4 mM.

3) Rigor solution with the same composition as contraction solution excepting the ATP.

The next step included incubation with ^{22}Na (cat.NENRes. Products, Du Pont) according to Zak et al. (1979) method of skeletal and cardiac rat muscle of different ages.

Characteristics of ^{22}Na radioisotope:

- It is found in aqueous solution of NaCl; the half life time = 2.602 years;
- type of gamma radiation; the energy of radiation 511,1275; specific activity between 100-2000 Ci/gram; the radionuclide power>99%. Concentration approximative 1-10 mCi/mil; it was used the isotope concentration of 0.5 mCi/ml.

This concentration was diluted I: 500 mCi in 5 ml bidistiled water to a concentration of 100 mCI/ml;

Dilution II: 0.1 ml (10 mCi) + 20 ml bidistiled water = 0.5 mCi;

The working dilution was 0.05 mCi/0.1 ml.

The incubation of biological samples was performed for 2 hours at 37°C, then the samples were washed two times for a minute each in the corresponding solutions of Contraction, relaxation and rigor in order to remove ^{22}Na non-specifically absorbed.

Then the tissue fragments were placed in extraction medium (1 ml HCI), following then the recording of radioactivity in incubation and extraction media with a Gamma Counter Beckman, with a multiplier with NaI crystals.

The results of 5 experiments (of 24 samples each, total-120 samples) from which (60 were incubations and 60 extractions) were calculated using the same formula as for Beta isotopes used in our previous studies (Revnic et al., 2018).

E/I x G = cpm/gram tissue, where: E = extraction, I = incubation, G = weight in grams.

RESULTS AND DISCUSSIONS

The distribution of charges at the level of contractile filaments in contraction, relaxation and rigor following the uptake of ^{22}Na (Figure

1) in striated muscle from 6 and 37 months old rats in the presence of ionic strength of 150 mM KCl pointed out an increase uptake of Na22 quantity in young rat skeletal muscle in comparison with 37 months old rat skeletal muscle which is accounted for the presence of high quantity of negative charges at the level of contractile proteins in young rat at the skeletal muscle in comparison with old rat skeletal muscle .

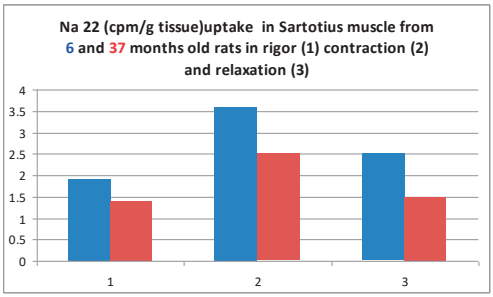

Figure 1. The uptake of ^{22}Na in striated muscle from 6 and 37 months old rats in the presence of ionic strength of 150 mM KCl

Molecular movement of actin on myosin is of Brownian type motion. In Ri the cross bridges are relatively mobile (mobility is strictly Brownian). The water from the surface of proteins is different from that of total water in different ways. Proteins include oxygen which arranges water molecules at the level of protein surface. Chemical change of protons between water and ionisable proteins groups from the around protein molecules have a preferential orientation to the protein surface and a quick movement identified through local water liberated from the lateral side chains.

According to Offer (1984) studies in the presence of high ionic strength there is a higher quantity of water retention because of the binding of Cl ions to myofibril proteins increasing in such a way the negative charges on muscle filaments.

Other studies of Millan et al. (1980) pointed our that the repulsive forces have increased when there is an increase in charge density, taking into account that a certain density of charges there is at the level of untreated muscle. On the other hand, the salt concentration will increase the shielding of these charges according to Leward (1983). It seems that the only way in which the repulsive forces will increase will be is the ray of these charges toward the filament axis will be increased, because the repulsive forces are extremely sensitive to this parameter according to Elliot et al. (1982).

It is not clear at the moment if the increase or not in electrostatic force are partially responsive of the swelling contraction phenomenon (the lateral expansion of hexagonal lattice). The fact that swelling at the high concentrations of salt is highly cooperative suggests that the removal of transverse structure as could play a major role in determination of swelling.

Offer et al. (1983) studies have shown that the removal of cooperative restrictions due to cross bridges of myosin filaments heads it can be produced either by dissociation of myosin heads upon the actin filaments as it is achieved in the presence of pyrophosphate and of increase concentration of Cl ions, or by de polymerization of thick myosin filaments as is produced in the presence of Cl ions in high concentrations.

The effect of salt and pyrophosphate upon the structure of other potential candidates such as Z and M line is not yet known. The increase accumulation of ^{22}Na in contraction at the level of contractile proteins leads to the increase in electric charge on contractile filaments increasing their rigidity. The increase of fixed charges is due to mobilization of cations which are binding at the surface of myosin filaments. The increase in the ionic strength determines the reduction in thickness of double diffusion layer and the repulsive forces appear by redistribution of ions in diffusion layer close to another filament.

In 37 months old rat skeletal muscle it was pointed out a reduction in ^{22}Na uptake in striated muscle in contraction state (Figure 1).

In conditions in which the splitting capacity of ATP is retained in old rat skeletal muscle, the energy liberated by splitting of ATP is used for movement of sub fragment 2 at an angle of 45 degree on actin filament. It is possible that in aging skeletal muscle the presence of myosin with modified features to obstruct the coupling of myosin head at a 45 angle degree with actin and therefore, it is possible not to be achieved a long range electrostatic repulsive force between the filaments.

Aging evolves with a reduction in contraction due to number of fixed charges at the level of contractile proteins, accounted for an increase in superficial charges due to lability of polar groups of contractile proteins with modified features.

Our previous studies pointed out an increase in $C_{14}H_3$-COONa in cardiac muscle of young rats versus old rats.

In Figure 2 there are presented the results of ^{22}Na uptake in young and old heart muscle in contraction, relaxation and rigor.

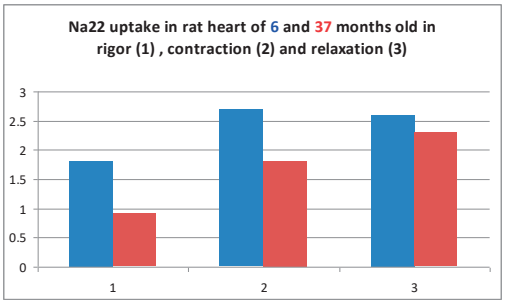

Figure 2. The uptake of ^{22}Na in young and old heart muscle in contraction, relaxation and rigor

In contraction there is an increase uptake of ^{22}Na in young rat heart muscle than in old rat heart muscle. The increase number of negative charges on contractile filaments explain the increase uptake of ^{22}Na in contraction due to excess accumulation of ^{22}Na within the contractile apparatus.

During aging there is a constant reduction of negative charges population in contraction due to the increase degree of dissociable electronegative groups at the level of proteins with modified features accumulated during aging.

Dragomir (1980) studies concerning the measurement of ^{22}Na in frog heart muscle in the presence of Deuterium oxide revealed modifications in ionic behavior of muscle suggesting the intervention of long range action force during contraction state. The water absorbed at the molecular level is decisive for ionic behavior and the functional state of cardiac muscle and this may explain the alterations in ^{22}Na accumulation in the presence of heavy water.

Our results concerning the ionic behavior of contractile apparatus from rat sartorius and papillary ventricular heart muscle in contraction, relaxation and rigor pointed out an increase in ^{22}Na uptake in contraction state in 6 months old rats which is accounted for the presence of an increased quantity of negative charges at the level of contractile filaments.

In 37 months old rat sartorius and papillary ventricle muscle there is a reduction in ^{22}Na uptake in contraction as an expression of changes in negative charges density at the level of contractile filaments implicated in muscle contraction phenomenon, according to the swelling theory.

Contraction and Relaxation in the presence of increased quantity of ^{22}Na are correlated with a reduction in muscle hydration in case of aging muscle, contraction being a function of ions binding to the proteins sites, those sites being important in achieving the hydration state of proteins.

CONCLUSIONS

During aging there is a reduction of negative charges population due to the high degree of dissociable electronegative groups at the level of proteins with modified features accumulated during aging.

Our ^{22}Na uptake studies pointed out another structural aspect of functional implication of contractile proteins with modified features from 37 months old skeletal and cardiac muscle and the data concerning the charges distribution at the level of contractile proteins in Contraction and Relaxation which bring new arguments in the favor of swelling theory(lateral expansion of filaments network) of muscle contraction in skeletal and cardiac muscle during aging process.

REFERENCES

Curtin, N.A., Kometani K., Woledge, R.C. (1988). Effect of intracellular pH on force and heat production in isometric contraction of frog muscles fibres. *Journal of Physiology*, 396, 93-104.

Curtin, N.A., Woledge, R.C. (1981). Effect of muscle length on energy balance in frog skeletal muscle, *Journal of Physiology*, 316, 453-468.

Dragomir, C.T. (1980). Deuterium oxide effects upon three parameters characterizing the activity of glycerol extracted muscle:phosphate release, ATP induced shortening. *Physiol.Chem Physics*, 12, 69-75.

Elliot, G.F., Bartles, E.M. (1982). *In muscle development, molecular and cellular control*. ML Pearson &HF Epstain (eds), Huntington, USA: Cold Spring Harbor Publishing House, 439-482.

Elliot, G.F. (1968). Electrostatic forces in muscle contraction. *Journal Theor.Biol*., 21, 71-76.

Levard, D. (1983). Meat Science. *Proceedings of European Meeting of Meat Research Workers*, Rome, Italy, 9, 315-320.

Marin, I., Tudose, V., Hadar, A., Goga, N., Doncescu, A. (2017). Improved adaptive resolution molecular dynamics simulation. *International Conference on Engineering, Technology and Innovation (ICE/ITMC)*, 173-176.

Marin, I., Goga, N. (2018a). Model checking the properties of an electronic healthcare system to facilitate the detection of preeclampsia through a smart bracelet. *eLearning & Software for Education (ELSE),* 2, 209-216.

Marin, I., Goga, N., Doncescu, A. (2018b). sentiment analysis electronic healthcare system based on heart rate monitoring smart bracelet. *IEEE 11th Conference on Service-Oriented Computing and Applications (SOCA)*, 99-104.

Millan, B., Nickel, B. (1980). Fibrous proteins and ind&medical aspects. DAD Parry&LK Creamer 2, 67-73, London.

Offer, G, Trinick, J (1983). Progress in biochemistry, physiology and structure of meat. *Meat Science*, 8, 245-281.

Offer, G.W. (1984). The nature of repulsive forces between filaments in muscle and meat. *Meat Science*, 10, 155-160.

Popa, A.M., Simion, V., Codreanu, I., Fernoagă, C., Cornilă, M., Codreanu, M. (2017). The prevalence of hematuria in dogs and cats. *AgroLife Scientific Journal*, 6(2), 155-160.

Revnic, C.R., Revnic, F., Voinea, S. (2018). Radioisotope studies of skeletal and cardiac muscle metabolism and ionic fluxes in rats with experimental hyperthyroidism. The impact of Gerovital H3. *Scientific Papers, Animal Science, Series D.*, Vol. LXII.

Revnic, F., Revnic, C.R. (2013). The management of water state in glycerinated rat heart the role of 1H NMR spectroscopy. *Scientific Papers, Animal Science, Series D*, LV, 127-30.

Rüegg, J.C. (1987). Dependence of Cardiac Contractility on Myofibrillar Calcium Sensitivity, *NIPS*, 2, 179-182.

Zak, R., Martin, A. (1979). Assessement of protein turnover by the use of radioisotope tracers. *Physiological Review*, 59, 407-412.

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NMR SPECTROSCOPY AND OPTICAL MICROSCOPY STUDIES OF PYSIOLOGICAL STATES OF RAT SKELETAL MUSCLE OF DIFFERENT AGES

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Abstract

Skeletal muscle is a very good model for fundamental research of aging process due to its postmitotic character. In order to obtain new data concerning muscle contraction at the molecular level, the polar groups of contractile proteins have been investigated by means of ^1H NMR Spectroscopy. Glycerinated Sartorius muscle from 6 and 37 months old rats has been used for proton transverse relaxation time measurements in Ri, Co and Re at different [ATP]. The distribution of negative charges in contraction and relaxation has been measured by exposing glycerinated muscle from 6 to 37 months old rats to different [Mn^{2+}]. Our data have pointed out the existence of two proton relaxation tines: T2s and T21 accounted for two water compartments. The modifications in water state are related with modifications in contractile activity. The elongation odd proton transverse relaxation time is associated with a decrease in the degree of water molecules aggregation. T2s and T2l are correlated with a reduction in muscle hydration, contraction being a function of ions binding to the protein sites. These sites are implicated in determination of protein hydration state.

Key words: *^1H NMR, aging, glycerinated skeletal muscle, contraction, relaxation, rigor.*

INTRODUCTION

Skeletal muscle is a very good model for fundamental research of aging process due to its postmitotic character. It has been supposed that during involution process of muscle tissue, involves proteins and especially those contractile (Allnaqueeb, 1984; Marin et al., 2017).

Literature data (Dragomir, 1987) concerning muscular contraction phenomenon, have pointed out the appearance of long-range repulsive forces within contraction state, which tend to repel the myofilaments one from each other (Elliot, 1982). These forces are converted into active shortening tension through passive intervention of transverse myosin crossbridges with an oblique orientation between myofilaments (Eisenberg, 1980). The repulsive forces that take place during contraction are the consequence of the increase in the electric charge of myofilaments (Offer, 1984). Nuclear magnetic resonance (NMR) spectroscopy is an attractive technique due to its noninvasiveness with an increasing importance in determination of chemical structures, but only nuclei capable of transitioning between energy states, in the presence of an intense and constant magnetic field, could be studied and this includes abundant nuclei such as proton (^1H) and phosphorous (^{31}P), as well as stable isotopes such as deuterium (^2H) and carbon 13 (^{13}C) and for the *in vivo* study of metabolism (Alves, 2012; Marin et al., 2016).

^1H NMIR is a very useful method in biology because we can obtain very important data about mobility of some groups at the level of protein molecules, which provide information about conformation changes which result from the chemical modifications (Dragomir, 1992).

Ischemia induces changes in the distribution and polarization of tissue water and this may influence Nuclear Magnetic Resonance (NMR) relaxation times (Revnic, 2015).

The aim of our study was related with: In order to obtain new data concerning muscle contraction at the molecular level, the polar groups from the contractile proteins have been investigated by means of ^1H NMR Spectroscopy, to test the water state from the close proximity of myofilaments in different experimental conditions in contraction, relaxation from Sartorius muscle of Wistar rat.

1. The investigation of proton transverse relaxation times of water from glycerinated muscle in Ri, Re, Co at different ATP concentrations.
2. The distribution of charges in contraction and relaxation by exposing glycerinated muscle from 6 and 37 months old rats to different [Mn^{+2}], by means of ^{1}H NMR spectroscopy.

MATERIALS AND METHODS

The study has been performed on 12 male Wistar rats divided in 2 groups of 6 rats each: young (6 months) and old (37 months).
The animals from boths groups have been anesthetized and then killed by cervical dislocation and then sample of fresh Sartorius skeletal muscle have been collected on ice bath and processed within one hour for optical measurements of sarcomere length and for (^{1}H) NMR measurements and the other Sartorius muscle samples were collected for glycerination procedure (kept for 3 weeks in glycerol 50% solution in order to remove biological membrane system and to expose the contractile apparatus which is a perfect biological working model for physiological studies of contraction and relaxation, according to the published technique (Revnic, 1992).
After 3 weeks, glycerinated muscle fragments of 2 cm long have been washed for 15 minutes in bidistiled water and then dried on filter paper. The next step was the placement of biological sample in solution at pH 7.2 in order to assure the ionic equilibrium for one hour according to the published method (Revnic, 1991).
Following preincubation, the muscle fragments have been incubated for 10 minutes in Ri, after that have been removed and have been dried on filter paper, and then introduced in special tested for (^{1}H) NMR measurements (Constantinescu, 1989).
The NMR measurements were performed in fresh skeletal muscle collected and kept on ice to prevent biochemical alteration within one hour from the end of experiment and on glycerinated samples after 3 weeks of glycerinating procedure, in contraction state (cross bridges are attached) and relaxing state (cross bridges are detached).
We tested glycerinated skeletal muscle fibrils in contraction and relaxing media, because these two situations imply different molecular surfaces toward the environment.
The measurements were performed with an AREMI Spectrometer, at 25 MHz frequency.
We used a Carr-Purcell-Meiboom-Gill pulse sequence with 32 spin echoes ranging from 8-256 milliseconds after 90 degree pulse.
After applying the natural logarithm to our experimental curves, the result has the aspect of a decay obtained from two exponential individual relaxations.
Therefore, we attributed the two relaxations times obtained from our data to the fractions of bound water that support the motion of the protein chain substrate and the free water in which are dissolved a large number of solutes.
Thus, the T2 values estimated from the relaxation curves obtained by NMR technique were calculated as two values: T2l (long) offering data about the state of free water and T2s (short) giving data about bound water. The values of T2l and T2s were calculated with a computerized program that could fit the relaxation curve with two exponentials.
The compartmentalization is not rigorously defined but, can help us in the interpretation of the damage generated by the attack of free radicals.
Statistical analysis. Data were expressed as means ± SE or as percentages from stabilization values. A one way analysis of variance was performed to test the differences among groups. If, the difference was significant, the groups were compared further. Significance was $p<0.05$.

RESULTS AND DISCUSSIONS

By optical measurements done with ML4 optical microscope on sarcomere lengths in contraction state, a decrease in the active shortening capacity of sarcomeres from 1.48 u in 6 months old rats to 1.69 u in 37 months old rats for 0.5 mM [ATP] has been recorded as we For 1 mM ATP, sarcomere length recorded in young rats was 1.64 u and for 37 months old 1.90 u. Concerning 2 mM ATP concentration, the mean value of sarcomere length was 1.62 u for young muscle and 1.89 for old muscle. The increase in sarcomere length with ageing is significant from statistical point of view for the three ATP concentrations.

There is a decrease in T2s proton transverse relaxation times in young rat versus old rat, as an expression of a decrease with aging in the active shortening capacity of sarcomeres.
Concerning relaxation for all three ATP concentrations, an age dependent reduction of sarcomere length without statistical significance has been recorded.

Table 1. Relationship between sarcomere length in Sartorius muscle of young and old rats in Contraction state and T2s and T2l values at three [ATP]

Speci-fication	Sartorius 6 months			Sartorius 37 months		
	T2s (ms)	T2l (ms)	Sarc. length (u)	T2s (ms)	T2l (ms)	Sarc. length (u)
Co (0.5 mM)	34	230	1.74	47	280	2.01
Co (1 mM)	33	180	1.72	45	270	1.74
Co (2 mM)	30	138	1.70	46	278	1.72

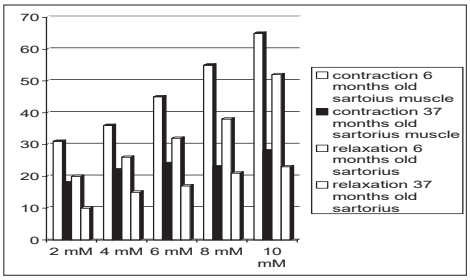

Figure 1. Relationship between sarcomere length in striated muscle of young and old rats in contraction and relaxation state at different ATP

Oxygen radicals may directly alter the proteins, causing cross linking and scission of aromatic amino acids and oxidation of SH groups.
If the 3rtiary and 4 ternary structure of the protein chains is effectively altered, H bonds became unshielded or exposed to the bulk phase water. As a consequence, we anticipate a decrease of T2s because of the increased polar sites directly exposed to the bulk phase water.
T21 is not as sensitive as T2s because only the fractions of random fluctuations that induces spin flip processes contribute to its values.
There is no great discrepancy between T2 values for fresh and glycerinated tissue, with the later about 18% shorter, may be because of the extra cellular fluids present in fresh tissue.
Our data emphasizes that muscle cell water is polarized and the contractile apparatus is responsible for this structure of polarized multi layers because of its proteins, which are partly or completely in the fully extended conformation and their NH and CO groups are free to interact with bulk phase water.
Our data considering T2 shortening in Co state show a possible increase of myofibril Ca^{2+} sensitivity that leads to more cross brides attached in CO groups.
We have calculated T2 by fitting the relaxation curve with two exponentials instead of one, which in our opinion is closer to its shape and to the water distribution in biological systems (free water having a longer T2, bound water a significantly smaller T2).
In such a way, T2s can give more information about the active centers distributed on proteins and responsible for water polarization
We investigated the behavior of contractile apparatus from sartorius muscle from 6 and 37 months old rats in the presence of different [ATP] concentrations.

Table 2. Mean values of sarcomere length (u) in Sartorius muscle from rats of different ages in relaxation state [ATP]

[ATP]	6 months	37 months
	$\bar{X} \pm S_{\bar{x}}$	$\bar{X} \pm S_{\bar{x}}$
0.5 mM	2.38±0.02	2.20±0.01
1 mM	2.40±0.02	2.22±0.0l
2 mM	2.24±0.02	2.24±0.02

There is a reduction in relaxation capacity of sarcomeres in old rats versus young rats for 0.5 mM [ATP] and 1 mM [ATP], but for 2 mM [ATP] there is no difference between the sarcomere length between young and old rat Sartorius muscle.
Another objective of our study was to measure the proton transverse relaxation times within intracellular compartment in the presence of water exchange between intracellular and extracellular compartment doped with paramagnetic ions such as Mn^{2+} obtaining the apparent relaxation time T_{2a}'.
The representation as a function of transverse magnetization M(t) is:

$$M(t) = A^* \exp(-t/T_{2a}') + B^* \exp(-t/T_{2B}) \quad [1]$$

where the slow component of magnetization with the apparently T_{2a}', is significantly separated from the decreasing one, after introducing experimental data in a filtration program with two exponentials.

Table 3. Proton relaxation times T2s in contraction in the presence of Mn^{2+} from Sartorius muscle from 6 and 37 months old rats

[Mn+2]	6 months old rat		37 months old	
	T2s	T2l	T2s	T2l
2 mM	50	100	99	150
4 mM	65	150	-	-

Our studies concerning ionic charges distribution in Contraction and Relaxation using glycerinated skeletal muscle from young and old rats exposed to different concentrations of Mn^{2+} have pointed out an increase in T2s in contraction in old rats.

As it can be seen, the elongation of proton transverse relaxation times is proportional with the concentration of Mn^{2+} which are accommodated supplementary in Contraction at the level of contractile proteins due to their fixation at the level of negative charges on contractile protein filaments.

In ageing muscle there is an elongation of proton transverse relaxation times T2s and T2l both for Contraction state in the presence of an increased quantity of Mn^{2+}. T2s and T21 are correlated with a reduction of muscle hydration in case of old muscle, contraction being a function of ions binding to the protein sites; these sites being important in determination of hydration of proteins.

Dragomir (1980) has studied the level of fixed charges in rabbit muscle, and he concluded that the level of fixed charges increases with the external electrolyte. For example, in the presence of 100 mM KC1 the concentration of fixed charges is approximatively 75 mM for rabbit psoas muscle in Rigor.

^1H NMR studies in presence of Mn^{2+} related with negative charge density in glycerinated sartorius muscle from 6 and 37 months old rats have revealed an elongation of T2s and T2l as a function of Mn^{2+}, this being more reduced in Co than in Re which accounts for supplementary accumulation of Mn^{2+} in Co at the level of contractile proteins negatively charged.

CONCLUSIONS

Our data have pointed out the existence in glycerinated muscle of two proton relaxation times: T2s and T2l accounted for two water compartments. The modifications in water state are related with modifications in contractile activity. The elongation of proton transverse relaxation times is associated with a decrease in the degree of water molecules aggregation.

T2s and T21 are correlated with a reduction in muscular hydration, contraction being a function of ions binding to the protein sites. These sites are implicated in determination of protein hydration.

REFERENCES

Allnaqueeb, M. (1984). Connective tissue changes and physical properties of development and aging skeletal muscle. *J. of Anatomy*, 139, 677-689.

Alves, C.T., Jarak, I., Carialho, A.J. (2012). NMR Methodologies for Studying Mitochondrial Bioenergetics. *Methods in Molecular biology*, 810, 281-309.

Constantinescu, A., Revnic, F., Dragomir, C. T. (1989). ^1H NMR of sartorius and cardiac muscle of Wistar rat of different ages. *Rom. J. Geront. & Ger*., Suppl. 1, 9, 23-28.

Dragomir ,C. T. (1980). Deuterium oxide effects upon three parameters characterizing the activity of glycerol extracted muscle: phosphate release, ATP induced shortening. *Physiol. Chemistry Physics,* 12, 699-756.

Dragomir, C. T. (1987). A new hybrid theory of muscle contraction. *J. Theor. Biol*., 61, 221-227.

Dragomir, C. T. (1992). Mobility of the contractile proteins in glycerol extracted muscle tested by NMR and EPR. *J. of Muscl. Res. & Cell. Motil*., 13 (2), 39-41.

Eisenberg, E. (1980). Crossbridge model of muscle contraction. *Quantitative analysis Biol. J*., 29, 12, 34-38.

Elliot, G. F. (1982). A type of contraction hypothesis applicable to muscle cells. *Nature*, 226, 412-419.

Marin, I., Goga, N., Goga, M. (2016). Benchmarking MD systems simulations on the graphics processing unit and multi-core systems. *IEEE International Symposium on Systems Engineering (ISSE)*, 1-5.

Marin, I., Tudose, V., Hadar, A., Goga, N., Doncescu, A. (2017). Improved adaptive resolution molecular dynamics simulation. *International Conference on Engineering, Technology and Innovation (ICE/ITMC)*, 173-176.

Offer, G. (1984). The nature of the repulsive forces between filaments in muscle and meat. *Meat Science*, 10, 155-160.

Revnic, C.R. (2015). ^1H NMR relaxometric studies of erythrocyte membrane permeability in patients with cardiovascular pathologies with different pathogenic mechanisms. *Proceedings of the 11th International Congress of Coronary Artery Disease Italy*, Florence, 41-44.

Revnic, F. (1991). A study of 1 H NMR studies of sartorius and cardiac muscle from rats treated with Gerovital H3. *Rom. J. Geront. & Ger*., 12, 1-2, 69-73.

Revnic, F. (1992). Sarcomere shortening peculiarities in glycerinated cardiac and skeletal muscle in aging rats. *Muscle res.& Cell Motil*., 13, 2, 219.

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BREEDING AGE OF MARES FROM THE HAFLINGER BREED, DURATION OF GESTATIONAL LENGTH AND THE INFLUENCE OF SOME PARATYPE FACTORS ON IT

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Abstract

A study of the gestational length (GL) in mares from the Haflinger breed and the influence of the age, mating season and sex of the foal on it was conducted. The records used for the study were from the breeding register of the breeding mares (15 mares and 49 pregnancies) from the State Stud farm "Kabiuk" near Shumen, Bulgaria, for a 9 years' period (2010-2019). It is established that the mares from the Haflinger breed are covered for the first time at average age of 3.98±0.78 years, average age at first fertilization - 4.02±0.80 years and average age at first foaling - 5.79±1.43 years. The GL is on an average 329.27±1.90 days. The mating season (P<0.05) and the sex of the foal (P<0.05) are significant sources of variation.

Key words*: covering, fertilization, Haflinger mares, horses, gestational length.*

INTRODUCTION

The gestation length in mares is about 11 months with a fluctuation from 307 to 400 days (Karaivanov and Barzev, 1994). According to the authors, it depends on the breed, but most often it is an individual peculiarity. The effect of breed on gestation length is important as a great variation can be found in the duration of pregnancy among different breeds, as reported by Allen et al. (2002). According to Macpherson and Paccamonti (2011), equine gestation ranges from 320 to 362 days, contradicting a concept that only fetuses with a gestational age of 330 days would be physiologically mature. Taveira et al. (2007) reported that the average period of first gestation in Thoroughbred mares was 337.83±9.47 days, with a fluctuation from 302 to 396 days. Aoki et al. (2013) established that the mean gestation length in heavy draft mares was 334.9±8.3 days, and according to Zuccari et al. (2002) for the Pantaneiro mares that length was 327 days. Popova et al. (2014) examined 37 mares of Purebred Arabian and 53 mares of Shagya-Arabian breeds and established that the average gestation length in purebred Arabian mares is 333.7±1.05 days and in the Shagya-Arabian mares - 333.1±0.83 days.

Studies on the gestation length and the influence of various factors on it were made by other authors (Davies-Morel et al., 2002; Zuccari et al., 2002; Perez et al., 2003; Sevinga et al., 2004; Winter et al., 2007; Cilek, 2009; Meliani, 2011; Satue et al., 2011a; 2011b; Langlois and Blouin, 2012; Walcowicz et al., 2014; Ferreira et al., 2016 etc.).

The efficiency of the horse breeding process is directly related to the reproductive performance of the horses. The reproductive performance of the mares is important for reducing the generation interval and increasing the accuracy of estimation of the breeding value.

In Bulgaria, purposeful studies on reproduction of Haflinger breed were not made. Such research is needed to optimize the use of the breed, which will contribute to the conservation and further development of this valuable genetic resource.

MATERIALS AND METHODS

The study was carried out in the stud farm "Kabiuk", Shumen. Stud book records and breeding registers were analyzed for all of the breeding mares from the Haflinger breed (15 mares with 49 pregnancies) during the period from 2009 to 2019. The gestation length is determined from the date of fertilization to the

date of foaling. The last cover date of the mare is used for date of fertilization. Records of age of first covering were available for 18 mares, for age of first fertilization - 18 mares and age for first foaling - 12 mares.

The multifactorial ANANOVA was used for data processing and establishment of the influence of some factors on the gestation length, the liner model being of the following statistical form:

Yijklm=μ+SZi+SXj+AGk+Bl+eijkl (m),

where: Yijklm - surveillance vector; μ- overall average constant; SZi, SXj and AGk are fixed effects of mating season (i = 3 Spring - March through May; Summer - June through August; winter - December through February); the sex of the foal (j = 2); age group of the mare (κ=7: 1- up to 4 years old; 2 - 5-7; 3 - 8-10.; 4 - 11-13; 5 - 14-16; 6 - 17-19; 7 - 20-23 years, respectively); eijkl - residual variance.

RESULTS AND DISCUSSIONS

The study indicates that the gestation length in Haflinger breed (HB) is 333.7±1.05 days (Table 1).

Table 1. Gestation length in mares of the Haflinger breed, days

Age group (in years)	LS ± SE	Min.	Max.
up to 4	323.72±5.33	312.89	334.55
5-7	332.21±2.24	327.66	336.76
8-10	325.15±3.19	318.67	331.62
11-13	334.15±2.43	329.21	339.10
14-16	328.35±3.41	321.42	335.27
17-19	326.72±5.33	315.89	337.55
20-23	334.61±5.82	322.79	346.43
Average for the breed	329.27±1.90	325.42	333.13

The results obtained are similar to those reported by Matassino (1962) for the same breed - 337.8±13 days.

Other authors reported a longer gestation length in mares from Haflinger breed such as: Bos and Van der Mey (1980) indicate an average duration of 341.3 days, and Heck et al. (2017) reported an average duration of 341.7±7.5 days for 130 mares of the same breed.

Satue. et al. (2011) have observed age differences in the gestation length in Carthusian breed, as the trait in mares between 8-12 years, gestation was 5.3 days shorter (P<0.005) than in mares between 13-17 years.

A significant influence of the age of the mare (Table 2) and a sustainable trend of variation of the gestation length with increasing age in the studied breed was not established (Table 1).

Table 2. Effect of the mating season, sex of the foal and age of the mare on the gestation length, F-criterion and degree of statistical significance

Factor	Df	Gestation Length
Age of the mare	6	1.357
Mating season	2	5.184*
Sex of the foal	1	5.223*

*P<0.05

There is a significant influence on the gestational length in HB (P<0.05) by the mating season and the sex of the foal (Table 2).

The shortest gestation length is in the mares fertilized in the summer (Figure 1) - 321.89±3.24 and the longest gestational is in mares fertilized during the winter - 335.82±3.60 days, as the differences in fertilization in summer are 13.93 days.

The average values of the age at first covering, age at first fertilization and age at first foaling were presented in Table 3.

Table 3. Mean values of the studied traits of mares from Haflinger breed in "Kabiyuk" (LS ± SD)

Traits	N	LS ± SD	Min.	Max.
Age at first covering (in years)	18	3.98±0.78	3.12	5.68
Age at first fertilization (in years)	18	4.02±0.80	3.21	5.77
Age at first foaling (in years)	12	5.79±1.43	4.11	8.34

It was established that the average age at first covering of the mares from the Haflinger breed in our country was 3.98±0.78 years, the average age at first fertilization was 4.02±0.80 years and the average age at first foaling was 5.79±1.43 years.

The age of first covering and its influence on the reproductive performance of the mares and the quality of the offspring has been a subject of a number of studies.

Taveira et al. (2007) reported that in Thoroughbred mares in Brazil, the average age at first covering was 4.93 years, with a standard deviation of 1.45 years, the minimum age being 2.07 years and the maximum one - 11.94. The average age at first parturition was 6.01 years, with a standard deviation of 1.53 years, the minimum age being 3.01 years and the maximum one - 12.9. In the same study, the authors cited slightly lower values for age at first covering for the Indian breeds Marwari and Katiavari - 4.25 years and 4.33 years, respectively. Rastija et al. (2005) reported for mares from the Lipizzaner breed, where the age at first fertilization was 1,157 days and at first foaling - 1,489 days.

Rodrigues et al. (2020) studied breeding and parturition records collected over a period of 35 years in the Alter Real stud of Lusitano horses. The authors reported that the 1027 gestations by 209 mares mated to 60 stallions had a mean GL of 338.1±9.26 days.

Talluri et al. (2016) reported average gestation length for Marwari foals born is 344.1±0.49 days. In the same study the range of gestation lengths was found to be 315-388 days all resulting in viable foals. The length of gestation was not significantly affected by age and parity of the mare. The only factors significantly affecting gestation length were foal gender and month of foaling ($P<0.05$).

Popova (2014) found that the average (LS) age of first covering in purebred mares was 1,584.2±29.5 days, the average age of first fertilization was 1,682.9±24.1 days, and the average age of first drawing - 2,117.7±69.9 days. For the Shagya-Arabian mares, the mean values of these indicators were 1,617.1±18.1, 1,673.9±18.8, 2,202.4±53.9 days, respectively.

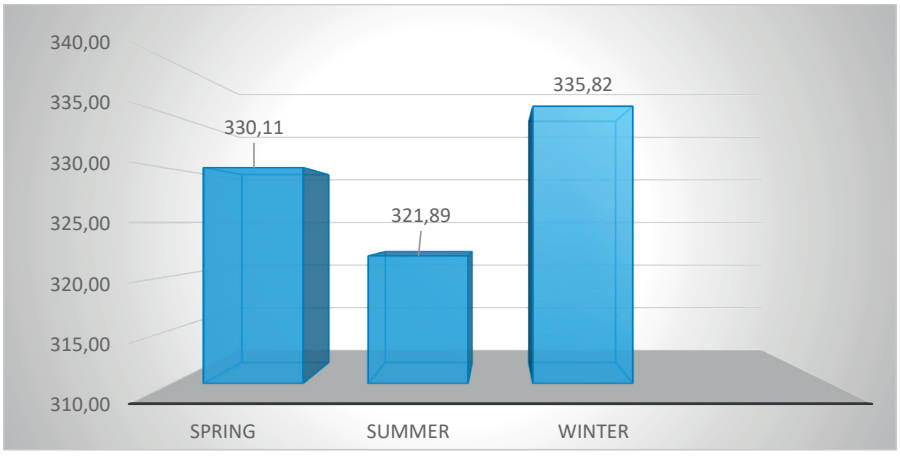

Figure 1. The average gestation length according to the season of mating, days

Data on the influence of mating season on the gestation length varies.

Bene et al. (2014) in a study of seven breeds in Hungary indicated that the mares fertilized in

March and April had the longest gestation length while the mares fertilized in later months of the year - shortest.

In Cartusian breed the gestation length in mares mated between May and July (320.3±9.7 days) was significantly (P<0.001) shorter than those mated between November and January (333.2±13.6 days) and between February and April (335.6±10.0 days) (Satue et al., 2011).

Mares from Friesian breed fertilized during the months of July through September, had 4 days shorter gestation length (329 days) from the ones fertilized earlier in the year (Sevinga et al., 2004).

In Bulgaria, Popova et al (2014) established that mares from the Purebred Arabian and Shagya-Arabian breed had the shortest gestation length when fertilized in the autumn.

Most studies report a longer period of gestation in male fetus: with 1.9 days (P<0.05) in Thoroughbred mares (Taveira et al., 2007); 5.7 days in Cartusian breed (Satue et al., 2011); 4.4 days (P<0.01) in heavy draft mares (Aoki et al., 2013); 0.81 days in Purebred Arabian breed and 1.74 days in Shagya-Arabian breed (Popova et al., 2014) etc.

The mares studied in this research are not an exception (Figure 2), as the difference being 6.89 days can be considered indisputably proven (Table 2).

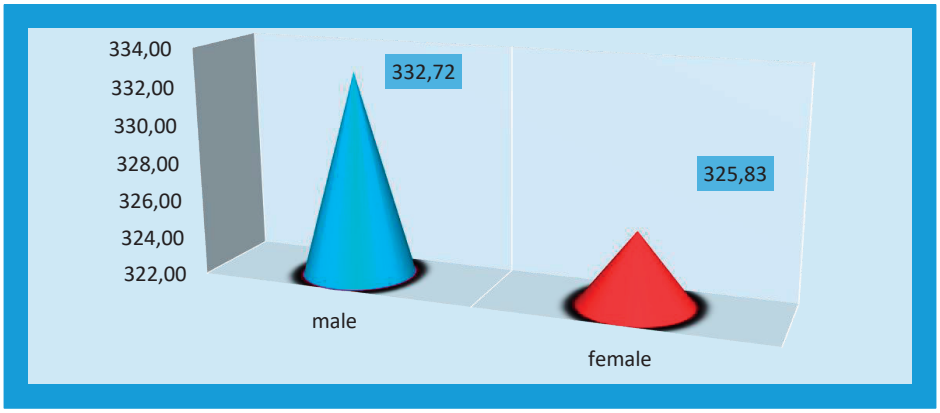

Figure 2. Average gestation length depending on the sex of the foal in mares from Haflinger breed, days

CONCLUSIONS

The average gestation length in the Haflinger breed in Bulgaria was 329.27±1.90 days.

The season of mating has a significant influence on the gestation length and for the Haflinger breed it is shortest in mares fertilized in the summer.

The mares of different ages did not differ significantly by gestation length.

The sex of the foal also has a significant influence on the gestation length, as the male foal has longer GL - 6.89 days.

It was established that the average age (LS) of first covering of the mares from Haflinger breed in our country was 3.98±0.78 years, the average age of first fertilization was 4.02±0.80 years and the average age of first foaling was 5.79±1.43 years.

REFERENCES

Allen, W.R., Wilsher, S., Stewart, F., Stewart, F., Ousey, J., Fowden, A. (2002). The influence of maternal size on placental, fetal and postnatal growth in the horse. II. Endocrinology of pregnancy. *J. Endocrinol*., 172, 237-246.

Aoki, T., Yamakawa, K., Ishii, M. (2013). Factors affecting gestation length in Heavy draft mares. *J. of Equine Veterinary Science*, 33, 437-440.

Bene, S., Benedek, Z., Nagy, S., Szabo, F., Polgar J. P. (2014). Some effects on gestation length of traditional horse breeds in Hungary. *J. of Central European Agriculture*, 2014, 15(1), 1-10.

Bos, H., Van der Mey, G.J.W. (1980). Length of gestation periods of horses and ponies belonging to different breeds. *Livestock Production Science*, 7(2), 181-187.

Cilek, S. (2009). The survey of reproductive success in Arabian horse breeding from 1976-2007 at Anadolu State farm in Turkey. *J. of Animal and Veterinary Advances*, 8(2), 389-396.

Davies-Morel M., Newcombe, J.R., Holland, S.J. (2002). Factors affecting gestation length in the Thoroughbred mare. *Anim. Reprod. Sci*., 74, 175-185

Ferreira, J.R.M., Meirelles, M.G., Guimarães, C.F., Alonso, M.A., Nichi, M., Fernandes C.B. (2016). Factors affecting gestational length in the Mangalarga Paulista breed. *Anim. Reprod., Belo Horizonte*, 13(2), 117-121.

Heck, L., Clauss, Marcus; Sánchez-Villagra, Marcelo R. (2017). Gestation length variation in domesticated horses and its relation to breed and body size diversity. *Mammalian Biology - Zeitschrift für Säugetierkunde*, 84, 44-51.

Karaivanov, R., Barzev, G. (1994). *Horse breeding*. Sofia, BG: Agropres Publishing House.

Langlois, B., Blouin, C. (2012). Genetic parameters for gestation length in French horse breeds. *Livestock Science*, 146, 133-139.

Macpherson, M.L., Paccamonti, D.L. (2011). *Induction of parturition*. In: McKinnon AO, Squires EL, Vaala WE, Varner DD (Ed.). Equine Reproduction. 2nd. Oxford, UK: Wiley-Blackwell Publishing House, pp. 2262-2267.

Matassino, D. (1962). Studio sulle statistiche vitali nelle cavalla di razza Avelignese nel Mezzogiorno: II intervallo interparto e sue frazioni, e loro ripetibilita. *Ann. Fac. Agr. Portici,* 28, 269-285.

Meliani S., Benallou, B., Abdelhadi, Si A., Halbouche, M., Naceri., A. (2011). Environmental factors affecting gestation duration and time of foaling of Pure breed Arabian mares in Algeria. *Asian J. of Animal and Veterinary Advances*, 6(6): 599-608.

Pérez, C.C., Rodríguez, I., Mota, J., Dorado, J., Hidalgo, M., Felipe, M., Sanz, J. (2003). Gestation length in Carthusian Spanishbred mares. *Livest. Prod. Sci*., 82, 181-187.

Popova, M. (2014). Duration of reproductive utilization of mares from Purebred Arabian and Shagya-Arabian breed. *Journal of Central European Agriculture*, 15(2), 129-136.

Popova, M., Nikolov, V., Barzev, G. (2014). Study of the gestation length in mares from the purebred Arabian and Shagya Arabian breeds and the influence of some factors on it. *Journal of Mountain Agriculture on the Balkans*, 17(6), 1346-1358.

Rastija, T., Baban, M., Antunović, Z., Mandić, I., Čači, M. (2005). Pripusna dozrelost mladih plotkinja lipicanske pasmine. *Poljoprivreda (Osijek)*, 11(1), 54-56.

Rodrigues, J.A., Gonçalves, A.R., Antunes, L., Bettencourt, E.V., Gama, L.T. (2020). Genetic and Environmental Factors Influencing Gestation Length in Lusitano Horses. *Journal of Equine Veterinary Science*, 84, 102850.

Sevinga, M., Barkema, H. W., Stryhn, H., Hesselink, J. W. (2004). Retained placenta in Friesian mares: incidence and potential risk factors with special emphasis on gestational length. *Theriogenology*, 61, 851-859.

Satue, K., Felipe, M., Mota, J., Munoz, A. (2011a). Gestational length in Carthusian broodmares: effects of breeding season, foal gender, age of mare, year of parturition, parity and sire. *Polish Journal in Veterinary Sciences*, 14(2), 173-180.

Satué, K., Felipe, M., Mota, J., Muñoz, A. (2011b). Factors influencing gestational length in mares: a review. *Livest Prod Sci*, 136, 287-294.

Talluri, T.R., Arangasamy, A., Singh, J., Ravi, S.K., Pal, Y., Legha, R.A., Alpha Raj, M., Ashish Chopra,.Singh, R.K., Tripathi, B.N. (2016). Factors affecting length of gestation in artificially inseminated Marwari mares of India. *Asian Pacific Journal of Reproduction*, 5(6), 481-489.

Taveira, R. Z., da Mota, M. D. S. (2007). Genetic and quantitative evaluation of breeding traits in thoroughbred mares. REDVET. *Revista electrónica de Veterinaria*, 8(5), 1-11.

Walcowicz, E., Unold, O., Maciejewski, H., Skrobanek P. (2014). The effect of selected factors on the length of gestation in Silesian mares. *Animal Science Papers and Reports*, 32, 55-64.

Winter, G.H.Z, Rubin, M.I.B, De La Corte, F.D., Silva, C.A.M. (2007). Gestational length and first postpartum ovulation of Criollo mares on a stud farm in Southern Brazil. *J. Equine Vet. Sci*., 27, 531-534.

Zuccari, C.E.S.N., Nunes, D.B., Correa Filho, R.A.C. (2002). Reproductive eficiency in Pantaneira mares during breeding season 1995/2000. *Arch. Zootec*., 51, 139-148.

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THE INFLUENCE OF THE MAINTENANCE SYSTEM ON THE PRODUCTIVE PERFORMANCES OF THE LOHMANN BROWN CLASSIC HYBRID HENS

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Abstract

The purpose of the present research was to analyze comparatively the productive performances of the Lohmann Brown Classic hybrid hens, kept in batteries with cages and on the ground, on permanent bedding. During the exploitation period (85 weeks in cages, 93 weeks on the ground), was monitored: intensity of egg laying, the dynamics of the production of eggs, egg weight, feeds consumption and the evolution of the body weight of hens. The hens grown in cages achieved superior productivity performances, in comparison to the ground grown hens: average egg laying intensity 87.83% versus 79.61%; average number of eggs at the age of 16 months 417.79 versus 349.23; the average egg weight 68.45 g versus 64.57 g; average body weight of hens 1947.65 g versus 1932.85 g. The daily consumption of feeds per bird varied between 124-171 g in the case of caged hens and between 130-150 g for the ground grown hens.

Key words*: egg laying intensity, egg production, egg weight, hen body weight, maintenance in batteries with cages and on the ground.*

INTRODUCTION

The productions of the birds are the result of the continuous action and interaction of the biological and environmental factors. The performance of an individual is conditioned by the genotype, environment and by the interaction between genotype and environment (Drăgănescu and Grosu, 2005; Popescu-Micloșanu, 2007). Among the environmental factors, the maintenance system, an integral part of the exploitation technology, plays a decisive role in achieving a performance (for example, egg production), as close to the biological potential, characteristic of each breed and hen hybrid. In the industrial system, egg-laying hybrids can be maintained in wide captivity (on the ground) or in close captivity (in cage batteries), both variants presenting advantages and disadvantages, which affect the productive performance and economic efficiency of the exploitation of hybrids for egg - consumption.

MATERIALS AND METHODS

The research was carried out in an industrial poultry farm in the south of the country, a unit specialized in the production of hen eggs for consumption. The biological material analyzed was represented by the hens belonging to the Lohmann Brown Classic hybrid, one of the most widespread and appreciated egg - laying hybrids in the world.

The purpose of the study was to analyze the impact of the maintenance system on the productive performances achieved by this hybrid, exploited superintensively (in cage batteries) and intensively (on the ground, on permanent bedding). The analyzed population was 22.299 hens operated in "blind" halls, for 85 weeks, in 4-level pyramidal batteries (BP4) and 8350 hens, kept on the ground, on permanent litter, operated for 93 for weeks. At the population of the hall where the chickens were kept in batteries, the density of 4 chickens/cage was ensured (the surface of a cage 2000 cm^2, returning 500 cm^2 cage/bird),

and at the population of the hall of exploitation on the ground, the density was 7 chickens/m^2.
During the exploitation period, the egg-laying intensity, the dynamics of the numerical egg production and the absolute weight of the egg, the dynamics of the feed consumption and the evolution of the body weight of the chickens were monitored.

RESULTS AND DISCUSSIONS

The intensity of egg-laying, a biological factor that influences and conditions the eggs production, expresses the number of eggs produced by a number of birds in a certain period of time. The percentage of egg-laying can be related to the number of chickens fed, to the number of chickens introduced into the hall or to number of females present in the hall. The egg-laying intensity can be calculated daily, weekly or monthly (Popescu-Micloșanu Elena, 2007). For more accurate monitoring of egg production, it is advisable to establish the egg intensity weekly and report it on the number of foddered hens (variant considered in this study).
The intensity of the egg-laying by the hens kept in the cage batteries, was high during the entire exploitation period, reaching the maximum value (the maximum value of the egg-laying) of 91.6% at the age of 27 weeks, then an extended plateau period, followed by another 92% egg-laying peak at the age of 52 weeks and slow production decline, reaching the minimum egg-laying percentage (73.7%) at the age of 104 weeks (Table 1, Figure 1).
In the case of the Lohmann Brown Classic hens operated in intensive system on the ground, the egg-laying intensity showed a continuous upward evolution from the beginning of the egg-laying, up to the age of 27 weeks when the egg-laying peak was reached (94.7%), the value being 3.1% higher compared to the batch maintained in batteries. The egg-laying intensity remained at a high level (90.1%) until the age of 37 weeks, then slowly decreased and reached a minimum value of 71.4%, at the end of the exploitation period (week 112) (Table 2, Figure 1).
During the entire period of operation, the hens exploited in the batteries achieved the average egg-laying intensity of 87.83%, and those kept on the ground on permanent bedding, achieved an average of 79.61%. Both batches showed a good egg-laying intensity, which is within the recommendations mentioned in the "Lohmann Brown Classic Hybrid Growth Guide", and the maximum values corresponding to the egg-laying peak are close to the maximum percentage (94.9%) that this hybrid can reach.
The values obtained in the analyzed groups are close to those specific to Lohmann Brown hybrids, which show a pronounced egg-laying precocity and reach the average egg-laying intensity of 88.5-81.9% at the age of only 24 weeks.
In the industrial system of breeding and exploitation, the birds that achieve early the maximum production of eggs, maintain their productive level for a long period (prolonged plateau phase) and show a slow decline in egg-laying intensity are preferred. Both batches analyzed showed high productive longevity (104 weeks hens maintained in batteries, 112 weeks hens kept on the ground), higher than the average longevity specific to hen egg-laying hybrids (77-80 weeks) (Vacaru-Opriș et al., 2002; Popescu-Micloșanu, 2007; Van et al., 2009; Usturoi, 2008).
In dynamics, the numerical production of eggs is on a certain egg curve, which is characteristic to each breed, line, hybrid and which comprises an ascending period (corresponding to the beginning of the egg-laying and the progressive increase of the percentage of egg-laying), a time when the number of eggs is maximal (the egg-laying peak), one plateau phase (production remains relatively constant) and one descendant period (the number of eggs gradually decreases until the egg-laying ceases) (Usturoi and Pădureanu, 2005; Usturoi, 2008).
In the industrial system, the exploitation of hens for the production of eggs for consumption is no longer profitable when the intensity of the egg-laying falls below the value of 65% (Popescu-Micloșanu, 2007).
The intensity of the egg-laying of the analyzed herd represented graphically in the form of the egg-laying curve is illustrated in Figure 1.

Table 1. The egg-laying intensity of Lohmann Brown Classic hens kept in cage batteries

Age (weeks)	The egg-laying intensity per hen foddered (%)	Age (weeks)	The egg-laying intensity per hen foddered (%)	Age (weeks)	The egg-laying intensity per hen foddered (%)	Age (weeks)	The egg-laying intensity per hen foddered (%)
21	7.2	42	90.2	63	91.2	84	90.3
22	38.1	43	90.1	64	91.0	85	89.9
23	81.9	44	89.9	65	91.2	86	89.8
24	91.2	45	90.1	66	91.2	87	89.5
25	92.8	46	90.4	67	91.3	88	89.3
26	91.4	47	90.3	68	91.3	89	89.2
27	91.6	48	90.6	69	91.7	90	88.7
28	91.3	49	91.1	70	91.5	91	88.7
29	91.1	50	91.0	71	91.7	92	88.5
30	90.8	51	91.8	72	91.6	93	88.4
31	90.6	52	92.0	73	91.9	94	87.8
32	90.6	53	91.9	74	91.4	95	86.8
33	90.8	54	91.9	75	91.0	96	86.3
34	90.5	55	92.0	76	91.2	97	86.1
35	90.0	56	91.9	77	91.1	98	85.2
36	90.3	57	91.8	78	90.7	99	85.4
37	90.3	58	91.5	79	90.3	100	82.7
38	90.6	59	91.7	80	89.9	101	74.4
39	90.9	60	91.5	81	89.5	102	74.6
40	90.5	61	91.4	82	89.9	103	74.7
41	90.4	62	91.2	83	90.1	104	73.7
Average intensity = 87.83							

Table 2. The egg-laying intensity of Lohmann Brown Classic hens kept on the ground

Age (weeks)	The egg-laying intensity per hen foddered (%)	Age (weeks)	The egg-laying intensity per hen foddered (%)	Age (weeks)	The egg-laying intensity per hen foddered (%)	Age (weeks)	The egg-laying intensity per hen foddered (%)
21	7.1	44	84.8	67	84.0	90	72.5
22	25.7	45	85.0	68	84.0	91	71.9
23	64.8	46	84.6	69	83.9	92	71.1
24	88.5	47	83.0	70	84.6	93	70.3
25	92.8	48	83.7	71	85.2	94	70.5
26	94.4	49	82.8	72	83.6	95	70.7
27	94.7	50	82.9	73	83.9	96	70.1
28	94.0	51	82.1	74	84.4	97	70.7
29	93.8	52	83.2	75	83,7	98	70.1
30	94.4	53	83.8	76	83.8	99	70.0
31	94.2	54	82.6	77	82.8	100	70.0
32	93.2	55	79.9	78	82.4	101	70.6
33	92.9	56	80.7	79	80.8	102	70.5
34	92.2	57	80.8	80	79.7	103	70.4
35	91.3	58	80.7	81	79.9	104	71.4
36	90.3	59	80.0	82	78.7	105	71.6
37	90.1	60	80.4	83	78.1	106	71.1
38	88.8	61	81.7	84	76.9	107	71.6
39	88.2	62	83.4	85	76.6	108	71.4
40	87.2	63	84.6	86	75.9	109	71.6
41	86.0	64	87.4	87	74.9	110	71.4
42	85.4	65	87.9	88	73.8	112	71.4
43	85.0	66	87.7	89	73.4	-	-
Average intensity = 79.61							

The upward part of the egg-laying curve, from the beginning of the egg-laying until the highest level of egg-laying intensity increased gradually, extending over a period of approx. 6 weeks for both lots. The plateau of the egg-laying curve, corresponding to maintaining the intensity of the egg-laying at a high level, lasted a long time, especially in the hens maintained in the batteries, and the downward part of the egg curve showed the slow decrease of the egg-laying intensity. The results show that the hens raised in close captivity, achieved a higher egg-laying intensity by 9.98% than the hens exploited on the ground. The birds that benefited from the freedom of movement in the hall, consumed some of the energy and protein of the food for the surplus of movement.

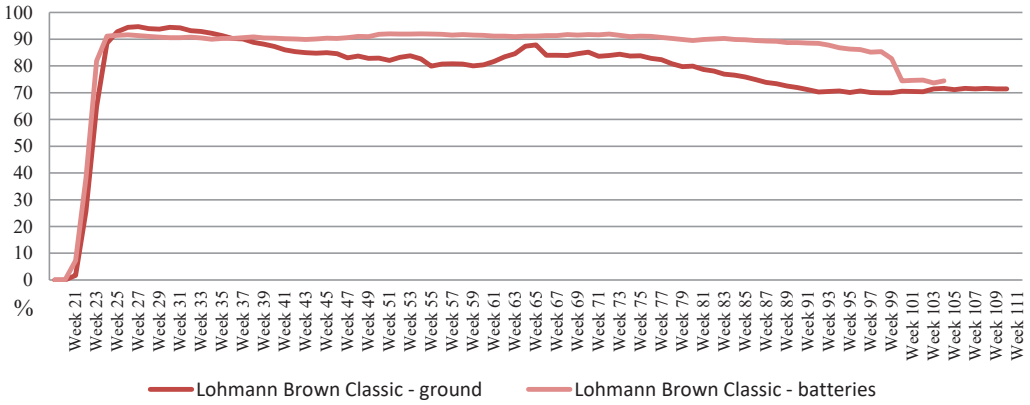

Figure 1. The egg curve of Lohmann Brown Classic hens kept on the ground and in the batteries

During the entire period of exploitation, the total numerical production of eggs made by the Lohmann Brown Classic hens kept in the batteries was 10,712,943 pieces, and the hens intensively grounded produced 3,692,607 pieces (Table 3).

Table 3. Monthly and total egg production of Lohmann Brown Classic hens kept in batteries and on the ground

Month of egg-laying	Number of eggs (pcs.)	
	Hens exploitation in batteries	Hens exploitation on the ground
1	385,551	12,953
2	626,655	210,757
3	600,079	224,611
4	615,468	226,761
5	612,396	209,151
6	589,532	206,391
7	613,430	201,127
8	595,668	190,213
9	611,459	191,521
10	606,392	182,720
11	546,940	200,441
12	604,797	187,243
13	578,902	166,419
14	587,277	180,004
15	565,496	164,432
16	576,224	161,331
17	540,847	147,709
18	439,623	147,435
19	349,157	145,443
20	67,050	140,254
21	-	195,691
TOTAL	*10,712,943*	*3,692,607*

The average weekly production of eggs produced by the hens maintained in the batteries was 126,034.62 pcs., and the hens kept on the ground made 39,705.45 pcs. In both batches analyzed, the exploitation period was longer than that provided in the "Lohmann Brown Classic Hybrid Growth Guide" (up to 90 weeks of age). Therefore, in order to determine the average egg production per hen, the number of eggs obtained in 16 months of eggs was taken into account, in order to make a comparison with the productive potential with which this hybrid is credited. The average egg production per hen exploited in the batteries was 417.79 pcs. and per hen exploited on the ground was 349.23 pcs. The average individual egg production achieved in 16 months of eggs-laying by the Lohmann Brown Classic hens exploited in batteries, is superior by 68.56 pieces to the production obtained from the same hybrid exploited on the ground, on permanent bedding. The average individual production of eggs made by the hens exploited in batteries is higher than the one mentioned in the "Growth Guide" (400-405 eggs in 16 months of laying), and the egg production of the hens exploited on the ground, is below the genetic potential of the hybrid. For the total period of exploitation, the hens kept in the batteries achieved the average individual production of 480.42 eggs in 85 weeks, and the hens kept on the ground produced each 442.23 eggs in 93 weeks.

The weight of the eggs is the main criterion on which the consumption eggs are marketed. Depending on the size of the eggs and how the hens from which the eggs come from were kept, the cost price of the consumption eggs differs. The weight of the eggs is a character with high genetic determinism (h^2 = 0.55 - Usturoi, 2008), and its value increases with the aging of the birds.

The weighing of the eggs produced by the hens of the analyzed batches, showed an upward evolution of the weight of the eggs throughout the period of exploitation of the hens, registering higher values in the batch exploited in batteries (50.95 g/egg at the age of 20 weeks, 73.20 g to 104 weeks) than in the case of the ground exploited lot (50.87 g/egg at 20 weeks, 71.95 g at 112 weeks), even higher than those stipulated in the morpho-productive standard of the Lohmann hybrid Brown Classic (Table 4). During the entire period of exploitation, the average weight of eggs produced by the hens maintained in the batteries was 68.45 g, and that of the eggs produced by the hens maintained on the ground was 64.57 g. Regardless of the maintenance system, most of the eggs they were in category L (large eggs, weighing between 63.0-72.9 g), followed by category M (medium eggs, weighing between 53.0-62.9 g) (Beate and Peitz, 2008).

Table 4. Average weight of eggs produced by Lohmann Brown Classic hens

Age (weeks)	Weight egg of hens kept in batteries (g)	Age (weeks)	Weight egg of hens kept in batteries (g)	Age (weeks)	Weight egg of hens kept on the ground (g)	Age (weeks)	Weight egg of hens kept on the ground (g)
20	50.95	66	70.95	20	50.87	66	65.05
22	52.30	68	71.02	22	50.90	68	65.05
24	56.60	70	71.30	24	55.65	70	65.68
26	62.20	72	71.58	26	56.23	72	65.73
28	63.30	74	71.90	28	57.48	74	66.20
30	63.55	76	72.00	30	58.33	76	66.28
32	64.00	78	72.00	32	59.70	78	66.50
34	64.80	80	72.20	34	60.95	80	66.78
36	65.63	82	72.85	36	61.38	82	67.05
38	65.83	84	72.98	38	61.92	84	67.35
40	65.83	86	73.00	40	62.28	86	67.95
42	65.90	90	72.85	42	62.98	90	67.02
44	66.63	92	73.05	44	62.95	92	67.58
46	67.05	94	73.40	46	63.48	94	67.80
48	67.15	96	73.50	48	63.70	96	68.20
50	67.95	98	73.65	50	63.85	98	68.38
52	68.60	100	73.87	52	63.95	100	68.93
54	68.70	102	73.15	54	64.30	102	70.90
56	69.38	104	73.20	56	64.33	104	71.00
58	69.63	106	-	58	64.58	106	71.15
60	70.02	108	-	60	64.60	108	71.65
62	70.12	110	-	62	64.82	110	71.81
64	70.20	112	-	64	64.95	112	71.95
Average: 68.45				*Average: 64.57*			

The egg-laying hens in the analyzed groups were fed phasically: Phase 1 of egg-laying (approximately weeks 19-45), Phase 2 of egg-laying (approximately weeks 46-65), Phase 3 of egg-laying (after 65 weeks). The food was administered 4 times daily, ensuring the energy and protein level corresponding to each combination feed recipe, depending on the expected egg intensity. The automatic feeding system allowed the rigorous monitoring of food intake, the control of the body weight of the hens being carried out periodically. The amount of feed consumed was permanently monitored and recorded monthly, in both the hens operating facilities. The feed consumption is significantly influenced by the microclimate factors, but also by the quality of the combined feed. The birds in the two analyzed batches achieved the lowest feed consumption in the first feeding stage (age 19-45 weeks), respectively in the first 7 months of exploited, the lowest average consumption of 130 g feed/hen/day was recorded in the 5th month in the hall where the hens were exploited on the ground, at which point, the chickens exploited in the batteries consumed 145 g of feed/hen/day (Table 5). Feed consumption increased gradually from the first month of egg-laying up to the 9th month (171 g/hen/day) for hens exploited in batteries, respectively until 9-10 months (150 g/hen/day) for hens kept on the ground. After reaching the maximum level of consumption in the aforementioned months, the intake gradually regresses until the last month of exploited (124 g/hen/day in the hens operated in batteries, respectively 135 g/hen/day in the hens kept on the ground). For the total period of exploitation, the

consumption of feed is higher in the case of hens exploited in batteries, due to the larger number and the larger body weight (Table 5). The analysis of the dynamics of food consumption shows lower feed consumption in the first 9 months of operating for the chickens belonging to the Lowmann Brown Classic group operated in wide captivity (on the ground), and in the next period of operation (from 10 months to the end of the cycle exploitation), the hens kept on the ground consumed higher amounts of feed than the hens exploited in close captivity (in batteries). In both variants of the maintenance of hens (on the ground and in the batteries), the consumption of feed was higher than mentioned in the "Lohmann Brown Classic Hybrid Growth Guide". Under optimum maintenance conditions, during the production period, Lohmann Brown Classic hens consume 110-120 g combined feed per feed day per bird.

Table 5. Feed consumption of Lohmann Brown Classic hybrid hens kept in ground and in batteries

Exploitation period	Specification	Hens exploited on the ground	Hens exploited in batteries
Month 1 (31/30 days)	Average lot (heads)	8320	22220
	Fodder consumed (kg/lot/period)	31882	89176
	Average consumption (g/head/day)	132	133
Month 2 (31 days)	Average lot (heads)	8260	22120
	Fodder consumed (kg/lot/period)	34598	105513
	Average consumption (g/head/day)	135	140
Month 3 (30 days)	Average lot (heads)	8200	22010
	Fodder consumed (kg/lot/period)	35725	96030
	Average consumption (g/head/day)	140	145
Month 4 (31 days)	Average lot (heads)	8130	21910
	Fodder consumed (kg/lot/period)	27607	101102
	Average consumption (g/head/day)	134	148
Month 5 (31 days)	Average lot (heads)	8060	21820
	Fodder consumed (kg/lot/period)	29745	87820
	Average consumption (g/head/day)	130	145
Month 6 (31 days)	Average lot (heads)	8000	21730
	Fodder consumed (kg/lot/period)	35034	95763
	Average consumption (g/head/day)	141	146
Month 7 (31 days)	Average lot (heads)	7950	21630
	Fodder consumed (kg/lot/period)	32357	99680
	Average consumption (g/head/day)	145	148
Month 8 (31/30 days)	Average lot (heads)	7890	21560
	Fodder consumed (kg/lot/period)	32041	116703
	Average consumption (g/head/day)	149	160
Month 9 (30/31 days)	Average lot (heads)	7830	21490
	Fodder consumed (kg/lot/period)	40008	114480
	Average consumption (g/head/day)	150	171
Month 10 (31 days)	Average lot (heads)	7760	21420
	Fodder consumed (kg/lot/period)	32736	94553
	Average consumption (g/head/day)	150	142
Month 11 (30 days)	Average lot (heads)	7730	21340
	Fodder consumed (kg/lot/period)	33230	81778
	Average consumption (g/head/day)	138	136
Month 12 (31 days)	Average lot (heads)	7370	21230
	Fodder consumed (kg/lot/period)	35822	88873
	Average consumption (g/head/day)	146	135
Month 13 (31/30 days)	Average lot (heads)	7300	21120
	Fodder consumed (kg/lot/period)	31149	91867
	Average consumption (g/head/day)	146	144
Month 14 (28 days)	Average lot (heads)	7230	21000
	Fodder consumed (kg/lot/period)	31385	71204
	Average consumption (g/head/day)	140	139
Month 15 (31/30 days)	Average lot (heads)	7160	20900
	Fodder consumed (kg/lot/period)	33722	76990
	Average consumption (g/head/day)	148	132
Month 16 (30/31 days)	Average lot (heads)	7080	20810
	Fodder consumed (kg/lot/period)	33893	90991
	Average consumption (g/head/day)	144	141
Month 17 (31 days)	Average lot (heads)	7005	17180
	Fodder consumed (kg/lot/period)	30643	80555
	Average consumption (g/head/day)	145	151

Continue table 5

Exploitation period	Specification	Hens exploited on the ground	Hens exploited in batteries
Month 18 (30 days)	Average lot (heads)	6930	17080
	Fodder consumed (kg/lot/period)	32007	69270
	Average consumption (g/head/day)	149	145
Month 19 (31 days)	Average lot (heads)	6860	17015
	Fodder consumed (kg/lot/period)	27690	65810
	Average consumption (g/head/day)	130	124
Month 20 (31 days)	Average lot (heads)	6790	16990
	Fodder consumed (kg/lot/period)	30006	65500
	Average consumption (g/head/day)	138	124
Month 21 (31 days)	Average lot (heads)	6720	-
	Fodder consumed (kg/lot/period)	28900	-
	Average consumption (g/head/day)	135	-

Body weight is one of the biological factors that significantly influences the number of eggs of farm birds. The largest egg productions are obtained from birds whose body weight is close to the average of the breed or hybrid to which they belong. Overweight birds produce smaller eggs, even than those whose body weight is below the average of their population (Usturoi, 2008; Vacaru-Opriş et al., 2000). The body weight also determines the specific consumption of feed, an important indicator of the economic efficiency of the process of exploitation of birds for egg production. The hens from the analyzed groups were monitored regarding the evolution of the body weight and its uniformity from the population of the halls (age 19 weeks), for a period of 85/93 weeks, as long as the operating process lasted, depending on the maintenance system. The determination of the body weight of the hens was achieved by the individual weighing about 10% of the herd, every two weeks. Each week during the exploitation period (with minor exceptions), the hens exploited in the batteries showed body weights slightly higher than those specific to the hens kept on the ground, due to the hight captivity (movement limitation) (Table 6).

Table 6. Body weight of Lohmann Brown Classic hens during the period of exploitation

Age (weeks)	Body weight hens maintained in batteries (g)	Age (weeks)	Body weight hens maintained in batteries (g)	Age (weeks)	Body weight hens maintained on the ground (g)	Age (weeks)	Body weight hens maintained on the ground (g)
20	1610	68	2010	20	1532	68	2010
22	1640	70	2010	22	1630	70	2012
24	1720	72	2010	24	1660	72	2015
26	1770	74	2015	26	1692	74	2016
28	1760	76	2020	28	1719	76	2021
30	1800	78	2024	30	1720	78	2022
32	1840	80	2026	32	1728	80	2024
34	1860	82	2028	34	1717	82	2026
36	1870	84	2032	36	1780	84	2029
38	1880	86	2035	38	1820	86	2031
40	1880	88	2038	40	1822	88	2036
42	1902	90	2041	42	1860	90	2035
44	1920	92	2045	44	1888	92	2037
46	1922	94	2050	46	1902	94	2042
48	1930	96	2050	48	1915	96	2043
50	1950	98	2055	50	1925	98	2044
52	1955	100	2058	52	1932	100	2043
54	1960	102	2060	54	1934	102	2045
56	1970	104	2070	56	1941	104	2048
58	1978	106	-	58	1950	106	2050
60	1980	108	-	60	1992	108	2050
62	1980	110	-	62	1998	110	2052
64	1995	112	-	64	2001	112	2055
66	2000			66	2000	-	-
Average: 1947.65				*Average: 1932.85*			

At the beginning of the operation (week 20), the body weight of the hens raised in the batteries was slightly higher (1610 g), than the body weight of the hens raised in the ground (1532 g) (Table 6). During the period when the maximum egg-laying intensity was reached

(week 27), the average body weight of the hens kept in the batteries reached 1760 g, of the hens raised on the ground was 1719 g, and in the last week of operation, the body weight of the hens kept in the batteries recorded the average value of 2070 g (week 104) *versus* 2055 g (week 112) in the case of hens exploited on the ground (Table 6). For the total production period, the average body weight of hens exploited in batteries was about 14.8 g higher than that of hens kept on the ground (1947.65 g *versus* 1932.85 g). The body weight of the hens from the analyzed groups was less than the standard body weight specific to the Lohmann Brown Classic hybrid, with small differences between the groups.

CONCLUSIONS

Both batches showed a good percentage of eggs, the average intensity of the egg-laying achieved during the entire period of operation of the hens maintained in cage batteries, being higher than that obtained by the hens kept on the ground (87.83% *versus* 79.61%). Both groups reached the egg-laying peak (91.6% and 94.7%, respectively) early, at the age of 27 weeks, the plateau of the egg-laying curve was long and the descending phase was slow (over 71% egg intensity at the end of the operation).

The total numerical production of eggs made during the entire period of exploitation of the hens maintained in the batteries, was higher than that obtained from the hens kept on the ground (10,712,943 pieces, compared to 3,692,607 pieces). And the average number of eggs per hen kept in batteries was higher than per hen exploited on the ground (417.79 pieces versus 349.23 pieces at the age of 16 months).

The weight of the eggs produced by the hens of both groups, showed an upward dynamics from the beginning of the egg-laying (50.95 g/egg hens exploited in cages, 50.87 g/egg hens exploited on the ground), until the birds reformed (73.20 g/egg *versus* 71.95 g/egg). In the dynamics of the exploitation, the hens kept in the batteries produced larger eggs: for the total period of exploitation, the average weight of the eggs produced by the hens maintained in the batteries was 68.45 g, and that of the eggs produced by the hens kept on the ground was of 64.57 g. Regardless of the maintenance system, most of the eggs belonged to category L (large eggs). The feed consumption increased from the first egg-laying month to the 9th month for the hens exploited in captivity, respectively until the 9th to 10th months for the hens exploited on the ground, and then the feed intake progressively regresses until the last month of exploitation. The average daily feed intake per bird varied between 124-171 g in hens kept in batteries and between 130-150 g in hens kept on the ground.

The body weight registered an upward dynamic from the population of the halls (1610 g/hen exploited in batteries, 1532 g/hen maintained on the ground), until depopulation (2070 g/hen maintained in the batteries, respectively 2055 g/hen maintained on the ground). At all stages of exploitation (with minor exceptions) and for the entire period of operation, the hens maintained in the batteries showed a higher body development than those maintained on the ground (during the entire period of operation, the average body weight of the hens kept in the batteries was higher by approx. 14.8 g to that of hens kept on the ground -1947.65 g *versus* 1932.85 g).

REFERENCES

Beate, T., Peitz, L. (2008). *Raising hens*. Bucharest, RO: M.A.S.T. Publishing House.

Drăgănescu, C., Grosu, H. (2005). *Animal breeding*. Bucharest, RO: Agrotehnică Publishing House.

Popescu-Micloșanu, E. (2007). *Raising birds for egg production*. Bucharest, RO: Printech Publishing House.

Usturoi, M.G., Păduraru, G. (2005). *Poultry breeding technologies*. Iași, RO: Alfa Publishing House.

Usturoi, M.G. (2008). *Raising birds*. Iași, RO: Ion Ionescu de la Brad Publishing House.

Van, I. (coordinator) (2009). *Breeding and exploitation of laying hens for eggs.* Bucharest, RO: Total Publishing Publishing House.

Vacaru-Opriş, I. (coordinator) (2000). *Poultry treaty (vol. I)*. Bucharest, RO: Ceres Publishing House.

Vacaru-Opriş, I. (coordinator) (2002). *Poultry treaty (vol. II)*, Bucharest, RO: Ceres Publishing House.

* Lohmann Brown Classic hybrid growth guide.

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A STUDY OF THE FARM FACTORS IN BUILDINGS USED FOR FARMING DAIRY COWS

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Abstract

A study of the farm factors was conducted in three buildings used for farming dairy cows. They were kept free in individual boxes or as a group living on deep litter bedding. Two of them were open and the third was closed structure. The average monthly and seasonal values of temperature, humidity and air movement were calculated. It was found that temperature, humidity and air movement in the buildings depend on the season, type of building and environmental factors. The daily thermo-hygrograms made during typical summer and winter days show the actual state of the farm factors in the buildings. The topographical distribution of floor temperatures in open buildings, compared to closed ones, confirmed their higher degree of dependence on ambient temperatures. The results were statistically processed using IBM, SPSS-21.

Key words*: farm factors, floor temperature, thermo graphic profile.*

INTRODUCTION

The formation of optimal microclimate in the building used for breeding dairy cows is of essential importance for the maintenance of sustainable health status and realisation of maximum productivity (Gauly et al., 2013). The diversity of buildings with different technical and technological solutions used in the cattle breeding is often a hindrance for the establishment and keeping up of an optimal temperature-humidity and light regime in them. The microclimate which is specific for each production building not only influences the food assimilation (Gaughan et al., 2002; Mader and Davis, 2004), the health and productivity of the cows (Solan and Jozwik, 2009; Darwin, 2001), their comfort (Heidenreich et al., 2004; Broucek et al., 2009; Velecka et al., 2014; Trofimov et al., 2016), but also their reproduction (Ravagnolo and Mistzfal, 2002). The temperature, humidity and air currents are leading in this respect (Gregoriadesova and Dolezal, 2000). In their publication, Ventura et al. (2015) examine the "cows comfort" as a combination of the environmental factors, design and construction characteristics of the building and their technological equipment which all lead to a certain behavioural reactions of the animals. When it comes to the influence of the light on the metabolism, reproduction, health and milk productivity of the cows, Trofimov et al. (2016) believe that the studies are still insufficient. A regular control and assessment of the barn environment will provide the opportunity for timely correction and forecasting of the health and productivity of the cows in the long run.

The maintenance of the microclimate in the being now constructed semi-open buildings without longitudinal side walls is regulated by curtain walls which open and close depending on the air flow. Using the behavioural indicators, Dinev (2007) ascertains that these building can ensure suitable temperature, humidity and air flow speed in compliance with the physiological needs of the cows.

The task we set to ourselves was to examine the variable factors of the production environment in three buildings used for breeding dairy cows during transitional, summer and winter periods.

MATERIALS AND METHODS

The studies were carried out in the course of one year in three cattle breeding farms with different capacity in the region of Plovdiv. The breeding technology in two of them is free in individual boxes, and as a group living on deep litter bedding in the third one. We nominated

the farms provisionally with numbers (№ 1, 2 and 3) in view of keeping their confidentiality.

Farm № 1 has a capacity of 500 lactating cows kept free in separate boxes. 200 lactating cows divided into 4 groups are bred in the controlled building. Its total area is 2310 m², and the individual space per animal 11.5 m². The building is a concrete construction with concrete walls and roof panels.

The individual boxes are set on both sides of the longitudinal walls and have dimensions of 1.10/2.10 m. between them and the feeding zones are the manure alleys. The floor of the building and the individual boxes is made of cement, and that in the boxes is covered with a soft rubber bedding. There are no partition systems or chest restrain belts in the front. The feeding is via cattle feed mechanical mixer in the morning and in the evening.

The natural light in the barn is ensured by a total of 30 windows with an area of 220.5 m² and 12 ridge vents with an area of 62.5 m². The artificial lighting is fluorescent and is performed via 97 double-tube fluorescent lamps 40 W each. The side windows and the ridge vents are covered with a polyethylene sails during the winter. The mechanical ventilation is performed via 10 ventilators - 5 on both sides of the feeding lanes, above the movement and feeding zone; each ventilator has a power of 0.55 kW and productivity of 60,000 l/h.

The manure is cleaned with a delta scraper every 3 hours. The watering is performed with 16 nipple drinking troughs divided into 4 per each group of 50 animals. The milking is twice a day in a cow milking parlours type «Fish-bone» 2 x 8.

Farm № 2 has a capacity of 250 dairy cows bred freely in separate boxes. The controlled building accommodates 130 dairy cows divided into two groups of 65 cows each. It is an open metal construction with a thermopanel roof. The side walls are made of concrete with width of 0.25 m and height of 1.5 m. The end walls are also made of concrete and are 3.0 m high. The feeding lane zone has no doors and is entirely open. The total area of the building is 1248 m² and each animal has 9.4 m² individual area ensured.

The feeding lane is centrally positioned. On both of its sides are mounted rows of two-sided individual boxes (1.25/2.20) which are separated by manure lanes at the side walls and the feeding lanes. The floor is made of cement and that in the boxes is covered with a rubber bedding.

The natural light is ensured by the open spaces with a total area of 170 m². The artificial light is provided by 14 three-tube fluorescent lamps 40 W each. The mechanical ventilation is performed by 8 ventilators placed under angle of 45º (4 on both sides of the feeding lane), above the feeding zones. Each of the ventilators has a wattage of 0.55 kW and productivity of 60,000 l/h. When the temperature is up to 18ºC only half of them work, and when it is above 25ºC- all do.

The cleaning of manure is made with a delta scraper every 6 hours. The feeding is unlimited with a total mixed ration with a permanent access to water. Milking is performed twice a day in milking parlours 2 x 12 type «Fish-bone» equipped with a herd management software product.

Farm № 3 has a capacity of 110 cows bred free in a group living on deep litter bedding. The building controlled is for 67 dairy cows and has a total area of 598.5 m². The movement and rest area are 540 m². Each cow is ensured 8.06 m². The building is semi-opened, the roofs are made of double bricks without inner or outside coating. The feeding lane is situated in the east side of the rest and movement area. The open parts of the building provide natural ventilation close to the tunnel type. In addition, 8 ventilators (DeLaval) are mounted under 45º above the rest and movement area, each with a wattage of 0.55 kW and productivity of 60,000 l/h. The same are turned on in stages in temperatures above 18 and 25ºC.

The feeding is unlimited with a total mixed ration and a permanent access to water.

The cleaning of manure is made twice a year with periodic addition of hay. The milking is performed twice a day in a milking parlours «DeLaval» 2 x 5. Apart from the natural lighting there are also 5100 W lamps fixed above the feeding lane and 3200 W lamps - above the rest and movement area.

All microclimatic factors both outside and inside the buildings were measured at 10, 12, 14, 16 and 18 h in the course of three days every month. The temperature of the floor and

the air outside and inside the premises was measured with a multifunction thermometer. Compact infrared thermometer 105,518 with a scope from -50 to +550ºC and resolution of 0.1ºC, we measured the relative humidity (%) through an aspiration psychrometer by Assmann, the air flow speed (m/s) with a catathermometer, the atmospheric pressure (hPa) with an aneroid barometer type 103, Germany, and the illumination - with a lux meter PU 150 PRAHA. The ammonia concentration was ascertained with detector tubes manufactured by the company "Hygitest" Bulgaria. In the course of 3 to 5 days during each of the controlled months, we registered the daily fluctuations in the air temperature and the relative humidity with the help of weekly thermo hygrographs. The results were statistically processed via IBM, SPSS-21.

RESULTS AND DISCUSSIONS

To a large extent, the optimal microclimate or its maintenance within tolerable norms depends on whether the requirements for: stocking density, the volume available per animal, feeding and watering front are met (Dimova et al., 2012; Dinev, 2007). Our results indicated that each dairy cow is ensured 11.5 m², 9.4 m², 8.06 m² instead of the 6 m² required under Ordinance 44 (2006) and the Technological requirements for building of livestock and poultry farms and complexes (1982).

The farms subject to our studies are situated in the Upper Thracian Plain which is characterized by a transcontinental climate. Table № 1 clearly shows that the registered average daily temperatures, humidity and air flow in the region of each of the farms are approximately the same.

With minor exceptions, the region of farm № 3 is a bit hotter during the summer and cooler during the winter. The differences, however, are statistically unsubstantiated.

Table 2 features the average values of the examined parameters with no view of the farm and the season. It can be seen that the outdoor temperatures vary between 3 and 26.8ºC, and the inside ones - between 5.2 and 28.8ºC. The relative humidity values in the premises are mainly within the hygiene norms.

Table 1. Average temperature-humidity regime values in the region of the examined farms

Parameters	Transitional period			Summer			Winter		
	Building 1	Building 2	Building 3	Building 1	Building 2	Building 3	Building 1	Building 2	Building 3
Temperature, ºC	19.3	19.6	20	28.8	27.5	28.5	3	3.2	3.6
Relative humidity, %	70.6	69.1	68.5	58.2	55.4	59.2	55.2	67.4	68.5
Air flow speed, m/s	0.28	0.26	0.25	0.21	0.25	0.24	0.61	0.72	0.68

Table 2. Average values of the examined parameters with reference to the three seasons

Parameters	№	LSM	±SE	SD	Minimum	Maximum
T 1, ºC	54	16.29	1.25	9.21	3.0	26.8
T 2, ºC	54	18.94	1.22	8.98	5.2	28.8
T 3, ºC	54	15.10	1.16	8.50	2.2	25.9
H 1, %	54	68.31	0.73	5.33	58.2	75.2
H 2, %	54	74.44	0.90	6.61	65	88
AM, m/s	54	0.35	0.025	0.18	0.15	0.66

Note: T 1 - outside temperature; T 2 - inside temperature; T 3 - floor temperature; H 1 - relative humidity of the outside air; H 2 - relative humidity of the inside air; AM - movement of air in the buildings.

According to Petkov and Baikov (1976), natural factors such as temperature, humidity, air flow, which characterize the atmosphere and topography total circulation, are also major components acting upon formation of the microclimatic parameters of the closed (№

1) and the two open (№ 2 and 3) dairy cows production buildings (Table 2) studied by us.

The suitable temperature and relative humidity in the production premises guarantee the cosiness and the comfort of the animals bred there. They are also a prerequisite for good health condition and maximum productivity of the animals (Gaughan et al., 2000; Miteva, 2012; Hansen, 2007). According to Regulation 44, the temperature comfort zone of dairy cows is between 10 and 15ºC, at a minimum of 5ºC and a maximum 28ºC. Hanus et al. (2008) state that the thermal neutral zone of the dairy cows is between 3 and 13ºC because phylogenetically, cows fall in the group of the arctic animals.

As presented in Table 3, the average values of the examined hygiene parameters in the controlled buildings demonstrate their own dynamics with reference to each building but at the same time some dependence on the factors of the outside environment. When a comparison is made with the values recommended in Regulation № 44 it can be seen that in the winter, the cows from these farms live in an environment with temperatures below the lower limit (5ºC) and during the summer - in close or exceeding the upper limit (28ºC).

Table 3. Average values of the microclimate factors in the controlled buildings

	Transitional period			Summer			Winter		
Parameters	Building 1	Building 2	Building 3	Building 1	Building 2	Building 3	Building 1	Building 2	Building 3
Temperature, ºC	22	21.8	22.5	28.2	27.8	27.6	7.1	5.8	6.9
Relative humidity, %	73	68	70	79.0	64.8	75	85	73	76
Air flow speed, m/s	0.28	0.22	0.36	0.56	0.49	0.55	1.2	1.5	0.9
Cooling variable, mJ/cm²/s	9.5	8.8	10.2	4.5	3.1	4.8	9.5	13.8	7.8
Illumination, Lx:	400-600	350-750	200-450	400-1200	400-700	250-700	250-550	220-700	180-450
Ammonia content, mg/l	14.4	8	15.2	0.25	0,22	0.28	0.21	0.18	0.24
Bedding temperature, ºC	18.5	16.2	12.5	25.9	25,7	22.6	9.8	6.3	2.5

The data in Table 4 show the high correlation dependency of the air and floor temperature, and the air flow on the architecture-constructional and technological solutions in the production buildings. The relative humidity has a negative correlation not only with the type of building but also with the temperature maintained in it. The season of examination also highly affects the temperature of the air and the floor, and the air flow in the production buildings but not the humidity inside them.

The use of the average temperature and humidity values which are usually measured during the daytime (7, 14 and 21 h) does not provide precise but general idea about the temperature-humidity regime in the buildings. The amplitudes and their duration usually are not revealed. Namely these features are of extreme importance upon unlocking of a certain stress reaction.

Table 4. Correlation between the examined parameters and reliability degree

	T 2, ºC	T 3, ºC	H 2, %	AM, m/s
Season	0.97***	0.91***	-0.1	0.90***
Farm	0.99***	0.93***	-0.47***	0.8***

Note: ***P<0.001; **P<0.01; *P<0.05

T 2 - indoor temperature; T 3 - floor temperature; H 2 - indoor air relative; AM - air flow in the buildings

The thermo-hygrograph diagrams (Figures 1, 2, 3, and 4) made by us provide a more precise idea about the real condition of these ecological factors during typically hot and typically cold days of the year.

The thermo-hygrograph diagrams show that in farm № 1, the temperatures during the summer vary from 20 to 40ºC, and the amplitudes sometimes exceed 20ºC. The relative humidity at the same time varies between 30 and 90%. In the winter, the amplitudes both of the temperature and the relative humidity are

almost two times lower. The registered winter temperatures in the premises of farm № 1 are always above 0ºC.

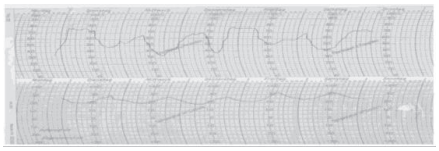

Figure 1. Thermo-hygrograph diagram from farm № 1 taken in the summer

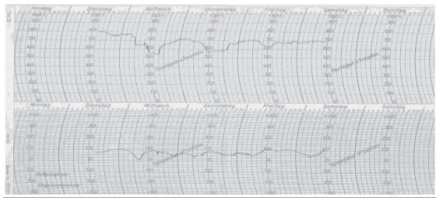

Figure 2. Thermo-hygrograph diagram from farm № 1 taken in the winter

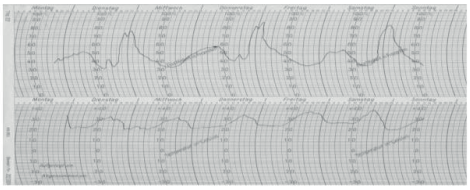

Figure 3. Thermo-hygrograph diagram from farm № 2 taken in the summer

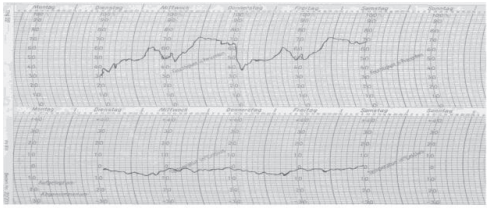

Figure 4. Thermo-hygrograph diagram from farm № 2 taken in the winter

It can be observed that during the summer the temperature and relative humidity fluctuations in the premises of the three farms are within close values. The thermo-hygrograph diagrams taken in the open buildings during the winter indicate fewer variations or entirely overlap with those of the outside temperature. This comes to prove that to a large extent, the temperature- humidity regime in them is more dependent on the atmospheric factors than the closed building is.

It is well known that the dairy cows are more tolerant to low than high temperatures. However, the continuous effect of the low temperatures may prove to be stressful. Nardone et al. (2006) observe reduction in the milk productivity at temperatures of minus 4ºC. They point the temperature of minus 23ºC as a critical lower level. Bianka (cited by Petkov and Baikov, 1976) considers that the dairy cows can also be bred in conditions close to 0ºC. It is also added that provided the feeding is good, the low temperatures increase the resilience of the animals, while the high temperatures have a negative effect on them.

To what extent the temperature and humidity in the buildings may be considered corresponding to the physiological needs of the animals also depends on the airflow and its cooling ability. The results of our studies indicate that the air flow and the cooling variable during the summer are quite low in all three farms - from 0.49 to 0.56 m/s and from 3.1 to 4.8 mcal/cm²/s. In the winter the air flow speed in two of the farms exceed the accepted norm 4 times. When this speed is maintained for a longer period of time, especially in temperatures under 0ºC, we can say that a cold stress prerequisite arises. Under the norms, acquired in our country, the air flow speed during the winter must not exceed 0.3 m/s, and the cooling variable- 5-8 mcal/cm²/s. Even at temperatures under 10ºC, no flows should be allowed. In their overview material, Petkov and Baikov (1976) cite authors according to whom the air flow must not exceed 0.6 m/s, and that it could exceed even 4 m/s according to others. Therefore, we reasonably support the conclusion of Gregoriadesova and Dolezal (2000) stating that the cows' welfare, their health and productivity during the summer are mainly affected by the temperature and humidity of the air, and during the winter - by its speed.

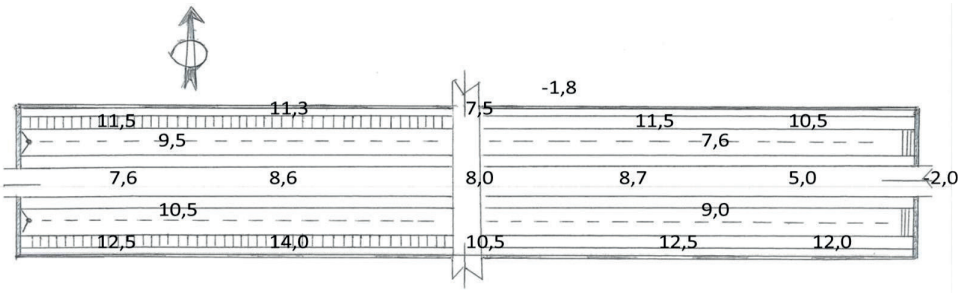

Figure 5. Topography of the floor temperatures in building № 1 during the winter

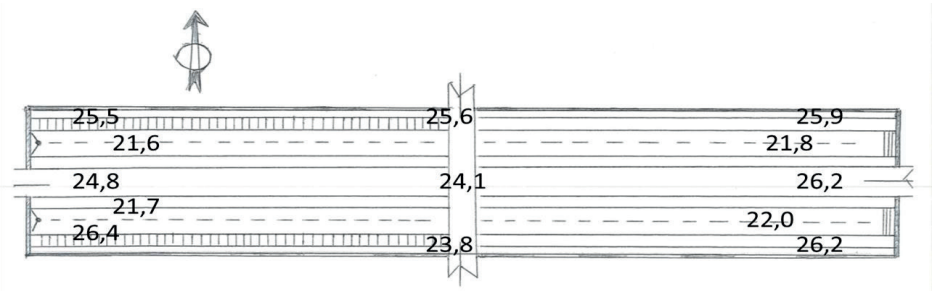

Figure 6. Topography of the floor temperatures of building № 1 during the summer at an outside temperature of 35ºC

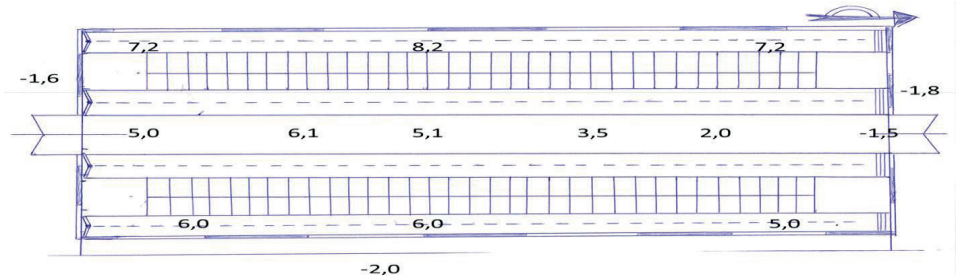

Figure 7. Topography of the floor temperatures in building №2 during the winter

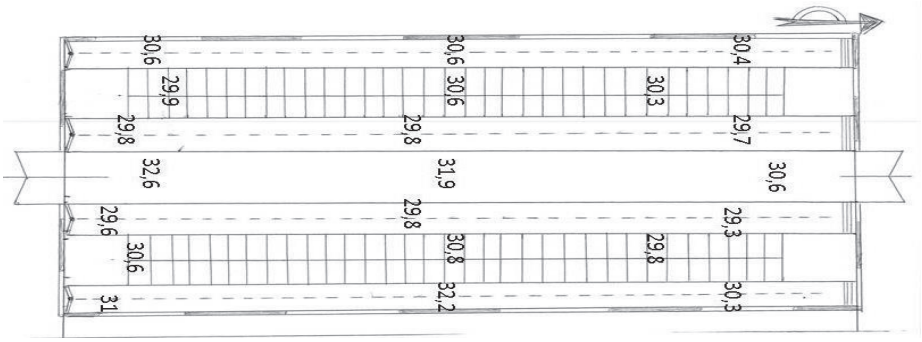

Figure 8. Topography of the floor temperatures in building № 2 at an outdoor temperature of 35ºC

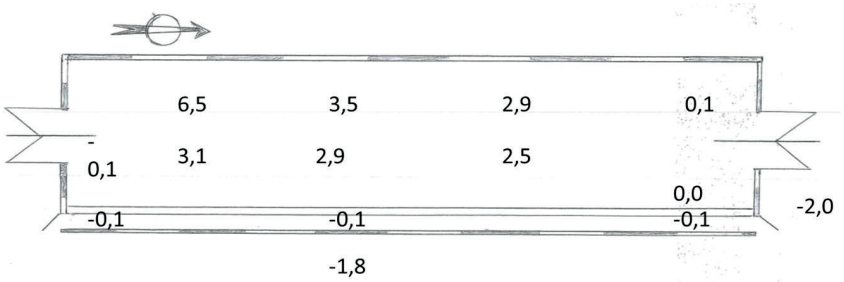

Figure 9. Topography of the floor temperatures in building № 3 during the winter

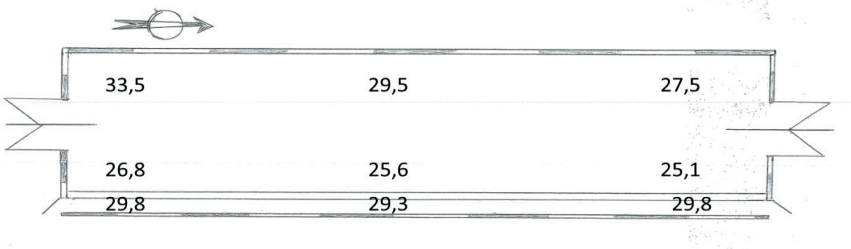

Figure 10. Topography of the floor temperatures in building № 3 during the summer at an outdoor temperature of 35 ºC

The microclimatic factors determine the welfare and the behaviour of the animals, including their lying time. The topographies of the floor temperature during the summer and the winter we made (Figures 5, 6, 7, 8, 9 and 10) characterize the differences in the breeding technologies and the behavioural reactions of the cows. The studies of O'Driscoll et al., (2009) prove that the cows spend more time lying during the winter regardless of the box bedding and the breeding technology. The percentage of lying cows in building № 3 reaches 41 at an average temperature of 2.5ºC. The temperature measured under those cows was 12.5ºC. Overton et al. (2002) report the highest relative share of lying cows at a temperature of minus 15ºC.

Data presented by Plyashtenko and Hohlova (1976) indicate that cows lie down for approximately 40-50% of their time and the number of lying-downs and getting-ups varies between 12 and 14 per day. According to the authors, the cold floor quickly cools the body of the lying cow and its temperature decreases from 37.5ºC to 25ºC for a period of 10-15 minutes.

After the cow gets up, the floor releases up to 2ºC warmth in the air environment of the premises. The high temperatures we measured in buildings № 1 and 2 and in the beddings there during the summer are the reason why the cows mainly group in the zone of ventilator activity in an effort to prolong the time for cooling their bodies by standing up (Hristev et al., 2019). At the same time, 55.2% of the cows from building № 3 prefer lying so as to release the excessive body warmth to the deep and humid bedding.

The significance of the light in breeding dairy cows should not be underestimated, too. The level of illumination ascertained by us in all three farms corresponds to the physiological needs of the animals. According to Trofimov et al. (2016), the duration of the light part of the day during the winter must be 16 hours with an intensity of 200-300 lux. The lack of enough light, regardless of the balanced feeding of the animals may prove to be the leading factor for a weak sexual activity and low impregnation rate.

CONCLUSIONS

The specific microclimate which is formed in each of the buildings is a result of the different stocking density, breeding technology, constructive characteristics and the season. The microclimate in the open buildings is more dependent on the outside environmental factors when compared to the closed ones.

The topography of the floor temperatures reveals the differences in the breeding technologies and forecasts the behavioural reactions of the cows. Therefore, upon the overall assessment of the barn environment comfort, it is necessary to take into account not only the thickness and the nature of the bedding but also the floor temperature.

REFERENCES

Broucek, J., Novak, P., Vokralova, J., Soch, M., Kisac, P., Uhrincat, M. (2009). Effect of high temperature on milk production of cows from free-stall housing with natural ventilation. *Slovak J. Anim. Sci*., 42(4), 167–173

Darwin, R. (2001). Climate Change and Food Security; *Agriculture Information Bulletin*, 765–768; United States Department of Agriculture: Washington, DC, USA.

Dimova, V., Mitev, J., Miteva, Tch., Popova, Y., Vasilev, N. (2012). Evaluation of some zoohygienic parameters in a semi-open free-stall dairy barn. *Trakia Journal of Sciences*, 10(2), 102–108.

Dinev, D.(2007). Study of technological and construction solutions of dairy cattle breeding, buildings in the Republic of Bulgaria. *Habilitation Work*, 314.

Gaughan, J. B., Mader, T. L., Holt, S. M., Hahn, G. L., Young, B. A. (2002). Review of current assessment of cattle and microclimate during periods of high heat load. *Anim. Prod. Aust*., 24, 77–80.

Gaughan, J. B., Holt, S. M., Hahn, G. L., Mader, T. L., Eigenberg, R. (2000). Respiration rate - is it a good measure of heat stress in cattle? *Asian-Australian Journal Animal Science*, 13 (Supp C), 329–332.

Gauly, M., Bollwein, H., Breves, G., Brugemann, K., Danicke, S., Das, G., Demeler, J., Hansen, H., Isselstein, J., Konig, S. (2013). Future consequences and challenges for dairy cow production system arising from climate change in center Europe - A review. *Animal*, 7, 843–859.

Gregoriadesova, J., Dolezal, O. (2000). Vliv vysokých teplot prostředí na skot. Revue, řada C (technologie, technika, welfare, ekologie), VÚŽV Uhříněves, Praha, 106 pp.

Hansen, P. J. (2007). Exploitation of genetic and physiological determinants of embrionic resistance to elevated temperature to improve embryonic survival in dairy cattle during heat stress, *Theriogenology*, 68, 242–249.

Hanus, O., Vyletelova, M., Gencurova, V., Jedelska, R., Kopecky, J., Nezval, O. (2008). Hot stress of Holstein dairy cows as substantiv factor of milk composition. *Scientia Agriculturae Bohemica*, 39, 310−317.

Heidenreich, T., Büscher, W., Cielejewski, H. (2004). Vermeidung von Wärmebelastungen bei Milchkühen. *Deutsche Landwirtschafts Gesellschaft, Merkblatt*, 336.

Hristev, H., Ivanova, R., Tasheva, S. (2019). Special features used when forming the microclimat and comfort conditions of buildings used for breeding dairy cows in summer. *Scientific Works*, Agricultural University of Plovdiv, in press.

Mader, T. L., Davis, M. S. (2004). Effect of management strategies on reducing heat stress of feedlot cattle: feed and water intake. *J. Anim. Sci*., 82, 3077-3087.

Miteva, Ch. (2012). Hygienic aspects of the production activity of free-range dairy cows. Thracian University of Stara Zagora, *Scientific Works*, 222.

Nardone, A., Ronchi, B., Lacetera, N., Bernabucci, U. (2006). Climatic effects on productive traits in livestock. *Veterinary Research Communications*, 30 (Suppl. 1), 75–81.

O'Driscoll, K., Boyle, L., Hanlon, A. (2009). The effect of breed and housing system on dairy cow feeding and lying behaviour. *Applied animal behaviour science*, 116(2-4), 156–162.

Ordinance No. 44 of April (2006). On veterinary requirements for livestock, breeding establishments. St. G., 41/19.05.2006.

Overton, M., Sischo, W., Temple, G., Moore, D. (2002). Using time-lapse video photography to assess dairy cattle lying behavior in a free-stall barn. *J. Dairy Sci*., 85(9), 2407–2413.

Petkov, G., Baikov, B. (1976). Importance of environmental factors in industrial cattle breeding, 140.

Pliashchenko, S. I., Khokhlova, I. I. (1976). *Microclimate and Productivity of Animals*. Leningrad, RU: Kolos Publishing House, 208.

Ravagnolo, O., Misztal, I. (2002). Effect of heat stress on nonreturn rate in Holsteins: Fixed- model analysis. *J. Dairy Science,* 85, 3101–3106.

Solan, M., Jozwik, M. (2009). The effect of microclimate and management system on welfare of dairy cows (in Polish). *Wiad. Zoot*., 1, 25–29.

Trofimov, A. F., Musica, A. A., Moskalev, A. A. (2016). Features of the formation of the microclimate of livestock buildings, depending on design decisions. *National Academy of Sciences of Belarus,* 2, 80–86.

Velecka, M., Javorova, J., Falta, D., Vecera, M., Andrysek, J., Chladek, G. (2014). The effect of temperature and time of day on welfare indices in loose-housed Holstein cows. *Acta universitatis agriculturae et silviculture mendelianae brunensis*, 62(3), 565–570.

Ventura, B. A., von Keyserlingk, M. A. G., Weary, D. M. (2015). Animal Welfare Concerns and Values of Stakeholders Within the Dairy Industry. *J Agric Environ Ethics,* 28, 109–126.

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PRELIMINARY DATA ON INFORMATION SOURCE FOR THE FARMERS - THE CASE OF ALBANIA

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Abstract

This is an exploratory survey, aiming at assessing farmers' sources from which they receive information, and analyzing the extension methods that are most valuable to them. A questionnaire-based survey was conducted to assess the farmers' knowledge on agriculture practices, as well as their information knowledge competencies. A total of 40 farmers were interviewed, and the method of data analysis used is the descriptive statistics. The main source of information and advice for agro-input (seed, fertilizers and pesticides) are the agro-input dealers and village input shops. 75% of farmers get the market price information from each other. Most of the farmers (65%) think extension activities are not in line with their requirements. Out of 10 sources of information analyzed, the main source of information and knowledge for farmers are themselves. About the competence knowledge 2/3 of farmers think they have good or very good level of knowledge. 92.5% of the farmers are willing to participate in the trainings. The public advisory service should plan well other activities to provide more up-to-date technical information, as the knowledge of most farmers is now outdated.

Key words: *extension methods, farmers' competencies, information source, survey.*

INTRODUCTION

One of the main sectors of the Albanian economy, accounting for about 40% of total employment (INSTAT, 2017), and one of the main sources of income for rural households, which has generated, in recent years, 20-23% of GDP (World Bank, 2017; World Factbook, 2017), is agriculture. This sector is also important in terms of alleviating poverty (where the majority of the poor are in rural areas) and improving the standard of living.

In addition to the problem of competitiveness, agriculture faces several challenges, which we think are: (i) small farm size (1.2 ha) and fragmentation of land (about 4 plots per farm)[1]; (ii) malfunctioning of associations, cooperatives or product groups; (iii) poor marketing of products; (iv) improper irrigation and drainage systems; (v) low interest in investment in agricultural activities; (vi) low quality of agricultural inputs; (vii) lack of agricultural credit; (viii)inefficient farm management practices. Some of these weaknesses have continued to be the same over the last 20 years, such as the low technology level of farmers, or the public and private Extension Service not at the level required by farmers.

According to Frashëri (1936) the beginning of the advisory service in Albania dates back to 1936[2]. After 1945, this service was covered by specialists in muncipalities and collection centers, and with the establishment of agricultural cooperatives and agricultural state farms were the agronomist and the livestock experts of those entities who were in charge to train the workers for the daily work and new technologies.

The Advisory Service in Albania underwent major changes after 1991 when agriculture began its privatization and land was distributed to families working in centralized agriculture state farms and cooperatives. The advisory

[1] The land reform implemented after August 1991, in which the state agricultural land was equally distributed to the rural population, resulted in small and fragmented farms that hamper the growth and competitiveness of agriculture.

[2] The government then set up 5 pilot groups consisting of 5 specialists each (agronomist, zoo technician, veterinarian, forest engineer and economist) to assist Albanian farmers with agricultural advice.

service in Albania, as it stands today, started in 1992, and for the period 1994-2001 was supported by the EU and Dutch Government, with technical assistance in training the agriculture specialists with the concepts and principles of extension and communication. During this period private extension services have also emerged. Despite of improvements in some private and public services, most services are poorly provided or non-existent. Skreli et al. (2014), emphasis that the impact of government/public extension service on farm performance is limited, and the coverage of public extension services is limited, while the private advisory services are the main source of advice for most progressive medium and large farms.

After 2001 the extension service went through several "reforms" and since march 2018 the structure is as it shown in Figure 1[3].

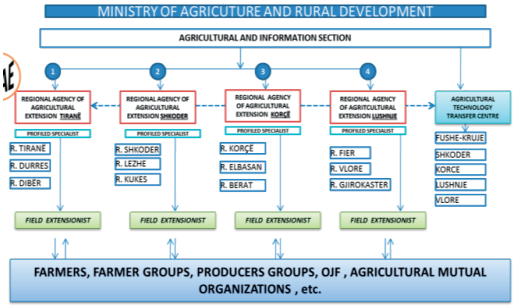

Figure 1. Farm Advisory Service in Albania

Based on the Rural and Agricultural Development Strategy (2014-2020)[4] and Extensive Service policy, the product that this service should provide is measured by the indicator "Percentage of farmers and agro-processing businesses that have been informed, against the total of farmers and agro-processing businesses"

The demand and supply sides of extension services are undergoing a substantial change, in Albania. According to the Rural Development Strategy of Albania (MAFCP, 2007), approximately 30 % of the farm holders have an agricultural education background. While the young generation of farmers has limited relevant experience and know-how. While on the supply side, a major issue is the shortage of young professionals, as the most qualified experts are more interested in other activities.

One of the key factors in the extension process is the education and through it the farmers receive technical knowledge and information, which helps farmers to make decisions about the future of the farm.

According to Ingenaes (2015), over the past century, extension education is developed as a discipline with its own philosophy, goals, methods and techniques that should be understood and used by most extension workers if they are to be effective in meeting the needs of all farmers, especially small farmers and women farmers.

It is also argued that co-production knowledge, for example, between farmers and advisers, is a new form of knowledge, combining scientific evidence and training, technical information, experience-based knowledge, information on household goals and interests, the unspoken knowledge of farmers, etc. This shows that agricultural advisory services are characterized by diversity and complexity. It is therefore argued that it is necessary to combine extension methods to increase knowledge transfer and improve learning in agriculture (Labarthe and Laurent, 2013).

However, to improve learning is required a level of farmers' competences. Competence is often considered as the sum of knowledge and skills, where knowledge is something theoretical or academic, while skills are about the ability to solve problems in practice. For the agricultural sector, with regard to competence, more emphasis should be placed on people's attitudes and motivations, both in gaining new knowledge and applying skills. Thus, an important part of extension and extension services is raising awareness of good practices and motivating farmers (Karbasioun, 2007)

[3] The Albania Government Decision no. 147, date 13.3.2018 "The establishment, organization and the functioning of Regional Agencies of Extension Service".

[4] Strategjia ndersektoriale per zhvillimin rural dhe bujqesor ne Shqiperi (2014-2020). Vendim i Këshillit të Ministrave nr. 709, datë 29.10.2014 Fletore Zyrtare, Viti: 2014 – Numri:169
Tiranë – 5 Nëntor 2014.
https://bujqesia.gov.al/wp-content/uploads/2018/02/STRATEGJIA_NDERSEKTORIALE.pdf

Except the public extension service, a source of information for farmers are the agro-input dealers. They are interested in maintaining good business relationships with farmers and on the other hand farmers are interested in information on the use of inputs. The trader that conducts extension activities is valued by farmers (Schwartz, 1994).

MATERIALS AND METHODS

The purpose of the survey was to identify sources from which the farmers receive information. It will also analyze the extension methods that are most valuable to them.

The realization of the survey has been made possible by the use of primary, secondary sources and literature data related to extensive service in the field of agriculture.

The survey was conducted on 40 farms of Vora, Maminas, Bërxullë and Preza administrative units of Vora municipality[5], which are known for their production of vegetables and olives.

For the purpose of this survey, a questionnaire is designed for interviewing farmers and collecting the data needed. The questionnaire consists of a series of questions. There are questions about the farmer's personal background, such as age, gender, and family. Other variables in the dataset relate to farm characteristics such as size, production types, and location; and socioeconomic aspects such as experience in agricultural production, education etc. Of particular interest to this paper is a set of questions related to education, knowledge, competence and use of advisory services.

To check the validity of the questionnaire it was subject of review by a panel of three agricultural experts. Their remarks/suggestions were reflected in the improved questionnaire. In addition, the questionnaire was pre-tested with a pilot group of three farmers; in the case of inconsistent questions, it was modified accordingly.

Interviews were conducted at the farm, in most cases with the head of household and in few cases with family members. The questionnaire contained open-ended and closed-ended questions.

According to Jackson (2009) open-ended questions allow for a greater variety of responses from participants, but are difficult to analyze statistically because data has to be coded or reduced in some way. While, closed-ended questions are easy to analyze statistically, but they seriously limit the answers that participants can provide. We have also used a Likert-type scale (1932) because it is very easy to analyze statistically and it is very used in agricultural research (Clason and Dormody, 1994).

In this survey, the sample consisted of a total of 40 agricultural production farms, which were randomly selected from the list of potential farmers prepared by advisory service of the Tirana Regional Agricultural Extension Agency (TRAEA). These areas where the farms were selected were selected because of the convenience and assistance provided by the advisory service.

Only 40 farmers were included in the survey, because in the moment of the interviews in some media was reported that for vegetables and fruits, farmers use pesticides and growth stimulants improperly. This resulted in many farmers refusing to visit their farm and being interviewed. The farmers interviewed did not create any problems for the interviewer, especially as they were clarified about the purpose of the interview and the study.

The survey was administered during April - June 2019, using direct interviews, by the authors of the paper.

The data obtained were entered in Microsoft Excel and transferred into SPSS. The analysis is based on descriptive statistics, namely frequencies.

RESULTS AND DISCUSSIONS

1. General data on observed farms

The purpose of this survey was to identify the impact of the public advisory service on farmers operating in some of the administrative units of Vora municipality, in Tirana.

As can be seen from the data in Table 1, all interviewed farmers have 31.6 years (10-62 years) working experience in agriculture; satisfactory educational level, with 65% of

[5] Vora municipality is 18 km far from Tirana- capital of Albania.

them having completed secondary education and university (Figure 2); but on the other hand the age of farm managers is quite high 60.9 years (35-83 years).

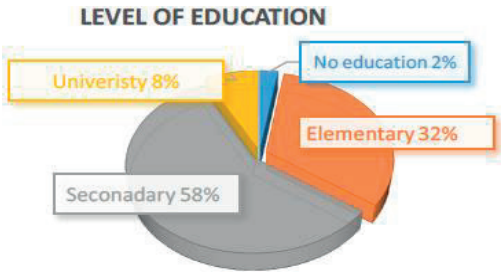

Figure 2. Farmers' education level

In 96.7% of the cases the head of the household decides how the farm will be run and the sale price of the products, and in 3.3% of the cases the decision is made as a family. The same result (94.5%) is reported by Androulidakis et al. (2002) in a study conducted in Albania.

Only 15% of the farmers have contracts with traders/collectors and 5% with processors.

As can be seen from Figure 3, the main activity for the interviewed farmers are the vegetables grown in greenhouse (35%), Olives orchards (17.5%) and open field vegetables (17.5%). This is a consequence of the proximity to the Tirana market in terms of vegetables and the hilly terrain itself planted with olives, most of them inherited from decades.

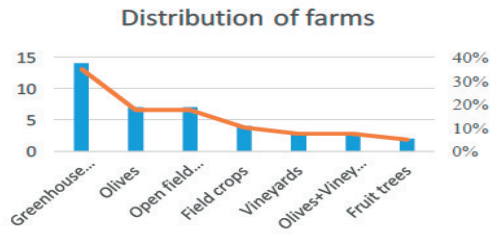

Figure 3. Distribution of farms

2. Agro-input purchase and products selling

Products are sold by farmers as follow: 35% sell by themselves their products; 20% only to wholesalers; 32.5% sell the products to traders and retailers; and 12.5% sell them to retailers. 62.5% of farmers have only one source of sales, the rest have 2-3 sources of sales.

81.8% of production is sold (P value = 0.083), 10.8% is consumed by the family, and 7.4% of production is damaged, which is statistically significant for P <0.05 (0.039) and the value of Pearson Chi Square is 13.298.

72.1% of farmers say that their business in the last three years has been at the same level; 15.2 report improvement; while 12.7% declaring business decline. As the P-value of the ANOVA table is more than 0.05 (0.814), there is no statistically significant business-level relationship in the last three years at 95.0% confidence level.

In terms of input purchasing, for seeds, fertilizers and pesticides there is a difference between farmers with slightly larger farms who are more aware of the consulting and training provided, so most of them buy them from agro-input sealers, whereas those who have very small farm are buying the inputs mainly in village shops (P = 0.003 to 0.038), which trade low quality inputs. Almost the same results are reported by Androulidakis et al. (2002), where 58% of seeds, 70% of fertilizers and 62% of pesticides the farmers bought at agro-input dealers (Figure 4). In addition, most of the farmers reported that level of input used was the same in the last three years (Table 2).

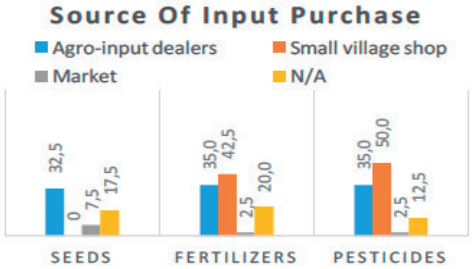

Figure 4. Source of input purchase by farmers

Most of the farmers pointed out that level of incomes and business in the last three years is the same (47.5-52.5%) or worse (5-17.5%) and only 35-42.5% report that is better.

Table 1. Main sample socio-demographic and farm indicators

No of farmers interviewed	Number of family member	Head of the Household						Farm area (ha)
		Age (years)	Working Experience (years)	Education level				
				No education	Elementary	Middle/high school	University	
40	4.7	60.9	31.6	1	13	23	3	0.81

No of farmers interviewed	Number of family member working in the farm	Rented workers	Farms with:						
			Olives	Vine yards	Greenhouse Vegetables	Open field vegetables	Fruit trees	Field crops	Olives+ Vineyards
40	2.13	1.18	7	3	14	7	2	4	3

Table 2. Use of agro-inputs in the last three years

No of farmers interviewed	Use of agro-inputs in the last three years (%)											
	Fertilizer				Manure				Pesticides			
	Same level	More	Less	N/A	Same level	More	Less	N/A	Same level	More	Less	N/A
40	62.5	2.5	12.5	22.5	65	12.5	0	22.5	62.5	15.5	10.0	12.5

Table 3. Distribution of answers regarding the source of information (%)

Information source	Seed varieties	Fertilizers and fertilization	Pesticides use	Irrigation
Public extension	20.0	5.0	10.0	7.5
Private extension	10.0	12.5		10.0
Agro-input dealers	30.0	20.0	25.0	
Village shops of inputs	25.0	40.0	45.0	
Other farmers	7.5	2.5		32.5
Other sources	7.5	20.0	20.0	50.0
Total	100	100	100	100

Information source	Farm management	Business plan preparation and business planning for the future	Prices and Market	Environment protection
Public extension	10.0			100
Private extension				
Agro-input dealers				
Village shops of inputs				
Other farmers		75.0		
Other sources	90.0	25.0	100	
Total	100	100	100	100

3. The relation farmer-advisory

The sources of information are different for each issue (Table 3). It is seen that the main source of information and advice for agro-input (seed, fertilizers and pesticides) are the agro-input dealers and village input shops.

Public extension should also focus on issues such as farm records keeping, pricing and market information. In addition, the public extension and Ministry of Agriculture and Rural Development should take more responsibility in providing the market price information because 75% of farmers get the information from each other. Private sector extension may be provided not only by companies wishing to sell to farmers, but also by those wishing to purchase from them. Extension advice may be provided both to increase product quality to the benefit of the purchasers and as a way of promoting contract farming with suppliers (Androulidakis et al., 2002).

Most of the farmer emphasis that they are satisfied mainly from agro-input dealers (Table 4).

Most of the farmers (65%) think extension activities are not in line with their requirements. In addition, 62.5% of the farmers evaluate the communication with the extension agent as good and very good, however the farmers that consider it not very good is considered high, and all the providers needs to think about it (Table 5).

From the 10 sources of information analysed, the main source of information and knowledge for farmers, the main source are themselves (Table 6). The public advisory service should therefore plan well for other trainings and activities to provide more detailed and up-to-date technical information and advice, as the knowledge of most farmers is now outdated, given the advanced age of the farmers.

The same can be said about the competence for the 10 sources analyzed where 2/3 of farmers think they have good or very good level of knowledge. However, seeing that 1/3 of farmers confirm that they do not know or know little about certain problems, the public advisory service needs to conduct training with farmers to increase their level of competence (Table 7).

The farmers' opinion is that the main methods most relevant to them are: (i) demonstrations, (ii) meetings with farmers, (iii) field days, and (iv) discussions with advisors, other farmers and dealers (Figure 5). The same is mentioned Lukkainen (2012), who states that farmers are keen to see how a new idea works and how it can affect their farm production and these can be done with a demonstration. Explaining why farmers say demonstrations are an effective method may be that they are able to see a particular technique or technology in practice. It also states that the farmer-to-farmer method is the most productive for farmers.

Asked if you used any of the new ones you learned from extension activities at your farm? Out of 40 farmers - 9 farmers responded that they applied drip irrigation, and 4 of them pruning. About 1/3 of farmers have applied what they have seen and learned in extension activities, which is a good indicator. Whereas for training courses very few farmers (7.5% of them) consider it as a valid method, while Lukkainen (2012) emphasizes training as a source of innovations implemented by farmers.

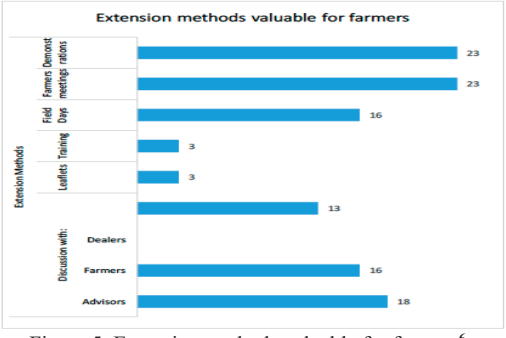

Figure 5. Extension methods valuable for farmers[6]

In terms of motivation to participate in demonstrations and trainings (Table 8), where farmers had to choose for each alternative one of the 5 Likert scale options (1 = not at all, 5 = strongly), we see the highest percentage of farmers, goes for the answer "To gain new knowledge" and "To get more services". While the answers to "To know other farmers" and "The Extensionist's Insistence" are not very well liked.

[6] The answers that are summarized in table 8 9, 10, 11, 12 and figure 5 the farmers checked more than one source.

The data in Table 9 give us a clear picture of how far trainings and demonstrations have met the needs of farmers. The answers of most of farmers is negative, and this probably means that trainings/demonstrations are not planned with a wide discussion with farmers and are not planned at the right time for them, that is, when low season of works in their farm.

When farmers are asked what needs to be improved in extension activities, 60% of farmers and say that more meetings with extension agents and trainings are needed.

To the question whether you will continue to participate in the trainings - 92.5% of the farmers answered yes (Table 10), which shows interest for training, and here it seems that the answers given to how competent the farmers are on issues related to farm activity (Table 8) are not very correct.

Farmers think that subsidies are the most important way to increase their farm production and income (Table 11).

When the farmers were asked to check their three main problems affecting competitiveness the answers were: low profit, high level of taxes and low level of subsidy support (Table 12).

CONCLUSIONS

The farmers interviewed long working experience in agriculture; satisfactory educational level, but on the other hand the age of farm managers is quite high.

In 96.7% of the cases the head of the household decides how the farm will be run and the sale price of the products. 62.5% of farmers have only one source of sales, the rest have 2-3 sources of sales.

72.1% of farmers say that their business in the last three years has been at the same level.

The large farms, who are more aware of the consulting and training provided, most of them buy the inputs from agro-input dealers, whereas those who have very small farm are buying the inputs mainly in village shops, which trade low quality input.

It is seen that the main source of information and advice for agro-input (seed, fertilizers and pesticides) are the agro-input dealers and village input shops; while 75% of farmers get the market price information from each other

Most of the farmers (65%) think extension activities are not in line with their requirements. From the 10 sources of information analysed, the main source of information and knowledge for farmers are themselves. The same answer is about the competence, for the 10 sources analysed, where 2/3 of farmers think they have good or very good level of knowledge.

The farmers opinion is that the main methods most relevant to them are: (i) demonstrations, (ii) meetings with farmers, (iii) field days, and (iv) discussions with advisors, other farmers and dealers (Figure 5).

About 1/3 of farmers have applied what they have seen and learned in extension activities, which is a good indicator. Whereas for training courses very few farmers (7.5% of them) consider it as a valid method. Most of farmers said that trainings/ demonstrations did not meet their needs, and this probably because trainings/demonstrations are not planned with a wide discussion with farmers and are not planned well.

The opinion of most farmers (60%) is that extension activities should be improved, and more meetings with extension agents and trainings are needed.

92.5% of the farmers have the willing to participate in trainings, and here it seems that the answers given to how competent the farmers are on issues related to farm activity are not very correct.

The public advisory service should therefore plan well for other trainings and activities to provide more detailed and up-to-date technical information and advice, as the knowledge of most farmers is now outdated, given the advanced age of the farmers. Seeing that 1/3 of farmers confirm that they do not know or know little about certain problems, the public advisory service needs to conduct training with farmers to increase their level of competence.

Table 4. Which source are you most satisfied with the advice received

Farmers	Public extension		Private extension		Agro-input dealers		No answer	
	No	%	No	%	No	%	No	%
40	11	27.5	5	12.5	18	45.0	6	15.0

Table 5. Farmers evaluation regarding the communication with advisor

Farmers	Very good		Good		Satisfactory		No good		N/A	
	No	%	No	%	No	%	No	%	No	%
40	8	20.0	17	42.5	6	15.0	4	10.0	5	12.5

Table 6. Sources of knowledge about farm work (%)

Information source	Crops knowledge	Fertilizer and fertilization	Pesticide use	Irrigation & drainage	Record keeping
Myself	55.0	57.5	47.5	57.5	75.0
Colleagues	17.5	15.0	17.5	17.5	0
Seminars/Trainings	15.0	17.5	20.0	17.5	25.0
School	12.5	10.0	15.0	7.5	0
Total	100	100	100	100	100

Information source	Farm management	Business plan & planning for the future	Prices and Marketing	Environment protection	Communication and cooperation skills
Myself	70.0	67.5	75.0	72.5	75.0
Colleagues	10.0	12.5	2.5	0	2.5
Seminars/Trainings	17.5	17.5	20.0	27.5	20.0
School	2.5	2.5	2.5	0	2.5
Total	100	100	100	100	100

Table 7. Knowledge competencies for farm work

Level of knowledge	Crops knowledge	Fertilizer and fertilization	Pesticide use	Irrigation & drainage	Record keeping
I do not know	17.5	12.5	12.5	15.0	15.0
Some	17.5	12.5	12.5	10.0	0
Good	27.5	37.5	32.5	17.5	10.0
Very good	37.5	37.5	42.5	57.5	75.0
Total	100	100	100	100	100

Level of knowledge	Farm management	Business plan & planning for the future	Prices and Marketing	Environment protection	Communication and cooperation skills
I do not know	12.5	15.0	17.5	12.5	22.5
Some	12.5	15.0	10.0	17.5	7.5
Good	30.0	22.5	32.5	20.0	12.5
Very good	45.0	47.5	40.0	50.0	57.5
Total	100	100	100	100	100

Table 8. Motivation to participate in demonstrations and trainings [7]

Farmers	To gain new knowledge		To get more services		Personal interest		The extensionist's insistence	
	No	%	No	%	No	%	No	%
40	29	80.5	22	66.7	16	48.5	11	33.3

Farmers	Get certificate		To know better the advisor agent		To know other farmers		Friend interest	
	No	%	No	%	No	%	No	%
40	13	43.3	11	31.4	7	21.2	2	16.7

Table 9. To what extent did the trainings/demonstrations meet your needs?

Farmers	How was the quality of the trainings/ demonstrations?		How do you assess the applicability of the issues addressed in trainings/ demonstrations?		How do you evaluate the place of trainings/ demonstrations?		Were the timing of the trainings/ demonstrations appropriate?	
	No	%	No	%	No	%	No	%
Positive answer	19	47.5	14	35.0	13	32.5	12	30

Table 10. Farmer needs for training

Farmers	Subsidies		Technology		To be known their needs		Farm management		No answer		Books	
	No	%	No	%	No	%	No	%	No	%	No	%
40	17	42.5	9	22.5	5	12.5	4	10.0	4	10.0	1	2.5

Table 11. Support required by farmers to increase their production

Farmers	Subsidies		Quality and better price of inputs		New Technology		No answer	
	No	%	No	%	No	%	No	%
40	29	72.5	2	5.0	2	5.0	7	17.5

Table 12. Problems that adversely affect competitiveness

Description	First problem		Second problem		Third problem	
	No	%	No	%	No	%
Low profit	25	62.5	3	7.5	3	7.5
Competition from import	8	20.0	10	25.0	2	5.0
High level of taxes	3	7.5	12	30.0	14	35.0
Lack of markets	1	2.5	0	0	5	12.5
Low level of subsidies	0	0	11	27.5	8	20.0
Others	3	7.5	4	10.0	8	20.0
Total	40	100	40	100	40	100

[7] Here are listed only those farmers who responded that there was strong motivation and the percentage is based on them for each answer.

REFERENCES

Androulidakis, S., Claude, C., Freeman III, C.C., Bicoku, Y., Peqini, I., Agolli, Sh., Korra, Ll. (2002). Private Extension in Albania: Impact of Albanian Fertilizer and Agri-Business Dealers Association on Farmers Adoption of Technology. *AIAEE*, 9(1), 47-55.

Clason, D.L., Dormody., J. (1994). Analyzing data measured by individual Likert-type items. *Journal of Agricultural Education*, 35(4), 31- 35.

Frashëri, M. (1936). Programi i kryeministrit mbi reformat ekonomike dhe financjare të konkretueme me projekt-ligjë dhe me raportet justifikuës. - Tiranë: Shtypur në Shtypshkronjën "Gutenberg", 126 f.

INGENAES (2015): Extension and Advisory Services - Terminology and Glossary. Feed the Future, The U.S. Government's Global Hunger & Food Security Initiative.

INSTAT (2017). Tregu i Punes, 2016.

Jackson, S.L. (2009). *Research Methods and Statistics: A Critical Thinking Approach,* 3rd edition. Belmont, CA: Wadsworth Publishing House.

Karbasioun, M. (2007). Towards a Competency Profile for the Role of Instruction of Agricultural Extension Professionals in Esfahan. PhD-thesis, Social Sciences Group, chair group of Education and Competence Studies, Wageningen University and Research Centre, The Netherlands.

Labarthe, P., Laurent, C. (2013). Privatization of agricultural extension services in the EU: Towards a lack of adequate knowledge for small-scale farms? *Food Policy*, 38 ©, 240-252.

Likert, R. (1932). *A Technique for the Measurement of Attitudes*. New York, USA: Archives of Psychology, No. 140.

Luukkainen, J. (2012). A Comparison of Extension Methods Used by Different Agricultural Extension Service Providers in Nyandarua County, Kenya. HAMK University of Applied Sciences, Bachelor thesis.

MAFCP (2007). Inter-Sectoral Rural Development Strategy of Albania, ISRDSA 2007-2013.

Schwartz, A.L. (1994). The Role of the Private Sector in Agricultural Extension: Economic Analysis and Case Studies. The Overseas Development Administration, Network Paper 48 0952-2468.

Skreli, E., Imami, D., Zvyagintsev, D., Gjeci, G. (2014). "Government Extension Service Impact Assessment". *142nd EAAE Seminar/conference*, Budapest, Hungary.

World Bank (2017). Country Snapshot. The World Bank in Albania. October 2017.

World Factbook (2017). Albania, 2017.

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THE INFLUENCE OF TREATMENTS WITH SYNTHETIC HORMONES ON THE REPRODUCTIVE PERFORMANCE OF YOUNG SOWS EXPLOITED FOR THE PRODUCTION OF BACON

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Abstract

The purpose of the paper is to highlight the reproductive performance obtained in a pig farm in Denmark following the treatment of sows with a hormonal preparation used to synchronize oestrus. The research involved the study of 105 YL (Yorkshire x Landrace) cross-breed females, all between the ages of 28 and 30 weeks, weighing between 100 and 120 kg, exploited for the production of bacon. The following indicators were followed: fertility, index of use of sows for reproduction, prolificacy, number of piglets weaned on female and piglets' mortality. The obtained results showed higher values for all the indicators analysed at the batch treated with the hormonal preparation compared to the control group (fertility 94.99 vs 91.66%; the index of use of sows for reproduction 2.47 vs 2.29; prolificacy 16.7 vs 15.6 piglets; number of weaned piglets on female 15.7 vs 15 piglets). The results of the study demonstrate that hormone treatment in sows leads to improvement of reproduction indicators in sows.

Key words*: fertility, mortality of the piglets, number of piglets weaned on the female, oestrus synchronization, reproductive performance.*

INTRODUCTION

Swine growth is of great economic importance as the branch of animal breeding in many countries (Rotaru, 2018). In Romania, the carcasses from pig's exploited in industrial systems, after classification, shows a very good quality, with a percentage of meat in the carcass of over 50% (Cărătuș et al., 2017, Găureanu et al., 2017). In the current breeding systems, it is considered that the proliferation is optimal when the number of piglets is equal to the number of sows' tits (approximately 14 piglets).

In order to optimize the efficiency of artificial insemination (AI) at sows the time of ovulation after weaning should be controlled (Pearodwong et al., 2019). For this reason, it is necessary to carry out multiple researches and to encourage various ovulation induction protocols (Driancourt et al., 2013).

In the last period protocols have been developed that use only GnRH (Baroncello et al., 2017) but also LH (Ulguim et al., 2014). Ovulation induction is of great interest to the entire pig breeding industry. It is a technology that, if well realized, leads to the improvement of pig production and implicitly to increased economic efficiency (Knox, 2015).

Hormone-inducing products can be administered at a fixed time after weaning (Cassar et al., 2005) or from the oestrus (Gooneratne et al., 1988). Treatment with hormonal preparations leads to synchronization of the oestrous period at sows. However, its effect on reproductive performance is inconsistent, scientific literature presenting contradictory data. Therefore, the purpose of the study is to determine if treatment with Progestin have positively or negatively effects of the reproductive performance of sows.

MATERIALS AND METHODS

The study was conducted on a commercial pig farm (i/s Petersmide v. Anne og Martin Jensen) in Denmark, with a capacity of 780 sows which specializes in breeding piglets up to 30 kg weight and can produce 25,000 piglets per year. The sows used on the farm come from the elite farms in Denmark, based on the Landrace and Yorkshire cross-breeds.

The research involved the study of 105 YL

(Yorkshire x Landrace) cross-breed females, all between the ages of 28 and 30 weeks, weighing between 100 and 120 kg, exploited for the production of bacon.

Progestin is a hormonal preparation that is mainly used for synchronizing oestrus in sows with a success rate of up to 90%. This hormonal treatment is intended to suppress ovarian development. Progestin administration has negative feedback on gonadotropin-releasing hormone (GnRH) released from the hypothalamus. This inhibits the release of follicle stimulating hormone (FSH) and the release of luteinizing hormone (LH) and extends the range from weaning to oestrus.

Compared to natural oestrus, synchronization allows for greater predictability of the period when the oestrus will occur.

Due to Progestin treatment, the level of progesterone in the blood decreases, this being the signal for starting a new cycle and obtaining a new ovulation within 5 to 6 days. For the administration of the product several rules must follow:

• It is administered orally for 14 or 18 days;

• Treatment should take place at the same time every day (+/- 15 min.);

• 5 ml of Progestin shall be administered daily to each sow.

To determine the performance of reproduction and to determine the optimal period for applying the treatment with Progestin, sows taken in the study were divided in three batches:

• L_1 - 35 sows treated with Progestin for 14 days, maintenance in boxes with a capacity of 10 heads;

• L_2 - 35 sows treated with Progestin for 18 days, maintenance in boxes with a capacity of 10 heads;

• L_m - 35 sows without treatment, maintenance in boxes with a capacity of 10 heads.

The main indicators followed were: fertility, farrowing index, prolificacy, number of weaning piglets for sow, piglet's mortality.

RESULTS AND DISCUSSIONS

For optimal artificial insemination of females, it was found that the best fertility was obtained when the females were inseminated 10 to 16 hours before ovulation, including the time of spermatozoon ascension through the uterine horns.

In the farm, gestation diagnosis is made at 18, respectively 35 days, with the help of the WED-2000AV ultrasonic wave based device.

A common feature of the two experimental batches is the synchronization of heat at sows with rates of over 90% (Table 1).

Table 1. Fertility of the sows from the experimental batches

Parturition	Batch	No of inseminated sows	No of pregnant sows of 18 days		No of pregnant sows of 35 days	
			No	%	No	%
I	L_1	35	33	91.42	32	91.42
	L_2	35	33	94.28	33	94.28
	L_m	35	32	88.57	31	88.57

The highest fertility value, at the first and the second control, was for the sows from the L_2 experimental batch (94.28%). Good values are also recorded for the L_1 batch (91.42%) at 35 day control. The differences between experimental batches L_1 and L_2 was 2.86%. If we also consider L_m where the values reach up to 88.57%, we can say that the sows from the experimental batches L_1 and L_2 have fertility values higher than those from L_m batch.

The farrowing index indicates the number of offspring that a sow has during a year. It is a breeding indicator that most effectively expresses the use of sows on the farm. The farrowing index is considered good when the values are between 2.3-2.5 (Table 2).

Table 2. Farrowing Index

Batch	Reproduction indicators (days)			Farrowing index	Average value in Romania
	Average preparation period for artificial insemination	Average period of gestation	Average lactation period		
L_1	6	117	30	2.41	2.2 – 2.3
L_2	5	118	29	2.38	
L_m	8	118	29	2.29	
Average (x)	-	117.66	29.33	2.38	

From the data presented in Table 2, we can see that the best value of this index was recorded at L_1 batch (2.41), followed by those from L_2 batch (2.38) and last L_m (2.36) batch. Comparing the values obtained we note the superiority of the results obtained by the batches treated with Progestin over the control batch.

Comparing the values from L_1, L_2 and L_m batches with the results from Romania (Păsărin, 1997; Hoha, 2009) we note the superiority of the results obtained in Denmark (Radu, 2015).

The reproductive performance of the sows is appreciated by prolificacy and is closely related to the number and quality of the weaned piglets during one year of production. The life of weaned piglets can be considered as an integrated measure of reproductive productivity throughout the life of sows. Therefore, a high fertility and prolificacy of sows is not sufficient, if they do not have a high capacity for breastfeeding, a maternal care for the piglets and a good management of the caregiver to remove the events that can cause the death of pre and postnatal products (Table 3).

Table 3. The average prolificacy of sows at parturition

Parturition	Batch	Total farrowed piglets (heads)	Piglets farrowed live		Piglets farrowed dead (heads)	Weaned piglets (heads)	% weaned piglets from total farrowed piglets
			Viable (heads)	Nonviable (heads)			
I	L_1	16.8	16.1	0.4	0.3	15.1	89.88
	L_2	16.7	15.9	0.4	0.4	14.7	88.02
	L_m	15.6	14.6	0.3	0.7	13.5	86.53

From the data presented in table 3, we notice that the number of piglets farrowed alive ranged between 15.6 and 16.8, values considered to be very good compared to the ones found in the literature (Nacu, 2005; Hoha 2009).

By comparison, the number of piglets farrowed and the total number of parturition on sows treated with Progestin increased by approximately 0.9-1.2 piglets compared to those of L_m batch. This increase may be due to increased ovulation rate, embryonic survival and higher uterine capacity.

The large number of piglets obtained on the parturition is due to the genetic value of the sows as well as the breeding technology that implies the introduction to reproduction of the sows at the age of 28 to 30 weeks with the weight between 100 and 120 kg, so the body is able to respond positive regarding gestation, improving reproductive performance since the first parity.

From the analysis of the data recorded in the weaned piglets it is observed that there were losses between 10 and 14% in all 3 batches. Compared with the data from the Romanian literature (Păsărin, 1997; Teiu, 2003; Hoha, 2009), one can observe the superiority of the values obtained in Denmark.

Dead piglets are those piglets that are alive at the onset of parturition, but die intrapartum or postpartum. In practice, dead piglets are those found dead behind the sow at first check after parturition, without any signs of decomposition. In the last decades, the biggest losses from the economic point of view have been observed in case of the piglets, where the losses can reach up to 20-25% of the number of piglets farrowed. The high number of dead piglets may also be related to other aspects of reproductive performance, for example, for sows with increased parity a higher risk of abortion and an increase in the number of dead piglets has been reported.

The mortality recorded during the experimental period of the three batches of pigs' shows that the percentage of losses, from the total pigs farrowed, was around 10.12 and 13.47%. The data recorded fall within the limits presented in the specialized literature (12-15%) (Table 4).

Table 4. Mortality recorded in maternity

Batch	Percentage of losses/ productive sow life (%)	% of dead piglets/sow during maternity (head)	Average in Romania (%)
L_1	1.4	10.12	12-15
L_2	1.3	11.98	
L_m	1.3	13.47	
Average (x)	**1.33**	**11.85**	

Average losses of 11.85% in all 3 batches can be taken into account due to the age and the special quality of the sows, as well as the breeding technology. The factors that determined these losses until the moment of weaning were caused by: crushes/accidents, diarrhoea syndrome, respiratory syndrome, hypothermia, anaemia, dystrophies and congenital anomalies.

CONCLUSIONS

The reproductive performance of sows are very important indicators for the economic efficiency of pig farmers. Improving reproductive performance, therefore, increases the economic efficiency of pork production.

Sows from experimental groups were treated with progestin for 14 and 18 days respectively, so the results were investigated by separate analysis. Both treatment periods with Progestin showed improvements for all values of reproduction indicators.

Therefore, the 14 and 18 days treatments resulted in a more precise synchronization of oestrus time in sows. Hormonal treatment is recommended to improve reproductive performance when the oestrus period is synchronized.

A treatment regimen that is 4 days shorter has the result of reducing non-productive days and simplifying the management of the herd. The treatment duration of 14 days is sufficient for synchronizing the oestrus period and improving the reproductive performance.

The conclusions of the study show that treatment with Progestin in sows has improved reproductive performance by obtaining a better reproductive use index, by increasing the number of piglets made and by the number of piglets weaned.

REFERENCES

Baroncello, E., Bernardi, M.L., Kummer, A.D., Wentz, I., Bortolozzo, F.P. (2017). Fixed-time post-cervical artificial insemination in weaned sows following buserelin use combined with/without eCG. *Reprod. Domest. Anim.,* 52, 76–82.

Cassar, G., Kirkwood, R. N., Poljak, Z., Bennett-Steward, K., Friendship, R.M. (2005). Effect of single or double insemination on fertility of sows bred at an induced estrus and ovulation. *J. Swine Health Prod.,* 13, 254–258.

Cărătuş, M., Găureanu, M. E., Burtea, M. C., Vidu, L., Vlad, I., Cărătuş, N. (2017). The dynamics of pig carcasses classification in Romania between 2009-2015, by manual method the zwei punkte. *Scientific Papers: Management, Economic Engineering in Agriculture & Rural Development*, 17(4), 85–89.

Driancourt, M.A., Cox, P., Rubion, S., Harnois-Milon, G., Kemp, B., Soede, N.M. (2013). Induction of an LH surge and ovulation by buserelin (as Receptal) allows breeding of weaned sows with a single fixed-time insemination. *The riogenology,* 80, 391–399.

Găureanu, M. E., Cărătușu, M., Cocîrcă, D.I., Vidu, L., Vlad, I. (2017). Technical aspects regarding the classification of pig carcasses in Romania. *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development*, 17(3).

Gooneratne, A.D., Kirkwood, R.N., Thacker, P.A. (1988). Effects of injection of gonadotropin-releasing hormone on sow fertility. *Can. J. Anim. Sci*., 69, 123–129.

Hoha, G.V. (2009). *Study of the production performance of the Pig Improvement Company (P.I.C.) hybrid exploited in an intensive pig breeding system*. Thesis, U.S.A.M.V. Iaşi.

Knox, R.V. (2015). Recent advancements in the hormonal stimulation of ovulation in swine. *Vet Med (Auckl),* 6, 309–320.

Nacu, G. (2005). *Research on some possibilities for optimizing the reproductive function in pigs*. Thesis, U.S.A.M.V. Iaşi.

Păsărin, B. (1997). *Contributions to the establishment of the optimal age and body weight of pig breeders according to breed, degree of interbreeding and reaction to the Halotan test.* Thesis, U.S.A.M.V. Iaşi.

Pearodwong, P., Tretipskul, C., Soede, N.M., Tummaruk, P. (2019). Factors affecting estrus and ovulation time in weaned sows with induced ovulation by GnRH administration in different seasons. *J. Vet. Med. Sci.,* 81(11), 1567–1574.

Radu, C., 2015. *Contributions to the knowledge of the production technology and the quality of the bacon produced in Denmark*. Thesis, U.S.A.M.V. Iaşi.

Rotaru, I. (2018). Evolution of pigs' livestock and pork production in the world. *Scientific Papers. Series D. Animal Science,* LXI(2), 189–193.

Teiu, St. (2003). *Research on the influence of mixed - breed sow maintenance technology on breeding and production rates.* Thesis, U.S.A.M.V. Iaşi.

Ulguim, R.R., Fontana, D.L., Rampi, J.Z., Bernardi, M.L., Wentz, I., Bortolozzo, F.P. (2014). Use of porcine luteinizing hormone at oestrous onset in a protocol for fixed-time artificial insemination in gilts. *Reprod. Domest. Anim*., 49, 756–760.

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CONSIDERATIONS REGARDING THE MOUNTAINOUS AREA OF ROMANIA: PRESENT AND PERSPECTIVES IN RELATION TO THE BREEDING ACTIVITY OF CATTLE

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Abstract

The paper aimed to present the situation of the mountain area in Romania between 2017 and 2019. This paper is based on statistical data provided by the National Sanitary-Veterinary Authority and for Food Safety and also by the National Institute of Statistics. These were processed within the National Agency of the Mountain Area in the following indicators: number of counties with mountain area, number of Administrative-Territorial Units in the mountain area, number of inhabitants in the mountain area, total area of the mountain area (km^2), area of pastures and meadows in the mountain area (ha), the number of animals in the mountain area (cattle, sheep, goats, pigs). Compared with 2017, the number of animals, in 2019, registered significant decreases, which leads to negative effects on maintenance of permanent grassland areas in the mountain area, milk and meat production from animals, milk processing activities, respectively meat and, implicitly, reduced possibilities of developing farms in the mountain area.

Key words*: indicators, livestock, mountain area, permanent grassland, Romania.*

INTRODUCTION

The mountain is a very important source of air, water and food all over the world. Over 80% of the fresh water comes from the mountain area. In the world, mountains occupy 24% of the earth's surface, respectively 40 million km². In the mountains, 12% of the world population lives, and 14% live in the immediate vicinity of the mountains. Europe is covered by 40% of the mountains, inhabited by 20% of the total population of this old continent (Ray R., 1985). Mountain areas are, in general, rural and are characterized by natural limitations of agricultural productivity, which lead to reduced agricultural production, caused by adverse climatic and biophysical conditions, under optimal conditions of agricultural activities (Law no. 197/2018 - Mountain law). The mountain area of Romania represents a disadvantaged national territory, with high economic, social, and cultural potential, which needs a different approach regarding the policies and strategy of development and protection of these mountain areas (Maruşca T., 2018). The mountain area has extensive areas of meadows, most of them with a high natural value, maintained in this condition due to the fact that, over time, a traditional, extensive agriculture based on the use has been practiced natural fertilizers (http://www.madr.ro).

In this context, the paper proposes an analysis of the evolution of the main indicators defining the mountain area of Romania, during the years 2017-2019, in particular, the emphasis is placed on the evolution of permanent grassland surfaces (meadows and pastures) and, respectively, on the evolution of livestock in the mountain area.

MATERIALS AND METHODS

In order to characterize the situation of the mountain area in Romania, the following indicators were used: number of counties with mountain area, number of Administrative-Territorial Units in the mountain area, number of inhabitants in the mountain area, total area of the mountain area (km^2), area of pastures and meadows in the mountain area (ha), the livestock in the mountain area (cattle, sheep, goats, pigs) (Statistical Yearbook of Romania 2016-2019, http://www.insse.ro). The period analyzed was 2017-2019, and the data were provided by the National Sanitary-Veterinary and Food Safety Authority and also by the

National Institute of Statistics; these data were processed within the National Agency of the Mountain Area.

RESULTS AND DISCUSSIONS

The mountain area of Romania has a total area of 71,381.48 km^2, and covers 658 Territorial Administrative Units, respectively 27 counties with mountain area, which represents 30% of the territory of the country, of which 577 communes, 81 cities and municipalities and 3536 villages. The population of the mountain area is 3,354,041 inhabitants, which represents 16.5% of the country's population. The number of animals decreased in 2019 compared to 2017 (Figure 1 and Table 1).

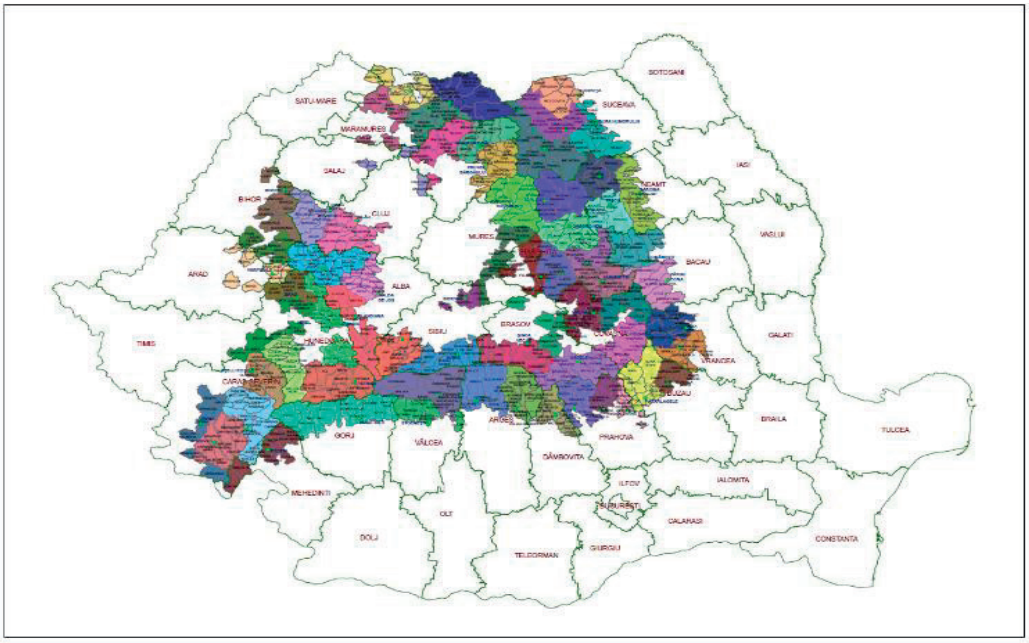

Figure 1. Representation of the mountain area in Romania with the 64 bioareas (mountain basins)

Table 1. Evolution of livestock and permanent grassland during the period 2017-2019 in the mountain area of Romania

Current number	Indicator name	2017	2018	2019
1.	Cattle (number)	653,069	634,882	614,303
2.	Sheep (number)	2,753,399	2,753,492	2,587,345
3.	Goats (number)	255,572	243,523	211,992
4.	Pigs (number)	260,054	198,848	254,509
5.	Area of pastures (ha)	1,232,415		
6.	Area of meadows (ha)	930,538		
7.	Agricultural land (ha)	2,738,428		
8.	Arable land (ha)	528,046		
9.	Orchards(ha)	43,789		
10.	Other (ha)	4,399,720		

Source: National Agency of the Mountain Area

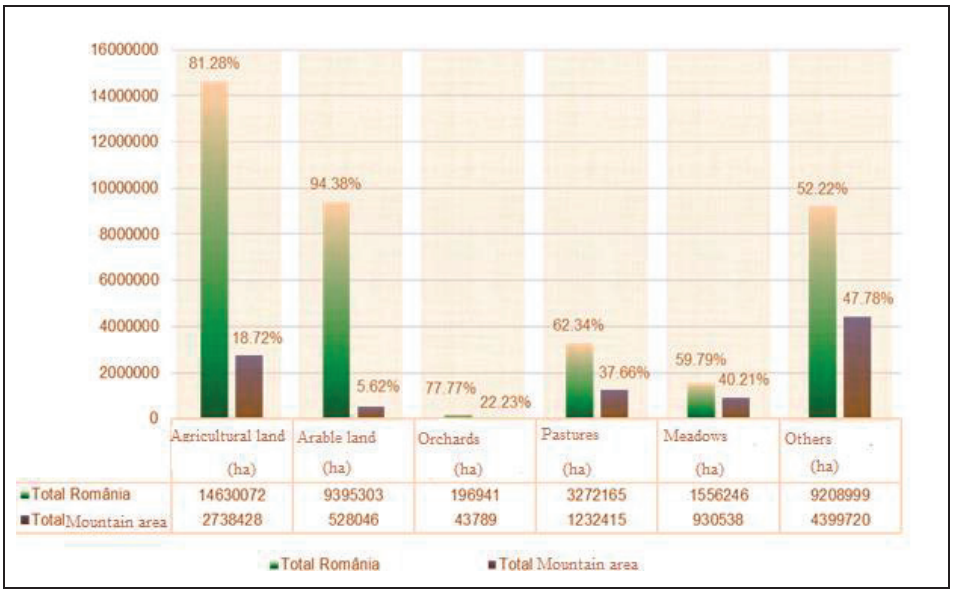

Figure 2. Use of agricultural land in the mountain area of Romania

The decrease of the livestock in the mountain area is based on several shortcomings, such as:
- the abandonment of the mountain localities by the young population and the migration to the urban areas of the country or abroad;
- lack of jobs;
- lack of counseling of young farmers who took over the farm from their parents;
- the lack of professional schools with agricultural profile in the mountain area, which will prepare the future generations of farmers;
- mountain farms are poorly equipped and mechanized, which leads to higher energy consumption, labor force and additional costs;
- reduced possibilities of capitalizing on the products obtained on the farm.

The surface of permanent grassland was maintained at the level of January 1, 2007, as provided in the first paragraph in art. 6 of Regulation (EC) no. 73/2009 of the Council of January 19, 2009. Local resources in the mountain area are represented by: forest resources, mineral deposits, mineral water, mountain tourism (ecotourism, agro-tourism, cultural tourism), pastoral heritage represented by:

a) permanent grassland meadows;
b) temporary or sown meadows in arable land;
c) clever arable streams, fruit tree plantations;
d) woody vegetation with shrubs and trees;
e) aquatic vegetation with hydrophilic plants.

According to the current statistical data, mountain agriculture is mainly practiced in small and very small farms (under 2 ha), the main activity being the raising of animals (cattle, sheep, goats) for milk and meat. The land fund (Figure 2) comprises about 7.3 million ha, of which, about 4.0 million ha forest fund and 2.09 million ha agricultural area, representing 15.44% of the total agricultural area of Romania (14, 6 thousand ha). The arable land in the mountain area occupies about 0.52 million ha, representing 5.62% of the total arable area of the country. The pastures located on the territory of the mountain area occupy approximately 1.2 million ha of the total area of the country, representing 37.66% of the total area of the pastures in the country. The meadows in the mountain area cover an area of approximately 0.9 million ha, representing 59.79% of the total area of the meadows in the country. Regarding permanent grasslands, it should be noted that they have several functions listed below.

1. Provides feed requirements for at least 60% of cattle and 80% of sheep.
2. The economic function, which refers to all the related activities that result from the use and capitalization of the pastures (processing of animal products, collection of medicinal flora,

beekeeping, energy biomass, etc.).
3. Pastures as a source of efficient and high quality animal production (milk, meat).
4. Pastures as a source of medicinal plants with phytotherapeutic properties.
5. Lawn as a source of biologically fixed nitrogen production (perennial or annual legume crops).
6. The grassland as a function of habitat for wild animals and for the conservation of the biodiversity of plant and animal species.
7. The ecological function of soil protection against erosion and conservation of natural space.
8. Landscape function, given by the diversity of plant species that ennobles and beautifies the environment.

Considering all these functions of the permanent grasslands, it must be said that their maintenance is vital for the mountain area; therefore, it is desired to manage them as efficiently as possible by tearing animals (sheep, goats) and administering the manure obtained from cattle within limits that do not negatively influence the grass carpet, the cleaning of cloves, mackerel, woody vegetation.

In the mountain area, a factor generating natural handicaps is the altitude at which the lands are located, which diminishes the possibilities of economic development in the case of certain relief steps, but this disadvantage can be compensated by the tourist potential, considering that these areas are holders of true values of natural and cultural heritage and, due to their isolation over time, have remained the keepers of the Romanian traditions.

The mountain area is recognized for its low pollution level, which gives food from this area an added value, already accepted on the market. The producers, growers and beekeepers who carry out their production activity in the mountain area will have to, but help in the activities of promoting food to the general public. The products from the mountain area, according to the specialists (Maciuc et al., 2003), are healthier and far superior to what is presently on the market, by the fact that, in this area, animal feed, pastures and meadows present a diverse range of valuable nutritious plants., including medicinal plants.

The main resource for the economic and social development of the mountain area is the product obtained "from the mountain". Mountain, traditional, ecological and quality products have a great capacity to contribute to the development of local communities, from an economic and social point of view, and to raise awareness of farmers and the general public about their economic potential and the importance of maintaining biodiversity (Maciuc, 2006). Traditional agricultural practices represent the starting point for the sustainable development of the mountain area.

In this sense, we propose some directions of action:

- improving the mountain legislation that will stimulate and support the population in the mountain area;
- promoting among the children the ancient teaching skills, crafts and old habits;
- equipping traditional households with modern equipment and machinery;
- support and concern for the development of agro-mountain education;
- providing consultancy and technical assistance for setting up professional organizations (associations, cooperatives, foundations);
- obtaining the mention of optional quality "mountain product" by a large number of farmers in the mountain area;
- rehabilitation of permanent grasslands degraded due to overgrowth, or delayed mowing of pastures;
- promoting ruminant species with economic interest, which does not compete with humans and especially cattle, with the two milk and meat productions in order to efficiently capitalize the pasture and increase the profitability of traditional households.

Cattle value and transform efficiently into milk and meat natural resources (pastures, meadows, agricultural by-products), as well as different residues (Maciuc et al., 2005), participate in the intensification and profitability of traditional households both through the productions we obtain, but also through the mountain product. It also represents an important source of convertible currencies (through the export of meat, meat and milk products, live animals, frozen semen (MSC) and frozen embryos).

The mention of optional quality "mountain product" is required for:
- to stimulate the development of the mountain area and to add value;
- avoids any deception on the consumer by misusing the word "mountain" and to remedy the market distortion caused by the sale of "mountain" products which are not in reality "mountain";
- increases the competitiveness of mountain agricultural systems.
The milk law project aims to regulate the marketing of dairy products, to increase consumer confidence in domestic dairy products and to eliminate falsified products (Coman et al., 2019).

CONCLUSIONS

Considering that the main agricultural activity in the mountain area is related to the breeding of animals, it can be observed that the number of animals (cattle, sheep, goats, pigs) decreased significantly during the period 2017-2019 with a negative impact on the agricultural economy, maintenance of permanent mountain pastures, milk and meat production from animals, milk processing activities, meat, biodiversity, environment, integrity of pastoral landscapes, population health and, implicitly, presents reduced opportunities for farm development in the mountain area.
As a consequence, we can say that, the mountain area of Romania needs special programs to support and encourage the breeding of animals, programs for installing young farmers in farms, to encourage the development of agricultural and non-agricultural activities in the mountain area.

REFERENCES

Apetrei, M., Surdu, I. (2017). *The mountain natural heritage, the foundation of the cultural diversity and the identity of the mountain communities,* "CE-MONT" of the National Institute of Economic Research "Costin C. Kiriţescu" - INCE, Romanian Academy.

Coman, I. T., Vidu, L., Marin, M. P., Ștefan, G., Mărginean, G. E. (2019). Proposals for legislative measures to improve the legal framework on the production and processing of milk, elimination of fake products from the milk market and growth of consumer trust. The milk law project. *Scientific Papers, Series D. Animal Science,* 62(1), 236-241.

Coman, I. T., Vidu, L., Trăistaru, C., Mărginean, G. E (2019). European legal framework in manufacturing and processing of milk. The milk package. elimination of milk quota. Case study: Germany. *Scientific Papers, Series D. Animal Science*, 62(2), 128-135.

Maciuc, V. (2006). *Cattle Breeding Management.* Iași, RO: Alfa Publishing House.

Maciuc, V., Nacu, G., Zaharia, V, Zota, D. (2003). *Animal Breeding*. Iași, RO: Alpha Publishing House.

Maciuc, V, Ujica, V, Nistor, I., Dascalu, C. (2005). *Management of dairy cow breeding*. Iași, RO: Alfa Publishing House.

Marușca, T. (2018). *Agrosilvopastoral Confessions*, Transilvania University Publishing House in Brașov

Marușca, T. (1995). Ecological gradient - the basis of sustainable development strategies in the mountain area. *VI National Ecology Conference*, Arad

Marușca, T. (2001). *Elements of gradient and mountain ecology,* Transilvania University of Brasov Ed.

Motcă, Gh., Oancea, I., Geamănu, L. (1994). *Romanian Meadows, Typology and Technology,* Agricultural Technical Publishing, Bucharest

Popa, D, Bozdog, C., Silvaș, E., Rusu, M. (2007). *The valorisation with the bullfights of the pastoral fund in the mountain and sub-mountain areas*, the Research-Development Institute for Cristian-Sibiu Montanology.

Rey, R. (1985). *The Mountain Civilization*, Bucharest, RO: Scientific and Encyclopedic Publishing House.

Șut-Gherman, M. (2006). *The effect of cattle breeding on the environment*, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca.

*** Law no.197/2018 - Law of the mountain area.

*** Law no.18/1991 - Land fund law.

***EU delegated regulation no.665/2014 to supplement Regulation (EU) no. 1151/2012 of the European Parliament and of the Council regarding the conditions of use of the optional mention of "mountain product".

*** Order no 52/2017 regarding the approval of the Procedure for verifying the conformity of the data contained in the specifications for granting the right to use the optional mention of "mountain product" quality and for verifying the compliance with the European and national legislation by the economic operators who have obtained the right to use the term.

***Regulation (EC) no. 73/2009 of the Council of 19 January 2009 establishing common rules for direct aid systems for farmers under the common agricultural policy and establishing certain aid schemes for farmers, amending Regulations (EC) no. 1290/2005, (EC) no. 247/2006, (EC) no. 378/2007 and repealing Regulation (EC) no. 1782/2003.

***Statistical Yearbook of Romania*, 2016-2018, National Institute of Statistics.

*** http://www.azm.gov.ro

*** http://www.insse.ro

*** http://www.madr.ro

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DYNAMIC OF BODY WEIGHT EVOLUTION OF CARPATHIAN KIDS GOAT AND KIDS BREED GOAT (♂SAANEN X ♀CARPATHIAN) FROM BIRTH TO WEANING

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Abstract

The application of modern goat breeding systems implies the addition of the classical methods used to improve the genetic potential with methods of appreciation of the hereditary base and their productive capacity. The purpose of this paper was to highlight the particularities of the evolution of the body weight from birth to the weaning of the kids goat Carpathian and kids goat breeds (Saanen male and Carpathian female), established according to three main factors: parturition type, the sex of the kid goat and the status physiology of the mother goats. The study was conducted in a farm in the Trascău mountains area, locality Rimetea, county of Alba, during January-June 2019, where we monitored daily: the body weight and sex of kids goat at birth, type of birth and the age of the parturients. The working method consisted of daily monitoring and weighing the kids goat studied with an electronic scale with an accuracy of: 5 g (0.5 g-10 kg); 10 g (10-50 kg). The obtained results have been statistically processed, being beneficial in making faster and more efficient decisions in the selection and improvement process.

Key words*: body weight, Carpathian goat, daily average gain, goat breeds (Saanen male and Carpathian female).*

INTRODUCTION

In Romanian agriculture goat breeding have a great economic importance, especially in mountainous and hilly areas where goats are raised, by improving their productivity through genetic selection and breeding (Răducuţă et al., 2007).

The Carpathian goat is a traditional breed of goats raised in most farms in Romania, being largely influenced by the climatic conditions of the breeding areas and represents 80% of the goat herd raised in Romania (Vlaic et al., 2016; Bacilă, 2014).

Analyzing the morphs-productive level of a nucleus of goats from the Carpathian breed, it was established that it is extremely heterogeneous, there being no selection procedure, which led to the decrease of number of animals on the farm (Dărăban, 2008).

The Carpathian goat is considered to be a breed with low performance, but nevertheless has a very good resistance and adaptability. The studies relive that performance of Carpathian goat have an modest production levels, with milk production from 220 to 350 kg/lactation, prolificacy of 130-160% and daily average gain for goats kids from 90 to 110 g/day (Pădeanu, 2001; Voia et al., 2010).

The Saanen breed is increased in purebred in largest farms, while in small farms it is increased for used to cross with the native Carpathian breed to improve milk production and the conformation of the udder (Kusza et al., 2018).

An improvement program is necessary from the goat populations in Romania in order to increase the productive parameters of goats (Cighi, 2007).

The Research and Development Institute for Sheep and Goat Breeding Palas has started a project to improve milk production in local goat populations by crossing with the best specialized breeds during the years 2006-2010 presented in Table 1 (ICDCOC Palas, 2010).

Table 1. The main morpho-productive indices for Carpathian breed and for cross-breed

	Weight at birth (kg/head)	*Weight at 28 days (kg/head)*	*Total average gain in first month (kg/head)*	*Daily average gain in first month (g/head)*	*Weight at 56 days (kg/head)*	*Total average gain in 28-56 days (kg/head)*	*Daily average gain in second month (g/head)*	*Total average gain at birth- to 56 days (kg/head)*	*Average gain to birth - to 56 days (g/head)*
Saanen x Carpathian	2.73	7.32	4.68	167	11.19	3.85	138	8.46	151
Carpathian	2.64	6.79	4.07	146	10.40	3.49	125	7.76	138
Difference ± between cross-breed and Carpathian goat									
Absolute values	+0.09	+0.53	+0.61	+21	+0.79	+0.36	+13	+0.7	13
%	+3.41	+7.81	+8.63	+14.38	+7.59	+10.3	+10.4	+9.0	+9.42

In the case of Saanen goats, their age and type of birth are significant on the weight birth kid goat ($P < 0.01$, $P < 0.05$). The type of birth and the sex of the product are significant for the weight at weaning and the growth rate of the kid goat ($P < 0.01$). The average birth weight recorded values of 3.06 kg, respectively 12.91 kg average weight at weaning (Duygu, 2010).

During lactation period (56 days) the cross-breed goats kids achieved an total average gain and an average daily gain higher by 9% and 9.42% compared to the Carpathian kids, respectively, proving a better conversion capacity of milk (ICDCOC Palas, 2010).

MATERIALS AND METHODS

The study was conducted in a farm, individual enterprise Cătălin Avram, in the Trascău mountains area, Rimetea, county of Alba, during January - June 2019, on Carpathian kids goat and cross-bred (Saanen male and Carpathian female) kids goat.

In order to assess the main characteristics regarding the morphological particularities of the kids goat, in relation to the type of birth and their sex, the body weights were determined by daily weighing, highlighting the weight at birth, at 28 days and at 56 days.

Measurements were made in the morning, every day, usually at the same time, using an electronic scale an accuracy of: 5 g (0.5 g-10 kg); 10 g (10-50 kg).

After birth, the goat kids were individualized: in the first 7 days of life with a Tyvek bracelet applied around the neck on which was inscribed the identification number of the mother and the type of birth (single, twin, triplet). After the first 7 days of life, the goat kid was individualized by a blind ear tag with the identification number of the mother goat. The growth evolution was estimated using the following growth indices:

- Growth energy (E);
- Growth rate: absolute (A) and relative(R);
- Growth intensity (I);
- Growth factor (F);

Growth energy represents the overall growth potential from birth to adult stage. The growth rate, absolute and relative, represents the average body mass accumulation recorded by the animal between two determinations (1) and (2).

The increase in body mass over a certain period of time (t) is growth intensity (3). The growth factor is the mass achieved in a given growing period (Mt) of the final animal mass (Mf) expressed as a percentage (4) (Dărăban, 2006).

$$A = \frac{M2-M1}{t} \quad (1);$$

$$R = \frac{M2-M1}{M1} * 100 \quad (2);$$

$$I = \frac{M2-M1}{M2+M1} * 2 * 100 \quad (3);$$

$$F = \frac{Mt*100}{Mf} \quad (4).$$

Where:

M_1 = body mass at t_1 (kg);
M_2 = body mass at t_2 (kg);
M_t =body mass accumulation in a period of time (kg);
M_f = final body mass (kg);
t = time period between t_1 and t_2 (days);

The obtained data were centralized and statistically processed with Excel program and GraphPad, T test and ANOVAs test was used to calculate the significance of the differences between the kids goat weigh.

RESULTS AND DISCUSSIONS

The analysis of the concentration of ruminal microflora in the fattening systems of small ruminants indicates differentiated values on the fattening phases, a large number of bacteria in the growth phase after which they register a decrease in the finishing phase, these being correlated with the accumulation of body mass (Mireşan et al., 2008).

The Carpathian goat analyzed in this study (N = 150) and the analyzed cross-breed goat (Saanen male and Carpathian female) (N = 70) were raised at the same farm, so they benefited from the same climatic conditions and the same type of food. From all parturients we identified simple birth, twin birth, triple birth (Figure 1).

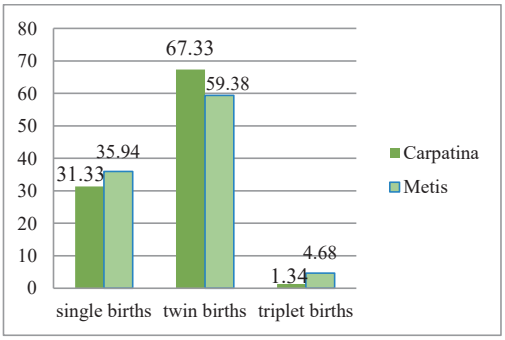

Figure 1. Number of single, twin, triplet births (%) for Carpathian goat and ♂Saanen x ♀Carpathian goat

Twin births are predominant for Carpathian goats, but also for cross-breeds goats. The literature reports in a study conducted on the Carpathian breed a percentage of 56.6% single births, 33.3% twin births, 6.6 triple births and 3.3% quadruple births (Răducuţă et al., 2007).

The high percentage of female products represents an advantage for the farm because the selection can be made much more rigorously, but also males obtained annually have an economic advantage for the farm because they accumulate body mass in relatively less time than females.

The number of female kids goat reported in this study for breed and cross-breed was higher than of males, predominantly for Carpathian breed than for cross-breed (Figure 2).

Figure 2. Percentage ratio of females (F) and males (M) for Carpathian and SaanenxCarpathian kids goat

Regarding the intensity of the growth kids goats, the same increasing trend is observed for the breeds and the cross-breeds, with similar values, having an average daily increase of 120.10 g for the Carpathian breed and 121.15 g for the ♂Saanen x ♀Carpathian cross-breeds in the 56 days (Figure 3).

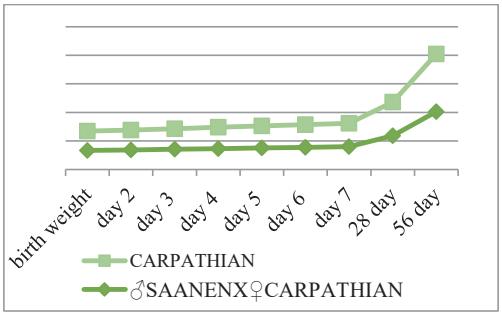

Figure 3. Daily average gain (0-56 days) for Carpathian and ♂Saanen x ♀Carpathian

During a lactation period (56 days) the cross-breed kids goat achieved a total weight gain and an average daily gain higher by 9% and 9.42% compared to the Carpathian kids goats, proving a better capacity to convert milk to body mass (ICDCOC Palas, 2010).

The birth weight of goats kids, the coefficient of variation, the minimum and maximum values and the significance of the statistical differences registered in 2019, for Carpathian breeds and cross-breeds Saanen x Carpathian are shown in the table below (Table 2).

Table 2. The average birth weight (kg), the coefficient of variation, the minimum and maximum values, confidence interval and the significance of the statistical differences recorded for Carpathian goat and ♂Saanen x ♀Carpathian goat according to the type of birth and sex of goat kids (kg)

	CARPATHIAN			♂SAANEN x ♀CARPATHIAN		
n	47	101	2	23	44	3
type birth	single	double	triple	single	double	triple
MIN	3.14	2.4	2.76	2.83	2.36	2.85
MAX	4.67	3.97	2.86	4.98	4.45	3.35
MEAN	3.936	3.138	2.81	3.715	3.188	3.037
SD	0.3687	0.3567	0.07071	0.5572	0.4717	0.273
CI 95%	3.828 - 4.044	3.068 - 3.209	2.175-3.445	3.474-3.956	3.045-3.332	2.358-3.715
V%	9.367%	11.37%	2.516%	15.00%	14.80%	8.990%
p value	<0.0001		****	0.0003		***
n	30		120	26		44
sex	M		F	M		F
MIN	2.89		2.4	2.43		2.36
MAX	4.67		4.35	4.98		3.98
MEAN	3.912		3.252	3.577		3.224
SD	0.5294		0.4245	0.6842		0.4127
CI 95%	3.715-4.110		3.175-3.328	3.3-3.853		3.098-3.349
V%	13.53%		13.05%	19.13%		12.80%
p value	<0.0001		****	0.0089		**

ns - p<0.5; *p>0,5;**p>0.1; ***p>0.01; M - male; F - female; V - coefficient of variation; CI - confidence interval; MEAN - average; SD - standard deviation.

Birth weight was influenced by the type of birth and the sex of the products, because was significant differences between the Carpathian breed and the Saanen and Carpathian cross-reed.

Birth weight recorded higher values for crossbreeds, with significant differences depending on the type of birth, a higher birth weight is recorded by kids goats resulting from single birth, then from twin birth and triple birth.

In addition to the factors studied, growth technology, maintenance level and microclimatic factors influence the growth and development of the kids goats from birth to weaning. The coefficient of variation recorded higher values for cross-breed goats than Carpathian goats.

The sex of kids goat significantly influenced the weight at birth, males birth weigh was more influnced than females, but also males obtained by cross-breeding had a higher birth weight than males obtained from the Carpathian breed.

The kids goats from simple birth have a better growth rate (Pascal et al., 2011).

The average value of weights, absolute growth rate (A), relative growth rate (R), growth intensity (I) and growth coefficient (F) in the first 28 days were analyzed because the maternal factor has a special importance in this period. The same parameters mentioned above were analyzed for a broader view of the results (Table 3).

Between day 28 and day 60, the influence of the maternal factor decreases even more compared to the first 28 days of life. Accumulation of body mass after the 28th day is obtained by consuming goat milk and additional feed (Călin et al., 2015).

Table 3. Variation of growth indices calculated according to type of birth, kids goat sex for Carpathian goat and ♂Saanen x ♀Carpathian goat to birth et 28 days and to birth at 56 days

	Trait		n	W (kg)	A (g)	R (%)	I (%)	F (%)
				X ± sX	X ± sX	X ± sX	X ± sX	X ± sX
				V%	V%	V%	V%	V%
CARPATHIAN	0-28 days		150					
	birth type	S	47	7.211±0.57	117±18.98	84.3±18.01	58.78±8.47	45.27±4.97
				7.97	16.23	21.37	14.41	10.99
		D	101	6.51±0.66	120±20.04	108.9±21.76	69.88±9.22	54.61±5.16
				10.21	16.64	19.98	13.19	9.99
		T	2	6.16±0.23	229.4±5.933	118.9±2.92	74.57+1.15	54.32±0.62
				3.79	4.97	2.45	1.54	1.13
	Level of segnificance			****	ns	****	****	****
	sex	M	30	7.32±0.67	121.5±19.56	89.11±21.17	60.95±9.74	46.51±5.64
				9.12	16.1	23.76	15.98	12.13
		F	120	6.58±0.647	118.8±19.63	104.4±23.14	67.84±10.04	50.45±5.68
				9.84	16.53	22.17	14.8	11.26
	Level of segnificance			****	**	ns	***	***
♂SAANEN X ♀CARPATHIAN	0-28 days		70					
	birth type	S	23	7.1±0.57	120.8±8.05	93.16±15.58	63.19±7.25	47.91±4.20
				7.99	6.66	16.72	11.48	8.77
		D	44	6.60±0.45	121.8±7.69	109.7±19.80	70.33±8.08	51.90±4.40
				6.88	6.31	18.05	11.49	8.48
		T	3	6.23±0.096	113.9±8.75	106±16.54	69.06±7.30	51.26±4.10
				1.55	7.68	15.59	10.58	7.99
	Level of segnificance			***	ns	**	**	**
	sex	M	26	6.91±0.69	119.1±7.08	96.89±21.02	64.65±9.10	48.69±5.12
				10.09	5.94	21.7	14.08	10.52
		F	44	6.65±0.41	122.3±8.19	108.3±17.84	69.86±7.36	51.67±4.02
				6.19	6.69	16.46	10.54	7.79
	Level of segnificance			ns	ns	*	*	**
CARPATHIAN	0-56 days		150					
	birth type	S	47	10.53±0.98	117.8±16.47	169.3±29.21	91.05±8.39	62.45±3.94
				9.28	13.98	17.25	9.22	6.32
		D	101	9.91±1.05	120.9±17.82	218.9±41.22	103.6±9.36	68.13±4.098
				10.63	14.74	18.83	9.03	6.02
		T	2	10.11±0.46	130.3±9.47	259.9±25.41	112.9±4.82	72.15±1.97
				4.55	7.27	9.78	4.27	2.73
	Level of segnificance			**	ns	****	****	****
	sex	M	30	10.82±1.08	123.3±18.21	180.4±39.3	93.81±10.43	63.69±4.79
				10.02	14.77	21.79	11.11	7.53
		F	120	9.93±0.98	119.3±17.11	209.8±43.91	101.3±10.42	67.08±4.64
				9.89	14.35	20.93	10.29	6.91
	Level of segnificance			****	ns	**	***	***

	Trait		n	W (kg)	A (g)	R (%)	I (%)	F (%)
				X ± sX	X ± sX	X ± sX	X ± sX	X ± sX
				V%	V%	V%	V%	V%
♂SAANEN X ♀CARPATHIAN	0-56 days		70					
	birth type	S	23	10.48±0.66	241.7±16.11	186.4±31.12	95.83±8.39	64.68±3.86
				6.31	6.67	16.7	8.76	5.97
		D	44	10.01±0.53	243.6±15.35	219.4±39.57	103.8±8.79	68.23±3.81
				5.3	6.3	18.04	8.48	5.59
		T	3	9.41±0.26	227.7±17.74	212.1±33.25	102.5±8.25	67.70±3.663
				2.78	7.79	15.68	8.05	5.41
	Level of segnificance			***	ns	**	**	**
	sex	M	26	10.25±0.77	238.3±14.19	193.8±42.02	97.38±10.24	65.34±4.59
				7.48	5.96	21.68	10.51	7.03
		F	44	10.08±0.52	244.7±16.34	216.7±35.63	103.3±8.04	68.05±3.49
				5.19	6.68	16.44	7.78	5.13
	Level of segnificance			ns	ns	*	**	**

ns - p<0.5; *p>0.5;**p>0.1; ***p>0.01; X - average; sx - standard deviation; V - coefficient of variation; S - single; D - twin; T - triple; M - male; F - female; W - body weight; A - absolute growth rate; R - relative growth rate; I - growth intensity; F - growth factor.

The values obtained are comparable to those presented by the Palas Sheep and Goat Breeding Research and Development Institute, with the mention that the goat studied in this paper had significantly higher values at birth weight. Goat kids from a single birth accumulated significantly higher body mass than goats kids from multiple birth. The kids goat male have a significantly higher body mass than kids goat females; Carpathian kids had superior results then cross-breed kids.

CONCLUSIONS

The improvement of current breeding and exploitation technologies have as a starting point the adaptive reaction of animals to the environmental conditions in which they live. Whatever the growing system are, a valuable biological material adds value to the farm, through a rigorous selection and improvement of genetic material.

The study of Carpathian kids goat and cross-breed kids goat provides us an informational basis for progress, because some of the studies showed the heterogeneity of the Carpathian breed.

Breed, type of birth, sex of kids goat, maintenance and feeding conditions have an important impact regarding the birth weight and weaning weight of the kid goat.

The data obtained are characteristic of the breed and cross-breed, resulting are compatible with those presented in the literature.

REFERENCES

Bacila, V. (2014). *Technical Informative Bulletin*, No.1, Ministry of Agriculture and Sustainable Development – National Agency for Breeding and Reproduction in Animal Husbandry "Prof.dr. G.K. Constantinescu" (ANARZ), Bucureşti.

Călin, I., Răducuţă, I., Dărăban, S., Vlad, I., Priseceanu, H., Pascal, C., Padeanu I. (2015). Research On Quantitative Skills in Meat Production Direction at Youth Goats from Carpathian Breed in Relation with the Rearing System. *Agriculture and Agricultural Science Procedia,* 6, 191–196.

Cighi, V. (2007). Study on the main body conformation traits of a Carpathian goat population. *Bulletin USAMV- CN*, 63–64.

Dărăban, S. (2006). *Sheep breeding technology.* Cluj-Napoca, RO: Risoprint Publishing House, pp. 70-75.

Dărăban, S. (2008). The morpho - productive characteristics of a Carpathian breed goat nucleus, bred in Toplita locality, Harghita county. *Bulletin UASVM Animal Science and Biotechnologies*, 65, 1–2.

Duygu, I. (2010). Reproduction performance of Saanen goats raised under extensive conditions. *African Journal of Biotechnology,* 9(48), 8253–8256.

I.C.D.C.O.C. Palas., (2008). Improving milk production in goat populations by crossing with the best specialized breeds. *Research report, Sector Stage Project V*, Constanţa.

Kusza, S., Toma, L., Ilie, D., Sauer, M., Padeanu, I., Gavojdian D. (2018). Kompetitive allele specific

PCR (KASP™) genotyping of 48 polymorphisms at different caprine loci in French Alpine and Saanen goat breeds and their association with milk composition, *PeerJ,*. 6, e4416.

Mireşan, V., Răducu, C., Dărăban S. (2008). Research concerning the ruminal microflora in intensive fattening young sheep. *Bulletin UASVM Animal Science and Biotechnologies*, 65(1-2).

Pascal, C., Pădeanu, I., Călin, I., Daraban, St., Nacu, G. (2011). Researches related to meat yield aptitudes of Carpatina breed reared in Romania - *Lucrări Ştiinţifice, Seria Zootehnie*, 55, 328–331.

Padeanu, I. (2001) Sheep and goat breeding technology. Timisoara, RO: Mirton Publishing House, pp. 373–375.

Răducuţă, I., Marmandiu, A., Stanciu, M., Cristian, C. (2007). The evolution of livestock, milk and goat cheese production in the European countries. *Acta Universitatis Cibiniensis, Agricultural Sciences*, 1 (7), 127–134.

Răducuţă, I., Marmandiu, A., Cristian, C., Grigoraş, G., Iftimie N. (2007). Research regarding the morpho-productive parameters to the Carpathine goat breed from Slobozia-Ialomiţa area. *Acta Universitatis Cibiniensis, Agricultural Sciences*, 1(7), 121–126.

Vlaic, A., Odagiu, A., Vlaic, B., Dărăban,S., Coşier, V.,(2016) Biometric Approach of Carpatina Goats Reared in South Romania Climate, *ProEnvironment,* 9, 192–196.

Voia, S., Padeanu, I., Daraban, S., Mot, T., Dronca, D., Pet, I., Gavojdian, D., Ivan, M. (2010). Study Regarding Goat Milk Composition and the Growth Rate in Kids of Carpatina Goat Breed. *Animal Science and Biotechnologies,* 43(2), 324–327.

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PRELIMINARY STUDY REGARDING THE ENVIRONMENTAL AND GENETIC FACTORS AFFECTING DAIRY CALVES HEALTH

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Abstract

In order to study the influence of calves sex, dams age and year of calving, it is very important to evaluate at farm level, the incidences of colibacillosis, coccidiosis, rickets, neonatal enteritis, respiratory diseases (BRD) and haemorrhagic enteritis in un-weaned dairy calves. The study was carried out at the dairy cattle farm of the Research and Development Institute for Bovine Balotesti, Romania between January 2018 and December 2019, with health datasets from 176 Romanian Black Spotted calves. Colibacillosis and coccidiosis incidences were not influenced ($p>0.05$) by the sex of the calves, while rickets incidence was significantly influenced ($p\leq0.001$), with 2.20 ± 1.55% of the male calves being affected and 10.59 ± 3.36% of the females, respectively. Year of birth had significantly influenced ($p\leq0.05$) the incidence of colibacillosis and rickets and had no effects ($p>0.05$) on coccidiosis incidence. Neonatal enteritis, BRD and haemorrhagic enteritis incidences were not influenced ($p>0.05$) by factors such as calves sex, dams age and year of birth. Colibacillosis and rickets incidences were the only health problems influenced by factors such as calves sex and year of birth.

Key words*: animal health, calves welfare, dairy cattle, environmental factors.*

INTRODUCTION

The first 21 days of life in calves are very important because of the sensitivity and the higher risks of mortality (Gulliksen et al., 2009). In addition to these aspects, long-term consequences of diseases as well as the high degree of morbidity in unweaned calves determine a reducing of the genetic potential as adults (Swali and Wathes, 2006; Urie et al., 2018). The breeding environment and infancy period among domestic animals play an essential role for future harmonious development (Bornstein, 1989).

The most common causes for health disorders among young calves are of infectious nature such as diarrhea and bovine respiratory diseases (BRD) (Perez et al., 1990; Olsson et al., 1993; Sivula et al., 1996; Virtala et al., 1996a).

The performance of the animal at maturity is affected by the presence of diarrhea and / or BRD in the first three months of life and is associated with sub-fertility (Warnick et al., 1994; Svensson et al., 2003).

In general, animals have a native ability to adapt to the climatic influences, however, extreme climatic situations are difficult to overcome by immunosuppressed calves. Direct economic consequences were reported due to the inability of thermoregulation in young mammals, given the brown adipose tissue fast metabolization during severe cold thermal stress, a major negative impact on their welfare (Silanikove, 2000; Snowder et al., 2006; Roland et al., 2016).

Due to a high incidence of diseases in dairy calves, a series of economic and production negative repercussions have been described (Rossini et al., 2004; Stanton et al., 2012). For instance, the average costs owed to dairy calves respiratory and gastrointestinal treatments in North America are on average 33.46 $/calf and 14.71 $/calf, respectively (Kaneene and Hurd, 1990). The array of variation in mortality risks for calves younger than 12 months varies between 2.1% and 14%, under the influence of

year of birth and breed (Gulliksen et al., 2009). Other factors such as farm geography, farm size, colostrum management, housing and feeding strategy were found to have a significant influence as well (Waltner-Toews et al., 1986a; Lundborg et al., 2005; Gulliksen et al., 2009; Windeyer et al., 2014).

Another common disease of unweaned dairy calves is represented by rickets, which is a disorder of bone epiphyses growth, with the main cause being a deficient supply of vitamin D, phosphorus and especially the lack of calcium in the body (Pugh and Baird, 2012). The skin and digestive tract are involved in the synthesis and absorption of vitamin D, which influencing of calcium and phosphorus in the small intestine (Holick et al., 2006).

Exposure of animals to medium-wave ultraviolet (UVB) solar radiation at wavelengths between 290 and 320 nm generates the production of vitamin D in the skin (Nelson et al., 2012). Thus, the main natural method by which the animal's body can produce vitamin D, is the direct exposure to sun. The factors that influence the exposure to UVB solar radiation are season, skin pigmentation and also geographical location, with little up-to-date research focus on such aspects in cattle (Pickworth et al., 2012; Hymoller et al., 2012; Casas et al., 2015).

The aim of our study was to evaluate at farm level the incidences of colibacillosis, coccidiosis, rickets, neonatal enteritis, respiratory diseases (BRD) and haemorrhagic enteritis in un-weaned dairy calves, in order to assess the influence of factors such as calves sex, dams age and year of calving.

MATERIALS AND METHODS

Animals and general management

The study was carried out at the Research and Development Institute for Bovine Balotesti (44°36′46″N 26°4′43″E) Romania, were health data was collected for two consecutive years, between November 2017 and October 2019, from a number of 176 purebred Romanian Black and White (Holstein Friesian group) calves, managed under identical conditions (91 males and 85 females, 89 born in the 1th year of study and 72 in the 2nd year, respectively).

After birth, the calf was separated from the dam and housed in the maternity compartment until the age of 10 days. In the first 3 days of life, calves are fed with colostrum minimum 4 kg of colostrum per day, in two meals at 12 hours intervals. The following 7 days they receive two meals per day of 3 kg per head. After 10 days of age, the calves were moved to outdoor individual hutches with straw bedding. Where they were fed with milk replacement, 6 kg/day in two meals. The calves diet was supplemented with *ad libitum* solid diet of starter concentrates and alfalfa hay until the age of 3 months, when the waning took place, regardless of sex. The concentrates feed contained 18.5% crude protein, 9% fibre, 0.36% methionine, 0.9% lysine, 2.96% calcium, 0.69% phosphorus, 0.9% salt and 1.00% fats. With nonrestricted access to clean water.

Dehorning was carried out at the age of two months, only on female calves because they remain on the farm for replacement, while the male calves were sold for further fattening soon after weaning.

Veterinary care

As veterinary prevention, anthrax vaccination was used at the age of two months and vitamin therapy was applied only to the sensitive and sick calves. The main treatments were applied for symptomatic effects such as diarrhoea and lung diseases, these being the most common diseases in the studied calves. Neonatal enteritis causes diarrhoea and associated fluid and electrolyte losses. Thus, fluid therapy was an important part in enteritis management. The deworming procedures were made after the age of weaning and only if needed earlier. The research activities were performed in accordance with the European Union's Directive for animal experimentation (Directive 2010/63/EU).

Statistical analyses

In order to assess the effect of the age group on the above-mentioned health traits, the MiniTab®18 software was used, with the statistical significance level set at values of $p \leq 0.05$.

RESULTS AND DISCUSSIONS

Results concerning colibacillosis, coccidiosis and rickets incidence in 0-3 months of age calves, based on calves sex, dams age and year of study are shown in Table 1. Our data showed that colibacillosis and coccidiosis incidences were not influenced ($p>0.05$) by the sex of the calves, while rickets incidence was significantly influenced ($p\leq0.001$), with 2.20 ± 1.55% of the male calves and 10.59 ± 3.36% of the females being affected, respectively. No supporting previously published data for comparison was available, concerning the influence of sex related factors on rickets incidence. This aspect, regarding the higher susceptibility of female calves to rickets is of outmost importance, given that in the dairy cattle industry only females are being kept for replacement reasons, males being most often sent for fattening or slaughter at an early age. Moreover, rickets impairs growth and future development of calves and negatively affects the immune functions (Adams et al., 2010; Nelson et al., 2012).

Dams age had no influence ($p>0.05$) on colibacillosis, coccidiosis and rickets incidences in un-weaned calves.

Year of birth significantly influenced ($p\leq0.05$) the incidence of colibacillosis with 14.71 ± 0.43% of the calves being affected in the first year of study and 5.38 ± 2.35% in the second year of study. Colibacillosis prevalence in commercial farms is strongly influenced by the bedding and fed hygiene, and also by heat and humidity conditions. These differences were observed in other commercial farms (Dubey and Rao, 1997; Tikoo et al., 2009; Shekhar et al., 2017). Coccidiosis incidence, however, was not influenced ($p>0.05$) by this factor.

Results relating to neonatal enteritis, respiratory diseases (BRD) and haemorrhagic enteritis incidences in 0-3 months of age calves, based on calves sex, dams age and year of study are shown in Table 2.

Neonatal enteritis, BRD and haemorrhagic enteritis incidences were not influenced ($p>0.05$) by factors such as calves sex, dams age and year of birth.

However, a sex related sensitivity could be observed for BRD, with an average incidence of 5.49 ± 0.24% in male calves and 2.35 ± 1.65% in females, respectively. This might be attributed to the higher growth rates of male calves, compared to females (Phyllis and Moss, 1986), with in return might lead to a prioritization of the nutrients towards the growth process, rather than allocating nutrients to support the immune system.

According to previous studies, total morbidity incidence in calves up to 3 months old was of 35% (Waltner-Toews et al., 1986b) and the highest risk was identified for neonatal diarrhoeas and bovine respiratory disease (BRD), with 29% and 39%, respectively (Van Donkersgoed et al., 1993; Donovan et al., 1998a; Windeyer et al., 2014). Conversely, current results shown the incidence for BRD to be significantly lower, of 3.98 ± 1.48%, while the incidence neonatal diarrhoea was similar as previously reported, of 29.55 ± 0.34%. Differences in BRD incidences could be attributed to different geographical and climatic conditions in which the calves were raised, previously published data concerned dairy calves raised in North America, especially in Canada, where severity of cold weather is much more prominent compared to temperate European climate.

Windeyer et al. (2014) found that BRD has a minimal impact on body weight loss in calves, while neonatal diarrhoea causes weight losses of 1.10 kg. In the same study, it was reported that BRD prevention strategies for un-weaned calves is much more complex and does not just refer to the general management of colostrum feeding, which is the first essential step, vaccination programs and antimicrobial administration represent major tools for the disease prevalence to be reduced. Calves with passive immunity transfer deficiencies (FTPI) had 1.6 times higher likelihood to develop BRD (Windeyer et al., 2017).

Our hypothesis, that age of the dam could influence calves health was not supported by current results. Although, in the literature there are mentions that multiparous cows produce a higher concentration of immunoglobulins in the colostrum and also the incidence of dystocia is much lower, compared to first calving cows, such aspects did not seem to have an influence on the overall health status of the resulting calves (Neamt et al., 2017).

In an extensive study Urie et al. (2018) reported a 5.0% mortality rate, also describing death causes, which were 32.0% of digestive related problems, 14.1% of respiratory nature, 7.0% a combined digestive and respiratory causes, while 13.3% of deaths were caused by infectious agents, various injuries or unknown causes. Given the relative low number of calves included in our study, for the future we plan to include more farms and a higher number of calves, following mortality losses and mortality causes.

The total morbidity incidence for calves studied was of 88.54%. None of the diseases has lead to mortality of calves included in our study. Conversely, other authors report a mortality rate in dairy calves between 5-6% (Seppa-Lassila et al., 2016) and 7.8% (Santman-Berends et al., 2019).

Table 1. Mean (± SEM) for colibacillosis, coccidiosis and rickets incidence in 0-3 months of age calves, based on calves sex, dams age and year of study

Factors/Disease	Colibacillosis (%)	Coccidiosis (%)	Rickets (%)
Cohort	8.52±2.11	39.20±0.36	6.25±1.83
Male calves	7.69±2.81	41.76±0.52	2.20±1.55
Female calves	9.41±3.19	36.47±0.52	10.59±3.36
Differences males vs. females (p value)	NS (0.686)	NS (0.475)	***(0.000)
Primiparous dams	11.36±4.84	43.18±0.75	4.55±3.18
Multiparous dams	8.42±2.86	45.26±0.51	7.37±2.69
Differences for dams age	NS (0.583)	NS (0.821)	NS (0.534)
1th year of study	14.71±0.43	36.76±0.58	11.76±0.39
2nd year of study	5.38±2.35	45.16±0.51	3.23±1.84
Differences year 1 vs. year 2	*(0.045)	NS (0.288)	*(0.034)

Table 2. Mean (± SEM) for neonatal enteritis, respiratory diseases and haemorrhagic enteritis incidence in 0-3 months of age calves, based on calves sex, dams age and year of study

Factors/Disease	Neonatal enteritis (%)	Respiratory diseases (%)	Haemorrhagic enteritis (%)
Cohort	29.55±0.34	3.98±1.48	1.13±8.01
Male calves	30.77±0.48	5.49±0.24	1.10±11.00
Female calves	28.24±0.49	2.35±1.65	1.18±11.80
Differences males vs. females (p value)	*NS (0.714)*	*NS (0.289)*	*NS (0.967)*
Primiparous dams	3.63±0.73	6.82±3.84	2.27±2.27
Multiparous dams	3.15±0.47	4.21±2.07	1.05±1.05
Differences for dams age (p value)	*NS (0.580)*	*NS (0.518)*	*NS (0.574)*
1th year of study	32.35±0.57	2.94±2.06	2.94±2.06
2nd year of study	29.03±0.47	5.38±2.35	n.a.
Differences year 1 vs. year 2	*NS (0.653)*	*NS (0.458)*	*n.a.*

CONCLUSIONS

Understanding the factors associated with calves morbidity is an essential part for improving calf health, welfare and performance.

We found that female calves have an overall susceptibility to rickets, when compared to male counterparts, which could translate in adapting future diets based on calves sex, e.g. diets with higher macroelements and vitamin D content, in order to promote better growth rates and health.

Coccidiosis and neonatal enteritis were the most prevalent health disorders found in our study, affecting altogether over 60% of the investigated calves.

Our initial hypothesis that dams age constitutes an influencing factor for overall calves health was not supported by the results. For our future studies, we plan to involve factors such as colostrum quality and calving year, in order to

better evaluate and differentiate between the innate and acquired organic resistance of dairy calves.

REFERENCES

Adams, J. S., Hewison, M. (2010). Update in vitamin D. *Journal of Clinical Endocrinology and Metabolism*, 95(2), 471–478.

Bornstein, R. F. (1989). Exposure and affect: Overview and meta-analysis of research, 1968–1987. *Psychological Bull*etin, 106, 265–289.

Casas, E., Lippolis, J. D., Kuehn, L. A, Reinhardt, T. A. (2015). Seasonal variation in vitamin D status of beef cattle reared in the central United States. *Domestic Animal Endocrinology*, 52, 71–74.

Centers for Epidemiology and Animal Health USDA (1994). Dairy heifer morbidity, mortality and health management focusing on preweaned heifers, Retrieved January 10, 2020, from https://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/ndhep/NDHEP_dr_Report.pdf

Donovan, G. A., Dohoo, I. R., Montgomery, D. M. (1998a). Association between passive immunity and morbidity and mortality in dairy heifers in Florida, USA. *Preventive Veterinary Medicine,* 34, 31–46.

Dubey, R. D., Rao, K. N. P. (1997). Some observations on neonatal diarrhoea in buffaloes. *Indian Veterinary Journal*, 74, 677–679.

Gavrila, M.I., Marginean, G.E., Vidu, L. (2015). Study on the interrelation between animal welfare and production in dairy cattle. *Scientific Papers: Series D, Animal Science,* LVIII, 278–283.

Gulliksen, S., Lie, K., Loken, T., Osteras, O. (2009). Calf mortality in Norwegian dairy herds. *J.ournal of Dairy Science*, 92, 2782–2795.

Holick, M. F. (2006). Resurrection of vitamin D deficiency and rickets. *Journal of Clinical Investigation*, 116(8), 2062–207.

Hymoller, L., Jensen, S. K. (2012). 25-Hydroxycholecalciferol status in plasma is linearly correlated to daily summer pasture time in cattle at 56 degrees. *British Journal of Nutrition*, 108, 666–71.

Kaneene, J. B., Hurd, H. S. (1990). The National Animal Health Monitoring System in Michigan III. Cost estimates of selected dairy cattle diseases. *Preventive Veterinary Medicine,* 8, 127–140.

Lundborg, G. K., Svensson, E. C., Oltenacu, P. A. (2005). Herd-level risk factorsfor infectious diseases in Swedish dairy calves aged 0–90 days. *Preventive Veterinary Medicine,* 68, 123–143.

Neamt, R. I., Gavojdian, D., Neciu, F. C., Cziszter, L. T., Ilie, D. E. (2017). Effects of some factors on calves viability and growth. *Proceedings of the 7th International Conference on the Assessment of Animal Welfare at the Farm and Group Level, 5*3.

Nelson, C. D., Reinhardt, T. A., Lippolis, J. D., Sacco, R. E., Nonnecke, B. J. (2012). Vitamin D signaling in the bovine immune system: a model for understanding human vitamin D requirements. *Nutrients,* 4, 181–96.

Olsson, S. O., Viring, S., Emanuelson, U., Jacobsson, S.O. (1993). Calf diseases and mortality in Swedish dairy herds. *Acta Veterinaria Scandinavica*, 34, 263–269.

Perez, E., Noordhuizen, J. P. T. M., Van Wuijkhuise, L. A., Stassen, E. N. (1990). Management factors related to calf morbidity and mortality rates. *Livestock Production Science,* 25, 79–93.

Phyllis, C. Lee, Moss, C. J. (1986). Early maternal investment in male and female African elephant calves. *Behavioral Ecology and Sociobiology,* 18, 353–361.

Pickworth, C. L, Loerch, S. C., Fluharty, F. L. (2012). Restriction of vitamin A and D in beef cattle finishing diets on feedlot performance and adipose accretion. *Journal of Animal Science*, 90, 1866–78.

Pugh, D. G, Baird, A. N. (2012). Diseases of the neurologic system. 2nd ed. *Sheep and goat medicine*. Elsevier. St. Louis, Mo.

Roland, L., Drillich, M., Klein-Jöbst, D., Iwersen, M. (2016). *Invited review:* Influence of climatic conditions on the development, performance and health of calves. *Journal dairy science*, 99, 2438–2452.

Rossini, K. L., McGillard, M., James, R., Pearson, R., Swecker, W. (2004). Effects of calfhood respiratory and digestive disease on calfhood morbidity and first lactation production and surivival rates. *Virginia Polytechnic Institute and State University, Blacksburg, Virginia (Unpublished master's thesis).*

Santman-Berends, I. M. G. A., Schukken, Y. H., Van Schaik, G. (2019). Quantifying calf mortality on dairy farms: Challenges and solutions. *Journal of Dairy Science,* 102, 6404–6417.

Seppa-Lassila, L., Sarjokari K, Hovinen, M., T. Soveri, T., Norring M. (2016). Management factors associated with mortality of dairy calves in Finland: A cross sectional study. *The Veterinary Journal*, 216, 164–167.

Shekhar, S., Ranjan, R., Singh, C. V., Kumar P. (2017). Prevalence, Clinicohaemato-Biochemical Alterations in Colibacillosis in Neonatal Calves. *International Journal of Current Microbiology and Applied Sciences,* 6(9), 3192–3198.

Silanikove, N. (2000). Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livestock Production Science,* 67, 1–18.

Snowder, G. D., Van Vleck, L. D., Cundiff, L. V., Bennett, G. L. (2006). Bovine respiratory disease in feedlot cattle: Environmental, genetic, and economic factors. *Journal of Animal Science,* 84, 1999–2008.

Stanton, A. L., Kelton, D. F., LeBlanc, S. J., Wormuth, J., Leslie, K. E. (2012). The effect of respiratory disease and a preventative antibiotic treatment on growth, survival, age at first calving, and milk production of dairy heifers. *Journal of Dairy Science*, 95, 4950–4960.

Svensson, C., Lundborg, K., Ulf Emanuelson, U., Olsson, S. O. (2003). Morbidity in Swedish dairy calves from birth to 90 days of age and individual calf-level risk factors for infectious diseases. *Preventive Veterinary Medicine*, 58, 179–197.

Swali, A., Wathes, D. C. (2006). Influence of the dam and sire on size at birth and subsequent growth, milk production and fertility in dairy heifers. *Theriogenology,* 66, 1173–1184.

Tikoo, A., Singh, R., Agrawal, R. (2009). Occurrence of cases of calf diarrhoea in Jammu. Indian. *Journal of Veterinary Medicine,* 29(1), 53-45.

Urie, N. J. (2018). Preweaned heifer management on US dairy operations: Part V. Factors associated with morbidity and mortality in preweaned dairy heifer calves. *Journal of Dairy Science,* 101, 1–16.

Van Donkersgoed, J., Ribble, C. S., Boyer, L. G., Townsend, H. G. (1993). Epidemiological study of enzootic pneumonia in dairy calves in Saskatchewan. *Canadian Journal of Veterinary Research*, 57, 247–254.

Virtala, A. M., Mechor, G. D., Gröhn, Y. T., Erb, H. N. (1996a). Morbidity from nonrespiratory diseases and mortality in dairy heifers during the first three months of life. *Journal of the American Veterinary Medical Association,* 208, 2043–2046.

Waltner-Toews, D., Martin S. W., Meek, A. H. (1986a). Dairy calf management, morbidity and mortality in Ontario Holstein herds. II. Age and seasonal patterns. *Preventive Veterinary Medicine*, 4, 125–135.

Waltner-Toews, D., Martin, S. W., Meek, A. H. (1986b). Dairy calf man-agement, morbidity and mortality in Ontario Holstein herds. IV: association of management with mortality. *Preventive Veterinary Medicine, 4*, 159–171.

Windeyer, M. C., Lesliea, K. E., Godden, S. M., Hodginsc, D. C., Lissemorea, K. D., LeBlanc, S. J. (2014). Factors associated with morbidity, mortality, and growth ofdairy heifer calves up to 3 months of age. *Preventive Veterinary Medicine*, 113, 231–240.

Windeyer, M. C., Timsit, E., Barkema, H. (2017). Bovine respiratory disease in pre-weaned dairy calves: Are current preventative strategies good enough? *The Veterinary Journal*, 224, 16–17.

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RESEARCH ON GROWTH INDICATORS IN ABERDEEN ANGUS YOUTH CATTLE, ACCORDING TO DIFFERENT INFLUENCING FACTORS

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Abstract

The official control of the performance for beef production on farms is the starting point in organization of genetic improvement of the Aberdeen Angus breed in Romania. The weighing with metrological approved equipment for young cattle at seven months, ten months and twelve months are to calculate different performance. The objectives of control of the performance for beef production department are to widen the selection base, to improve the management of farms, to apply the improvement breeding program of Aberdeen Angus cattle in Romania and to send all this information to herd book department to calculate genetic performance. All this activity gives the opportunity to research and follow the evolution of youth cattle in different growth influencing factors.

Key words*: Aberdeen Angus, growth factors, youth.*

INTRODUCTION

The official performance control for meat production on the farm is the starting point in organizing the genetic improvement of the Aberdeen Angus breed in Romania. One of the goals are the extension of the genetic selection base, estimation of breeding values and application of breeding program with the help of calculated performances for cattle registered in the herd book of Aberdeen Angus breed. Improving the management of the farms by using the technical reports obtained following the official performance control for the meat production, the development of the infrastructures and the breeding conditions of the cattle (Figure 1). The regional territorial organization of the official performance control for the meat production in the Aberdeen Angus breed calculate the performance of the youth cattle at different ages.

The registration in the official performance control for the meat production is given by the acceptance of the breeder with their cattle in the breeding improvement program of the Aberdeen Angus breed in Romania. The official performance control for meat production in the breeding farms from birth to weaning for Aberdeen Angus breed applies to cattle according the age category for seven months (G200) which is minimum age from 90 days to maximum 250 days. The official performance control for meat production in the finishing farms of Aberdeen Angus cattle in Romania are two categories like: ten months (G300) minimum age 251 days to maximum 319 days. Last one is the weight at twelve months (G365) minimum age 320 days to maximum 410 days. All this weight controls is performed according to the international regulations established by the ICAR - International Committee for Animal Recording (Grosu and Gociman, 2018).

Figure 1. O.P.C. weighing on the pasture

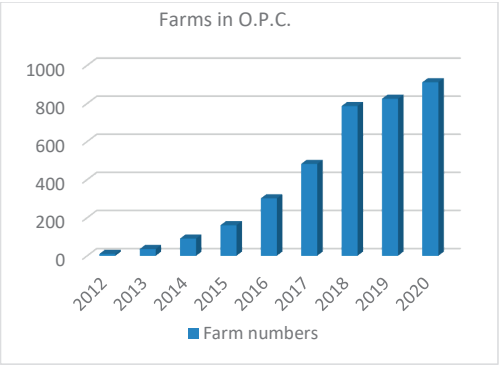

Figure 2. Aberdeen Angus farms in the official performance control in Romania

In Figure 2 is the evolution of Romanian Aberdeen Angus farms in official performance control for the meat production department.
In Figure 3 are the evolution of Aberdeen Angus cattle in the official performance control for meat production department.

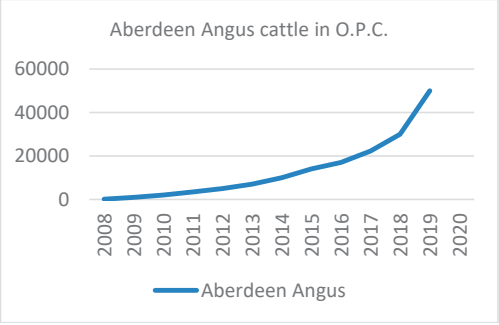

Figure 3. Aberdeen Angus cattle in the official performance control in Romania

MATERIALS AND METHODS

The official performance control for meat production is carried out by the control methods A, B and C. There are two mandatory controls weighing per year. Method A - all the controls are performed by an official representative of an association accredited to perform the official performance control for meat production by the herd book of the breed.
Method B - all controls are carried out by the breeder or his representative.
Method C - all checks are performed by the breeder or his representative and a representative of the accredited control association. The interval between two weighing is at least 60 days and not more than 210 days.

Aberdeen Angus Romanian Association who is the only accredited herd book society for Aberdeen Angus breed in Romania is using for the official performance control for meat production only method A. Using only method A increase the accuracy of the data.
The official performance control for meat production is performed by the Aberdeen Angus Romanian Association with legal approved trailer scales. The scales used to determine the weight, must have the following requirements: each scale used in the official performance control for meat production must be subjected to periodic metrological checks by the laboratories in the structure or subordinated to the Romanian Office of Legal Metrology or, as the case may be by the metrology laboratories authorized by them. The metrological checks of the scales are made annually, each verification is recorded in the metrological record that will be presented at each weighing control in farms. The scale must have a platform and a metal fence for the containment of the cattle.
Within the Aberdeen Angus Romania Association having member farms from the plain to the mountains region, the basic equipment for control official performance for meat production is off road cars and trailers with approved scale.
Before the official performance control for meat production in the Aberdeen Angus breed begins, the origin of each animal must be certified (validated) and entered only in the software program "BIDAA - Aberdeen Angus Data Base", granted by the Aberdeen Angus Romanian Association for calculation and estimation of performance for the genetic value of an animal (estimation method that contains the relation between the animals and the performances of each one - animal model Henderson). The identification and individualization are attributed to the breeder's to ensure the notification documents.
The farmer has the obligation to communicate in time all the events on the farm according to the notification documents: registration the natural breeding or artificial insemination (annex 3), registration of the calving (annex 8) and entries/exits, selling, bringing to slaughterhouse (annex 10) (Grosu and Gociman, 2018)

Figure 4. O.P.C. trailer scale equipment

This study was conducted in two farms, located in Calarasi county plain area in south region of Romania and in Sibiu county mountain area in the middle region of Romania. The research were made on a number of 50 calves of Aberdeen Angus breed with average age of seven months that have been studied for two years. We weighted the cattle at birth, 7, 10, 12 and 15 months (Grosu and Gociman, 2018)

RESULTS AND DISCUSSIONS

Romanian area is 237,500 km^2 wide, consisting of a symmetrical landforms, concentric and varied, with the main features of landforms proportioned as follows: 31% mountains, 36% hills and plateaus, and 33% plains. Large pasture lands, climate, precipitation variation, soil, quality feed are some of the great strengths of Romania to grow such an extensive cattle breed.

Second country from European Union with surface of pasture, the sixth country from European Union as agricultural area and the ninth country from European Union as number of cattle (more then 70,000 pure Aberdeen Angus cattle) (Gociman et al., 2019; Vidu et al., 2015).

Example: 237,500 km^2 x 36% hills = 85,500 km^2 hills - in Romania

85,500 km^2 hills x 1,000,000 m^2 (1 km^2) = 85,500,000,000 m^2 - in Romania

85,500,000,000 m^2/10.000 m^2 = 8,550,000 hectare - in Romania

Final result: 1 hectare for 1 cow - 8,550,000 breeding beef cows in Romania.

To have minimum: 1,000,000 beef cattle in Romania

Romania is a country with high agricultural potential given that the geographical configuration and favorable climate. Our country is not part of countries that have to import substantial amounts of food because, under normal circumstances, this land can produce more food for a population of three and a half times higher than the current population (Gociman et al., 2019).

Factors that influence the individual production of the Aberdeen Angus cattle:

Internal factors	**External factors**
- Genetic	*Environment
- Physiological	*Feeding
*Species	*Water
*Breed	*Breeding season
*Age	*Calving season
*Sex	* Technology

Figure 5. Influencing factors

Phenotype = Genotype + Environment

Having in Romania these variations of climate and landforms, the Aberdeen Angus cattle vary from point of breeding, increases from area to area (Figure 5). By selection according to performances, we aim to increase the quantities and qualities of romanian Aberdeen Angus beef production as well as the performances of the farms (Table 1).

Table 1. Average weight of Aberdeen Angus cattle in two farms (kg)

Farm	Age (month)	n	X ± Sx	Sẋ	V%
Sibiu	Birth	50	34±1.63	3.98	12
Calarasi			36 kg ± 0.62	1.51	4
Sibiu	7	50	211± 6.64	42.56	20
Calarasi			296± 8.9	23.49	8
Sibiu	10	50	282 ± 13.91	43.92	16
Calarasi			377 ± 8.1	37.78	10
Sibiu	12	50	316 ± 6.07	39.78	13
Calarasi			468 ± 32.77	65.53	14

From the analysis on the body dynamics weight, over a period of 12 months, on the 100 calves, from two farms located in different pedoclimatic areas, we observe the following:

- at birth the average body weights were close to the farm in Sibiu and Calarasi;
- at 7 months between the two farms we observe large differences in body weight, respectively in Calarasi farm, the average weight is 28.72 % higher than the bulls in Sibiu farm;
- it is worth noting that the group from Calarasi is very homogeneous (8% V), the average daily increase is 1308 g;
- the group from Sibiu achieved really small average daily gain increases (895 g ADG), has very high variability (20%) differences are explained by a less balanced of fodder diets;
- at 12 months the difference of the average body weight is accentuated, respectively the group from Calarasi have a greater body weight then from Sibiu 32.48%. Inside the batches the weights are balanced, they become really homogeneous (14-15% V)

Table 2. Average average daily gain (ADG) of Aberdeen Angus cattle in two farms (g)

Farm	Age (month)	n	$X \pm S_X$	Sẋ	V%
Sibiu	Birth	50	-	-	-
Calarasi			-	-	-
Sibiu	7	50	895 ± 33.18	215.3	24
Calarasi			1308 ± 42.16	111.3	8
Sibiu	10	50	835 ± 45.90	145.04	17
Calarasi			1143 ± 26.65	124.73	11
Sibiu	12	50	779 ± 16.55	108.43	14
Calarasi			1192 ± 92.05	184.09	15

Regarding the average daily gain increase (Table 2), the group from Călărași have average daily gain increases between 1143 g and 1308 g and the group from Sibiu have average daily gain increases between 779 and 895 g. Among the celebrities are firm and differential by 31.57%. The farm in Călărași follows a curve of good growth .

The Aberdeen Angus Romanian Association was in 2013 accredited the first association in Romania to do the official performance control for meat production (Figure 6).

After many weighing thounsands of calves of Aberdeen Angus breed in Romania, we discover that after weaning (approximate seven months) due to the maternal stress of the calf lose a lot of weight and they have no feeding appetite.

Figure 6. Aberdeen Angus young bulls before weighing

Worldwide from research conducted by large specialists in the animal welfare department, it has been found that there are two types of cattle, those that control more stress “reactive” and those that control less stress “proactive”(International Beef Cattle Academy, 2018/2019).This physiological cattle fact is controlled by the adrenal gland cortex, which controls the hormone cortisol responsible for stress. Below in the two figures you will find two examples of bulls within the association, the first being proactive and the second one reactive.

Bull 1008 comes from Aberdeen Angus farm in Sibiu county Transilvania Romania, was born in 10.07.2018 with a weight of 30 kg, his name is SOBIS U088 and is red color variety.

Bull 4849 comes from Aberdeen Angus farm in Calarasi county south Romania, was born in 02.09.2018 with a weight of 38 kg, his name is COCONI U849 and is black color variety.

In the table below (Table 3) we see two different types of feeding with more or less concentrate in diets.

It can be seen from Table 4 that the Călărași lot benefits from a ration structure with a higher proportion of high quality fibrous feed (hay and alfalfa) 53.33%, unlike the one from Sibiu (Table 3) where straws are used in proportion of 33.33%.

Table 3. Feeding diet in Aberdeen Angus farm from Sibiu county

Forage	Quantity
Straw	4 kg
Corn silage	4 kg
Corn	2 kg
Oat	0.5 kg
Wheat	0.5 kg
Soy bean	0.5 kg
Sun flower bean	0.5 kg
Total forage	12 kg

Table 4. Feeding diet in Aberdeen Angus farm from Calarasi county

Forage	Quantity
Hay	4 kg
Alfalfa	4 kg
Corn silage	4 kg
Corn	2 kg
Wheat	0.5 kg
Oat	0.5 kg
Total forage	15 kg

Bull "1008" is grown in the Carpathian mountains with intensively feeding (straw+concentrated) and the bull "4849" is grown in Romanian Dobrogea plains intensively with another feeding (hay+concentrated). In this case we can see in Figure 7 and Figure 8 the results of genetic and physiological potential of Aberdeen Angus breed (proactive vs. reactive).

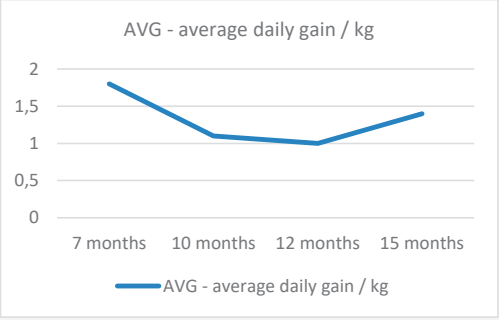

Figure 7. Bull 1008 weighing at different ages and the stress after weaning (proactive)

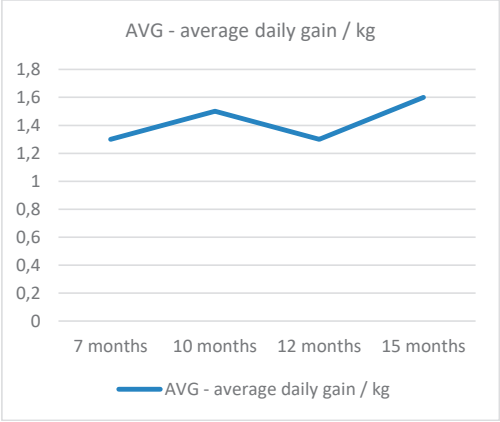

Figure 8. Bull 4849 weighing at different ages and less stress after weaning (reactive)

CONCLUSIONS

After all those years that Aberdeen Angus Romanian Association weighted thousands of youth Aberdeen Angus cattle and visit thousands of farms to achieve the main goal : genetic improvement of quality and quantity Romanian beef, we discover many interesting science results. We respect the improving Aberdeen Angus breeding program in Romania to make genetic progress.

All this internal and external factors have a big influence according youth Aberdeen Angus growth like: environment, climate, relief feeding, water, stress, welfare and genetics etc.

Romania should not be part of the countries forced to import substantial quantities of food, the pedoclimate configuration is favorable to produce the needs for the local population and much more. We need in European Union fresh beef with trasability not to import from another continents.

Romania has to become a European beef brand country.

ACKNOWLEDGEMENTS

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REFERENCES

Acatincăi, S. (2004). Cattle production / Producțiile bovinelor, second edition. Timișoara, RO: Eurobit Publishing House.

Gociman, I., Vidu, L. Mărginean, G., Bărăităreanu, S., Nicolae C. (2019). Research on the evolution of the Aberdeen Angus breed in Romania**.** *Scientific Papers. Series D. Animal Science*, LXII (2).

Grosu, H., Gociman, I. (2018). Aberdeen Angus Breeding Program in Romania. International Beef Cattle Academy, (2018/2019) Texas A&M University U.S.A.

Mărginean, G. (2012). *Cattle breeding treaty / Tratat de creștere a bovinelor,* vol. I + vol. II, Cluj-Napoca, RO: Risoprint Publishing House.

Vidu, L. (2006). *Meat chain*. Bucharest, RO: Printech Publishing House.

Vidu, L., Bacila, V., Udroiu, A., Vladu, M. (2015). Researches regarding the growing capacity and feed converting capacity in meat production at Romanian cattle breeds. *Annals of the University of Craiova - Agriculture, Montanology, Cadastre Series*, 45(1).

http://aberdeenangus.ro/

http://www.aberdeen-angus.co.uk/

www.fas.usda.gov/

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INFLUENCE OF TEMPERATURE-HUMIDITY INDEX AND FARM FACTORS ON SOME BIOCHEMICAL BLOOD PARAMETERS IN DAIRY COWS

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Abstract

A one-year study of the Temperature-humidity index (THI) was conducted in three buildings used for breeding dairy cows (farms). They were kept free in separate boxes or as a group living on a permanent litter bedding. Blood samples from 6 animals from each farm were taken and the following biochemical indicators were tested - blood sugar, total protein, urea, cholesterol, creatinine, cortisol, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), calcium(Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K) and chlorine (Cl). It was found that the season has a significant effect on THI, blood sugar, total protein, urea, cholesterol, creatinine, cortisol, ASAT, ALAT, Na and K ($P<0.001$), and a significant effect on Ca and P ($P<0.01$). The change in the values of THI, cortisol ($P<0.001$), urea and cholesterol ($P<0.01$) are related to the breeding technology. The blood sugar, creatinine, P, Mg, Na, K and Cl values are not affected by the type of building and are within physiological norms. Variations of the studied parameters are related to season and breeding technology.

Key words: *biochemical blood parameters, dairy cows, farm building, temperature-humidity index (THI).*

INTRODUCTION

The influence of the climate change on domestic animals is a global problem and is bound to lie even more challenges ahead of farmers in the years to come. The seasonal changes and temperature increases may affect the domestic animals' blood indicators (Bacalov et al., 2009). Hewett (1974) reported that the most productive high-yielding dairy cows are especially susceptible to high ambient temperature and this influences their blood profile. The seasonal fluctuations in the air temperature and the relative humidity as well as other environmental conditions affect the biological system of the animals (El-Nouty et al., 1990). The morphological and biochemical blood changes most objectively indicate any deviations from the basic biophysical and biochemical functions in the organism. These examinations are particularly useful due to the fact that they provide information about the condition of animals before any clinical symptoms of a disease or stress are displayed. The analysis of the blood parameters as early response indicators may serve as the basis for diagnostics, treatment and prevention of different diseases (Otto et al., 2000; Ndlovu et al., 2007). Studies carried out by Armstrong (1994), Kadzere et al. (2002), Dikmen and Hansen (2009) point that not only THI can be used as thermal climatic conditions indicator but also as a tool reporting stress levels in cows. With suitable temperature and humidity in production premises guarantee both the comfort inside, and the health and productivity of animals (Gaughan et al., 2002; Hansen, 2007). The optimal temperature zone under Ordinance No. 44 is 10-15ºC at a minimum of 5 and maximum of 28ºC. Temperatures above 18-20ºC could potentially cause heat stress in high-yielding dairy cows.

The studies show that the temperature and humidity index (THI) can be used as an indicator of heat stress in cows (Kadzere et al., 2002; Armstrong, 1994; Dikmen and Hansen, 2009). Mazzullo et al. (2014) have proved that environmental conditions are the main stress factors having effect on animals and take into account the seasonal influence on their hematology and blood chemistry parameters. Having in mind that environmental conditions affect animals the present study aims to trace the levels of some hematological indicators of

cows bred in different type of buildings during three different seasons.

MATERIALS AND METHODS

The present study comprises three periods-summer, winter and transitional one. The air temperature and humidity in the three controlled farms was measured every month of the respective period for 3 to 5 days. Farm 1 (F 1) has a capacity of 130 dairy cows, grown freely in individual cabins, divided into 2 groups of 65 animals each. The cabins are divided in four rows. The building is an open metal structure with a roof made of thermopanels. The longitudinal walls are made of concrete with a thickness of 0.25 m and a height of 1.5 m. The short walls are 3.0 m in height. The area of the feeding path has no doors and it is fully opened. The total area of the building is 1,248 m², walking area is 595.2 m² and rest area is 422.4 m².The space provided for one animal is 9.4 m².

The building is 48 m long, 26 m wide, 3 m in height to the roof structure and 8 m in height to the ridge. The floor is made of cement. The cabins are covered with a hard rubber mat with a thickness of 2 cm.

Lighting is provided through the opened space of the building with a total area of 170 m². Artificial lighting is provided by 14 luminescent laps, each consisting of 3 luminary pipes with a power of 40 W. Mechanical ventilation is provided by 8 ventilators mounted above the cabins and the walking area, at an angle of 45º. Each one of them has a power of 0.55 kW and a capacity of 60,000 L/h. Four of them are automatically started at temperatures above 18°C and the remaining four are automatically started at temperatures above 25°C.

The manure is cleaned with a delta scraper device that automatically turns on every 6 hours.

Farm 2 (F 2) has a capacity of 200 dairy cows, grown freely in individual cabins, divided into 4 groups. The total area of the building is 2,310 m² and the space provided for one animal is 11.5 m². The building is made of reinforced concrete and roof panels and its dimensions are 105/22 m. The height of the walls is 4.5 m and the height of the ridge is 5.8 m.

The individual cabins are located on both sides of the longitudinal walls with dimensions 1.10/2.10 m. There are no partitions between them. The manure paths are located between the individual cabins and the feeding area.

The floor of the whole building is made of cement. The natural light in the farm is provided by a total of 30 windows, 20 of which are with dimensions 5.80/0.90 m and 10 are with dimensions 5.80/2.00 m. At the ridge of the building there are also 12 ventilators (windows), each measuring 5.80/0.90 m. Artificial lighting is provided by 97 lamps, each consisting of 2 luminary pipes with a power of 40 W. In the winter period, the windows and the ridge ventilators are covered with polyethylene sheets. Mechanical ventilation is provided by 10 ventilators mounted above the walking and feeding areas. Each one of them has a power of 0.55 kW and a capacity of 60,000 L/h.

The manure is cleaned with a delta scraper device that automatically turns on every 3 hours.

Farm 3 (F 3) has a capacity of 67 dairy cows with a total living area of 598.5 m² and a 540 m² area used for walking and rest. The space provided for one animal is 8.06 m². The building is made of brick masonry, it is semi-opened and its dimensions are 45/12 m and a height of 3 m. The length of the short walls is 6 m and the rest 6 m are unclosed. The roof structure is made of galvanized sheet without insulation. The natural ventilation, achieved through the unclosed short walls, matches with the tunnel type. Eight rotary fans (DeLaval) are mounted above the deep litter area, at an angle of 45º, for additional mechanical ventilation. Each one of them has a power of 0.55 kW and a capacity of 60,000 L/h. Four of them are automatically started at temperatures above 18°C and the remaining four are automatically started at temperatures above 25°C.

The feeding is “at will” with an all-purpose fodder mix and constant access to water. The manure is cleaned twice a year, however litter is added periodically. Lighting is natural and its characteristics depend on the season. Artificial lighting is provided by five 100 W lamps mounted above the feeding path and three 200 W lamps mounted above the walking and rest zones.

The THI is calculated based on Thom. Blood samples were taken from six animals form each farm (in same age and physiological condition - in the first three months of lactation). The blood serum acquired was tested for: blood sugar, total protein, urea, cholesterol, creatinine, cortisol, ASAT, ALAT, calcium, phosphorus, magnesium, sodium, potassium chloride. The results were statistically processed via IBM, SPSS 21.

RESULTS AND DISCUSSIONS

In order to ascertain the extent to which the farm barn environment factors could form its comfort or discomfort, we used the temperature humidity index (THI) as defined by Thom (1959). To calculate it, we used the average temperature and humidity values.

Table 1 displays the THI values of the farms examined during the different seasons. The season has a plausible effect on the index (P<0.001) (Figure 1).

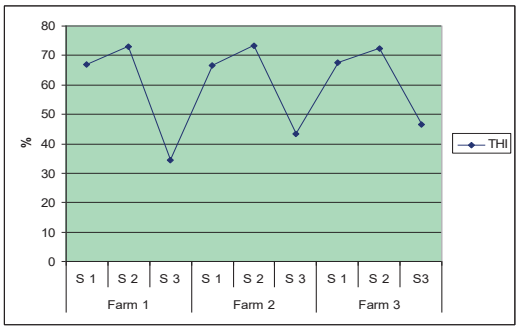

Figure 1. Seasonal dynamics in the THI values

The index values are dynamic throughout the day. The values between 77 and 87 are considered the critical THI point because it is then that the animals' lethal cases start to increase (Vitali et al., 2009).

Table 2 indicates that the blood sugar levels in the farms examined by us are close to the lower reference norms, however, the plausible influence of summer season on them is accounted for (P<0.001).

Table 1 Temperature humidity index of the buildings examined during the three seasons

Parameter	Farm	LSM	± SE	SD	Season	LSM	± SE	SD
THI	1	58.03	4.12	17.46	67.00	0.086	0.37	0.37
	2	61.07	3.11	13.21	72.80	0.105	0.45	0.37
	3	62.10	2.69	11.45	41.41	1.261	5.35	0.45

Note: LSM - average; SE - standard error; SD - standard deviation.

Table 2. Influence of the season and farm on some biochemical blood parameters in the studied cows

Parameter	F	LSM	± SE	SD	F-criteria and Sig	S	LSM	± SE	SD	F criteria and Sig
Glucose	1	2.61	0.17	0.74	0.88	1	2.82	0.024	0.10	300.21***
	2	2.64	0.22	0.93		2	1.56	0.063	0.27	
	3	2.71	0.23	0.98		3	3.57	0.086	0.36	
TP	1	70.68	1.40	5.95	5.72**	1	72.28	1.277	5.42	21.63***
	2	66.21	1.73	7.32		2	63.08	1.757	7.45	
	3	71.51	1.65	6.98		3	73.05	0.516	2.19	
Urea	1	5.23	0.36	1.54	8.89***	1	4.49	0.181	0.77	124.58***
	2	4.80	0.36	1.52		2	7.20	0.186	0.79	
	3	5.72	0.38	1.63		3	4.05	0.146	0.62	
Creatinine	1	108.8	14.74	62.54	0.53*	1	67.56	1.547	6.56	69.65***
	2	133.44	12.58	53.38		2	175.19	11.30	47.94	
	3	103.22	12.59	53.44		3	82.00	2.375	10.08	
Cholesterol	1	2.85	0.22	0.94	4.47*	1	3.59	0.082	0.35	185.41***
	2	2.76	0.24	1.01		2	1.67	0.078	0.33	
	3	3.08	0.22	0.93		3	3.42	0.097	0.41	
ASAT	1	97.66	7.90	33.52	0.47	1	86.77	3.627	15.39	10.87***
	2	91.99	6.08	25.80		2	129.67	14.047	59.59	
	3	103.61	14.30	60.67		3	76.83	2.154	9.14	
ALAT	1	22.44	1.53	6.48	4.37*	1	17.97	1.112	4.72	20.39***
	2	23.38	1.78	7.56		2	27.71	1.623	6.89	
	3	18.66	1.40	5.95		3	18.79	0.948	4.02	

Cortisol	1	65.26	4.96	21.04	51.55***	1	39.97	0.402	1.71	697.11***
	2	55.67	2.82	11.97		2	73.08	3.109	13.19	
	3	59.64	3.431	14.56		3	67.52	0.494	2.09	
Ca	1	2.44	0.03	0.13	3.96*	1	2.32	0.037	0.16	8.05**
	2	2.34	0.04	0.16		2	2.29	0.036	0.15	
	3	2.31	0.05	0.21		3	2.47	0.038	0.16	
P	1	1.93	0.06	0.27	1.22	1	1.73	0.039	0.168	8.22**
	2	1.97	0.08	0.32		2	2.04	0.062	0.265	
	3	1.85	0.05	0.21		3	1.98	0.064	0.270	
Mg	1	1.00	0.02	0.08	2.19	1	0.96	0.026	0.114	0.98
	2	0.94	0.03	0.11		2	0.97	0.023	0.099	
	3	0.99	0.02	0.09		3	0.99	0.016	0.065	
Na	1	146.50	1.75	7.44	3.62*	1	149.11	1.204	5.109	44.72***
	2	144.06	1.77	7.51		2	135.44	0.894	3.792	
	3	142.17	2.03	8.60		3	148.17	1.331	5.649	
K	1	4.78	0.11	0.48	0.19	1	5.23	0.061	0.258	108.2***
	2	4.74	0.09	0.42		2	4.27	0.030	0.128	
	3	4.75	0.11	0.44		3	4.77	0.042	0.177	
Cl	1	105.22	0.49	2.10	1.27	1	106.17	0.406	1.724	2.74*
	2	105.83	0.51	2.18		2	104.94	0.461	1.955	
	3	104.78	0.47	1.99		3	104.72	0.559	2.372	

Note: ***P<0.001; **P<0.01; *P<0.05.
Number of animals form each farm = 6, F - farm; S - season; LSM - average; SE - standard error; SD - standard deviation; F - criteria and Sig - ration of the mean sums of squares of the regression equation and the residual and significance level.

In a similar experiment Gorski and Saba (2012) also reported a decrease in glucose levels. The blood sugar is not a major source of energy for the ruminant animals, however, at the end of the pregnancy and at the beginning of the lactation, as it is in our study, a big amount of the blood sugar is used for lactose and milk fat synthesis so its values are indicative of some pre-pathological or pathological states. The studies of Darul and Kruczynska (2005) show that, the blood sugar levels decrease after birth, after beginning of active lactation and upon change in cows energy balance.

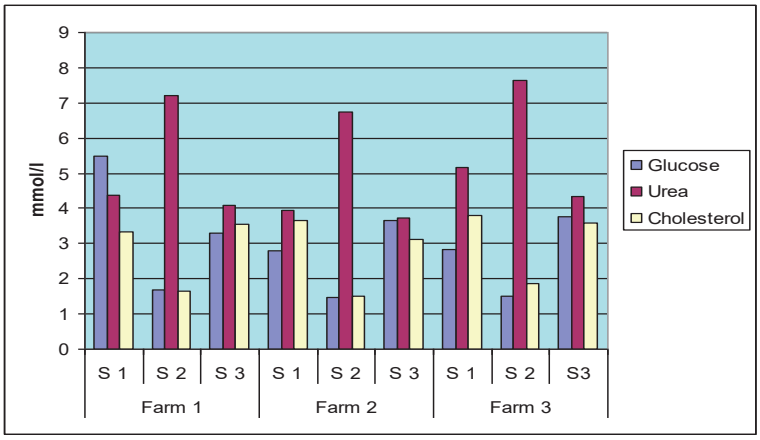

Figure 2. Seasonal dynamics in the blood serum content of glucose, urea and cholesterol of the cows from the farms examined

The blood serum urea levels (Figure 2) are a criterion for the organism protein self-sufficiency. Its high levels (P<0.001) along with these of the creatinine indicate increased kidney function, which in this case is related to the high summer temperatures and the increased metabolic activity associated with it. The season definitely affects the cholesterol levels (Figure 2) which are the lowest during the summer (P<0.001) while the farm does not

have influence on this parameter. When interpreting the lipid metabolism of ruminants, we must take into account the fact that they intake very little amount of lipids. Along with the glucose, the lipids turn into volatile fatty acids parts of which are present in the milk fat. The lactating cows have the unique quality to maintain high concentration of cholesterol in the plasma. The rich high-density lipoprotein content transports cholesterol to the liver due to which rarely does this type of animals experience any unfavorable consequences. The lower summer season values reported by us can probably be explained by the quality of the feeds or an initial stage of liver damage. This is evidenced by the higher ASAT and ALAT levels (Figure 3). Our study reveals that the enzyme activities are inherently high and affected both by the farm and the season ($P<0.001$). The less pronounced increase in ASAT when compared to ALAT levels is due to the fact that ALAT is present only in the cytoplasm while a significant part of the ALAT trans-fat is present in the mitochondria and is only released in case of very serious degenerative cell changes. Bhan et al. (2012) reported higher ASAT and ALAT levels during the winter which they explain as a result of these enzymes release form the liver cytosol into the bloodstream which in turn displays the damage and the dysfunction of the liver. The increased ASAT and ALAT function can also be associated with energy metabolism disorder in the body as well as stress (Krupczynski and Chudoba-Drozdowska, 2002; Darul and Kruczynska, 2005).

When interpreting the results, we should observe the fact that the cows which are subject to the study are highly productive animals and according to many authors their high trans-fat activity is related to the damage on their livers (Moore, 1997; Cozzi, 2011) claims that similar effect but with reference to healthy cows may be also a result of a higher productive stress. In one study, the authors Gorski and Saba (2012) presumed that the higher AST and ALT levels in cows are related to higher metabolic efforts caused by the intensified lactation.

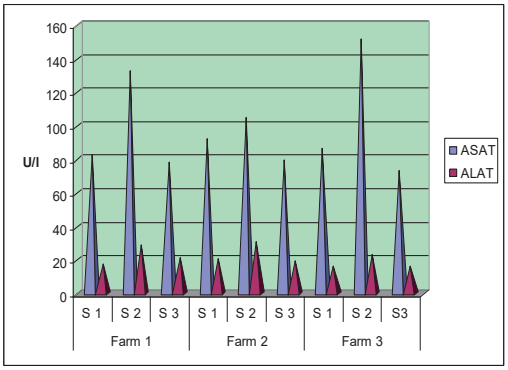

Figure 3. Seasonal dynamics of the blood serum ASAT and ALAT content of the cows from the studied farms

The total protein levels range within the lower reference values in all seasons and with reference to all farms but is considerably more noticeable in the summer. The feeding pattern is the major factor which influences the protein levels in the blood.

Creatinine is formed in the muscles and usually has a stable level which depends on the kidneys function. The creatinine levels show an interesting dynamics with the summer season influence being especially noticeable ($p<0.001$). The maintenance of high creatinine levels in this case probably has to do with the animals' physical activity. The increased creatinine levels after intensive muscle activity were observed by Sato et al. (2001), Nikolov et al. (2011; 2009).

Cortisol is considered a major biomarker upon unlocking of the animals adaptive mechanisms during heat stress. Our studies indicate that the season plausibly influences the cortisol values ($P<0.001$) (Figure 4). Johnson and Vanjonack, (1976) reported increased cortisol levels both at extremely low and very high temperatures.

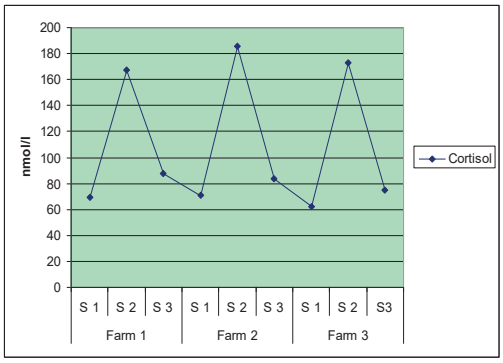

Figure 4. Seasonal dynamics in the blood serum cortisol content of the cows from the examined farms

The minerals are supplied with the food and play a structural and functional role; therefore, the change in their serum concentration affects the health and productivity of the animals.

The phosphorus takes part in the energy transport and is contained in the nucleic acids.

The potassium is involved in the muscle contractions. Our study shows that both microelements are affected by the season (phosphorus: P<0.01; potassium: P<0.001).

The sodium serves primarily for the maintenance of the osmotic stability. Its quantity in the organism is related to the water exchange. Its values (Figure 5) which are influenced dehydration state trying to compensate the low THI levels by increasing the evaporation through rapid breathing and sweating.

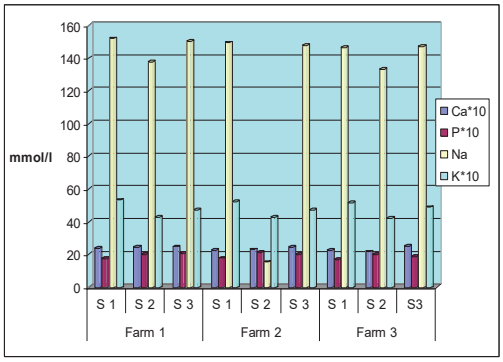

Figure 5. Seasonal dynamics of the blood serum content of calcium, phosphorus, sodium and potassium of the cows from the examined farms

CONCLUSIONS

It has been ascertained that the season has a significant effect on the THI, glucose, total protein, urea, cholesterol, creatinine, cortisol, ASAT, ALAT, sodium and potassium levels (P<0.001) and on the calcium and phosphorus levels (P<0.01). The changes found in the THI levels, cortisol, urea (P<0.001), total protein (P<0.01), creatinine, cholesterol, ALAT, Ca and Na (P<0.05) are connected with the breeding technology (farms). The glucose, ASAT, P, Mg, K and Cl in the blood of the cows are not affected by the type of building and are within the physiological reference norms.

The abovementioned gave us the reason to come to the conclusion that the variations in the examined parameters are related to the season, type of building, and the breeding technology (farms).

It was ascertained that the blood parameters examined are dependent on the THI in the premises and are mildly or not affected by the type of building and the manner of breeding.

Our results reveal differences in the hematology and blood chemistry parameters related to the changes in the ambient temperature and THI, although some of them are within their physiological diapason. Therefore, we can state that the seasonal changes may affect the metabolic processes of the dairy cows.

REFERENCES

Armstrong, D. V. (1994). Heat Stress Interaction with Shade and Cooling. J. *Dairy Sci*., 77(7), 2044-2050.

Bacalov, P., Nikolov, V., Hristev, H., Ivanova, R. (2009). Seasonal dynamics of some homeostatic indices in cows of Bulgarian Rhodope cattle breed raised under lowland conditions., *IV Balkan Conference of Animal science Balnimalcon, St. Zagora*, 264-268

Bhan, C., Singh, S. V., Hooda, O. K., Upadhyay, R. C., Mangesh, B., Mangesh, V. (2012). Influence of temperature variability on physiological, hematological and biochemical profile of growing and adult Sahiwal cattle. *J Environ Res Develop*, 7, 986-994.

Cozzi, G., Ravarotto, L., Gottardo, F., Stefani, A. L., Contiero, B., Moro, L., Brscic, M., Dalvit, P. (2011). Short communication: Reference values for blood parameters in Holstein dairy cows: Effects of parity, stage of lactation, and season of production. *J Dairy Sci*, 94(8), 3895-3901.

Darul, K., Kruczynska, H. (2005). Changes in some blood constituents of dairy cows: association with pregnancy and lactation Šin Polish, *Acta Sci Pol Med Vet*, 4(1), 73-86.

Dikmen, S., Hansen, P. J. (2009). Is the temperature-humidity index the best indicator of heat stress in lactating dairy cows in a subtropical environ- ment? *J Dairy Sci*, 92, 109-116.

El-Nouty, F. D., Al-Haidary, A. A., Salah, M. S. (1990). Seasonal variation in hematological values of high and average yielding Holstein cattle in semi-arid environment., *J King Saud Univ*, 2(2), 173-182.

Gaughan, J. B., Mader, T. L., Holt, S. M., Hahn, G. L., Young, B. A. (2002). Review of current assessment of cattle and microclimate during periods of high heat load. *Anim. Prod. Aust*., 24, 77-80.

Gorski, K., Saba, L. (2012). Changes in the level of selected haematological and biochemical parameters in the blood of dairy cows in Central-Eastern Poland, January, *Acta veterinaria*, 62(4), 421-428.

Hansen, P. J. (2007). Exploitation of genetic and physiological determinants of embryonic resistance to elevated temperature to improve embryonic survival in dairy cattle during heat stress, *Theriogenology*, 68, 242-249.

Hewett, C. (1974). On the causes and effects of variations in the blood profile of Swedish dairy cattle. *Acta Vet. Scand.* Suppl., 50, 1-152.

Johnson, H. D., Vanjonack, W. J. (1976). Effects of Environmental and Other Stressors on Blood Hormone Patterns in Lactating Animal. *Journal of Dairy Science*, 59(9), 1603-1617.

Kadzere, C. T., Murphy, M. R., Silanikove, N., Maltz, E. (2002). Heat stress in lactating dairy cows: a review. *Livestock Prod Sci*, 77(1), 59-91.

Kupczynski, R., Chudoba-Drozdowska, B. (2002). Values of selected biochemical parameters of cows blood during their drying-off and the beginning of lactation, *EJPAU Ser Vet Med*, 5, 1.

Mazzullo, G., Rifici, C., Lombardo, S. F., Agricola, S., Rizzo, M., Piccione, G. (2014). Large Seasonal variations of some blood parameters in cow. *Animal Review*, 20, 81-84.

Moore, F. (1997). Interpreting serum chemistry profiles in dairy cows. *Vet Med*, 92, 903-912.

Ndlovu, T., Chimonyo, M., Okoh, A. I., Muchenje, V., Dzama, K., Raats, J. G. (2007). Assessing the nutritional status of beef cattle: current practices and future prospects. *Afr. J. Biotech*., 6, 2727-2734.

Nikolov, V., Ivanova, R., Batcalov, P., Hristev, H. (2009). Influence of high temperature stress on behavior of mountain breed dairy cows, *IV Balkan Conference of Animal science Balnimalcon, St. Zagora*, 409-413.

Nikolov, V., Ivanova, R., Hristev, H., Alragubi, S. (2012). Study on some hematological indices of cows of the Rhodope shorthorn cattle breed, *Journal of Mountain Agriculture on the Balkans*, V 15(1), 74-92.

Ordinance No. 44 on Veterinary Medical Requirements to Livestock Sites. SG No. 41/2006 (Bg).

Otto, F., Baggase, P., Bogin, E., Harun, M., Vilel, A. F. (2000). Biochemical blood profile of Angoni cattle in Mozambique. *Israel Vet. Med. Assoc*., 55, 1-9.

Sato, S., Sugiyama, M., Yasue, T., Deguchi, Y., Suyama, T. (2001). Seasonal Change of land use strategy of free-ranging cattle in an agroforest setting without fencing. Proc. of the 35th Congress of the ISAE, Davis, California, 152.

Thom, E. C. (1959). The discomfort index. *Weatherwise*, 12, 57-59.

Vitali, A., Segnalini, M., Bertocchi, L., Bernabucci, U., Nardone, A., Lacetera, N. (2009). Seasonal pattern of mortality and relationships between mortality and temperature-humidity index in dairy cows. *J. Dairy Sci*., 92, 3781-3790.

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SEASONAL INFLUENCE ON HEMATOLOGICAL AND BIOCHEMICAL PROFILE IN DONKEY (*Equus asinus*)

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Abstract

The hematological and biochemical profile is important to study because it provides important information on animal health. Changing the parameters beyond the normal limits negatively affects the production and quality of milk. Hematological and biochemical parameters were influenced by season, age, sex, lactation, animal nutrition and maintenance system. The total protein was in average 55.44 ± 5.14g/l in the summer season, and 76.92 ± 2.82 g/l in the winter season. Creatinine registered an average values of 99.55 ± 3.57 µmol/L in summer, while in winter was 112.00 ± 6.59 µmol/L. Cholesterol is an important parameter for the body, and his changes give us important information about the major disturbances that occur in the body. This parameter was significantly influenced by the season and it was 1.82 ± 0.14 mmol/L for summer and 1.95 ± 0.06 mmol/L in winter. The LYM in the summer season had an average value of 49.96 ± 2.72%, while in the winter season was 59.92 ± 2.19%. The values of hematological and biochemical parameters obtained in our study fall within the characteristic limits of donkeys.

Key words*: donkey, hematological and biochemical parameters, season.*

INTRODUCTION

Seasonal changes in ambient temperature, relative humidity, and air velocity influence the physiological responses of animals (Ruiz et al., 2004). In donkeys, the hematological and biochemical parameters are significantly influenced by sex, age, muscle mass, nutrition, physiological status, donkey health (Mori et al., 2003; Laus et al., 2015; Yakubu and Chafe 2008). Hematological parameters (such as PCV, RBC, MCV and MCHC) are used as indicators to evaluate animals adaptability to the environment conditions (Koubkova et al., 2002). Physical activity and work of animals is a commonly recognized stress factor, which can influence hematological parameters. Extreme hot and cold ambient temperatures can affect the animals, and this could be evidenced in the fluctuations of physiological responses to combat environmental thermal stress (Pandey et al., 2012). All biochemical and metabolic signals serve to guide normal development. The differences among studies regarding to hematological and biochemical parameters could also be due to the different exploitation techniques, the different feeding level, the health norms and the diseases that animals can suffer (Michael et al., 2013).The purpose of this study was to establish the influence of the season on the hematological and biochemical profile in the donkey.

MATERIALS AND METHODS

The donkey metabolic profile was evaluated in two different seasons (summer *vs*. winter) by determining the hematological and biochemical parameters. Blood samples were used to analyze the hematological parameters. A total of ten blood samples were used for both summer and winter seasons.

The blood was collected from animals, and immediately was centrifuged in order to separate the serum.

The serum was separated by using the high performance centrifuge with 4000 rotations per minute. Then, samples were immediately frozen in Eppendorf type vials.

Biochemical analysis

The determinations for the biochemical parameters were performed with the semi-automatic analyzer for screen point biochemistry, with reagents-STAT-Fax 1904 Plus, Global Medical Instrumentation, Ramsey Minesota, USA. It is a general purpose photometer, controlled by a macroprocessor, with 6 filters and 37ºC incubation block.

Hematological analysis

The blood samples were analyzed in order to determine hematological parameters. Parameters were determinated by using the Abacus Junior Vet automatic device, Diatron, Messtechnik.

This device is an automatic analyzer made to count the blood cells and determining the hematological parameters by adding 25 µl of each blood sample. He selected automatically the species from which the blood comes.

For all analyzes, the quality control of the measurements is performed on 6 levels.

Parameters are calculated and presented on charts and in a separate database, and also appear on the device screen.

RESULTS AND DISCUSSIONS

Hematological and biochemical parameters are influenced by sex, age, season, lactation, animal nutrition and maintenance system (Plotka et al., 1988; Raymond et al., 2003).

Tables 1 to 6 present the average values and variability of hematological and biochemical parameters in the donkey, and how this was influenced by two different seasons (summer vs. winter).

The LYM had average of 49.96 ± 2.72% in the summer season, while in the winter season was 59.92 ± 2.19%. These values fall within the characteristic limits of donkeys, and are in agreement with the data published by (Sgorbini et al., 2013).

The season and the meteorological factors can influence the dynamics of the constituent elements of the blood. Satué et al. (2010, 2011) observed that the season may change certain hematological parameters such as: red blood cells (RBC), hemoglobin (Hb) and packed cell volume (PVC).

Table 1. The influence of the season (summer) on the hematological profile in the donkey

Parameter	Summer (n = 10)	
	X ± sx	V%
WBC (G/l)	9.426±0.63	14.91
RBC (T/l)	7.74±0.52	14.90
Hb (g/l)	134.38±3.05	5.07
HCT (l/l)	0.39±0.04	20.35
MCV (fl)q	53.56±3.23	13.50
NEU (%)	48.26±2.34	10.86
LYM (%)	49.96±2.72	12.16
MON (%)	1.618±0.14	19.59
MON (g/l)	0.20±0.03	37.07
NEU (g/l)	4.00±0.05	2.79
LYM (g/l)	8.44±0.29	7.78
EOS (%)	5.37±0.19	7.78
PLT (G/l)	2.62±0.25	20.95
MCHC (g/l)	351.36±5.77	3.67
BAS (%)	0.2±0.02	16.96

V - variability; X - average value; n - number of blood samples.

Table 2. The influence of the season (winter) on the hematological profile in the donkey

Parameter	Winter (n = 10)	
	X ± sx	V%
WBC (G/l)	10.38±0.60	13.03
RBC (T/l)	8.42±0.33	8.83
Hb (g/l)	138.52±3.71	5.99
HCT (l/l)	0.28±0.02	13.60
MCV (fl)q	69.54±2.60	8.36
NEU (%)	39.14±1.33	7.57
LYM (%)	59.92±2.19	8.18
MON (%)	1.80±0.06	7.67
MON (g/l)	0.15±0.02	23.57
NEU (g/l)	3.95±0.06	3.34
LYM (g/l)	8.32±0.28	7.59
EOS (%)	4.76±0.19	8.98
PLT (G/l)	1.63±0.15	20.19
MCHC (g/l)	313.20±6.81	4.86
BAS (%)	0.146±0.01	15.77

V - variability; X - average value; n- number of blood samples.

Satué et al. (2011) observed in her study that rabbits had higher PCV, RBC and mean corpuscular volume (MCV) in the summer season, compared to other seasons.

The results of our study showed that the season was an external factor that controls the dynamics of the constituent elements of the blood. Similar studies regarding the season influence on the hematological parameters were also reported by Shawaf et al. (2017). The differences that exist in the literature regarding to hematological and biochemical parameters are determined by several factors such as: health status, muscle mass, type of feeding and maintenance, if used in agricultural work (Shawaf, 2017; Rico et al., 1978; Cywinska et

al., 2015). Low temperatures reduce the number of red blood cells. In horses, alterations of the osmotic fragility of erythrocytes occur during physical exercise (Hanzawa and Watanabe, 2000). Physiological changes in hematological parameters appear in response to physical exercises and workouts (Fazio et al., 2011; Krumrych, 2009). Piccione et al. (2008) observed that platelet aggregation depends on the effort**,** and the physical activity or exercise exerts an effect on the daily rate of platelets. According to (Olaifa et al., 2012) the PCV and RBC of donkeys decreased significantly, while the neutrophil/lymphocyte ratio increased significantly after donkeys were subjected to agricultural work.

The oxygen consumption and the average heart rate in the assins that circulate at maximum speed, can significantly increase the heart rate, compared to the values obtained in the assins that did not exercise such work (Mueller et al., 1994). Changes in hematological parameters induced by physical labor have been reported by Hinchcliff et al. (2002) and Lorena et al. (2006). Our results presented in the Table 3 corroborated the previous findings, that seasons can affect significantly the haematological profile in donkeys.

Table 3. Statistical interpretation of hematological parameters of blood in donkey under the influence of season

Parameter	Summer	Winter	Significance
WBC	9.43	10.38	n.s
RBC (T/l)	7.74b	8.42a	*
Hb	134.38	138.52	n.s
HCT	0.39a	0.28b	*
MCV	53.56b	69.54a	**
NEU %	48.26a	39.14b	***
LYM %	49.96b	59.92a	*
MON %	1.61b	1.80a	*
MON (G/L)	0.19a	0.15b	*
NEU (G/L)	4	3.95	n.s
LYM (G/L)	8.44	8.32	n.s
EOS (%)	5.36a	4.75b	*
PLT (G/l)	2.62a	1.63b	*
MCHC (g/l)	351.36a	313.20b	**
BAS (%)	0.20a	0.15b	*

Our results obtained for the hematological and biochemical profile in the donkey are in agreement with those published by (Mori et al., 2003). Lemma and Moges (2009) in a study realized in Ethiopia observed that clinical and hematological values were not affected by the use of donkeys in agricultural work.

The osmotic fragility of erythrocytes is used to determine the level of stress in animals (Hesta et al., 2008).

In Figure 1, a-c are represented graphically the hematological parameters under the influence of the summer and winter season.

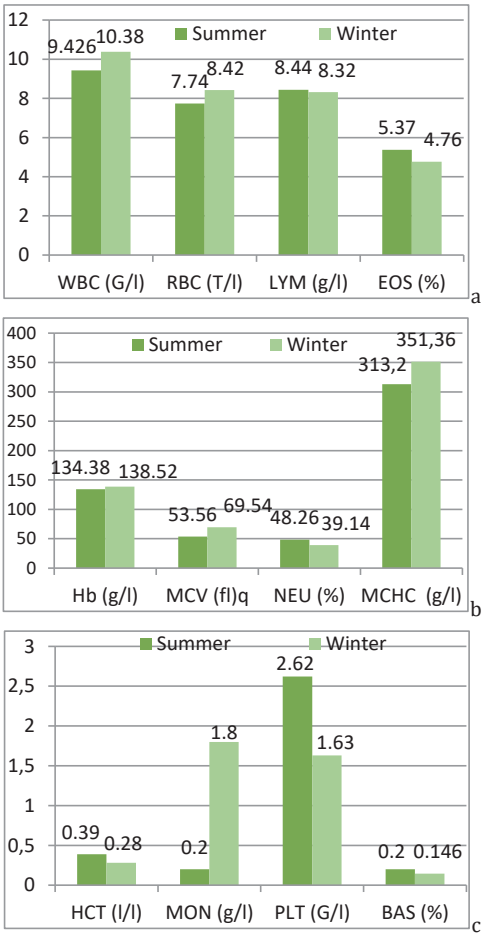


Figure 1 a-c. The average values for the hematological parameters in the summer and winter season in the donkey

The environment in which animals are raised may influence the animals' ability to maintain thermal equilibrium, which is also related to their thermal characteristics and in regulation of physiological mechanisms (Castanheira et al., 2010).

Urea was influenced by the season as follows: in the summer season urea varied between 5.93 ± 0.47 and 7.77 ± 0.59 mmol/L (Tables 4 and 5).

Table 4.The influence of the season (summer) on the biochemical profile in the donkey

Parameter	Summer (n = 10)	
	X ± sx	V%
Urea (mmol/l)	5.93±0.47	17.54
Total protein (g/l)	55.44±5.14	20.72
Glucose (mmol/l)	5.48±0.65	26.64
Albumin (g/l)	21.59±1.26	13.02
Creatinine (µmol/L)	99.55±3.57	8.02
Cholesterol (mmol/L)	1.82±0.14	16.99
Potassium (K) - (mmol/L)	4.29±0.20	10.21
Triglycerides (mmol/L)	0.45±0.05	24.23
Total calcium (mmol/L)	2.06±0.04	3.88
Sodium (Na) (mmol/L)	115.48±1.83	3.54
Total bilirubin (mmol/L)	7.82±0.12	3.45
Mg (mmol/L)	0.69±0.04	12.60
ALT (U/L)	12.96±0.46	8.00
AST (U/L)	252.02±2.60	2.31
ALP (U/L)	214.30±6.24	6.51
GGT (U/L)	84.10±2.74	7.29
CK (U/L)	196.00±2.33	2.66

V - variability; X - average value; n - number of blood samples.

Table 5. The influence of the season (winter) on the biochemical profile in the donkey

Parameter	Winter (n = 10)	
	X ± sx	V%
Urea (mmol/l)	7.77±0.59	17.10
Total protein (g/l)	76.92±2.82	8.19
Glucose (mmol/l)	6.01±0.81	29.96
Albumin (g/l)	22.5±1.53	15.22
Creatinine (µmol/L)	112.00±6.59	13.17
Cholesterol (mmol/L)	1.95±0.06	7.13
Potassium (K) - (mmol/L)	4.94±0.06	2.71
Triglycerides (mmol/L)	0.71±0.09	27.68
Total calcium (mmol/L)	2.21±0.10	10.36
Sodium (Na) (mmol/L)	135.26±2.53	4.19
Total bilirubin (mmol/L)	8.352±0.31	8.35
Mg (mmol/L)	0.71±0.09	27.74
ALT (U/L)	15.63±0.43	6.19
AST (U/L)	273.48±5.51	4.51
ALP (U/L)	230.48±3.27	3.17
GGT (U/L)	85.60±2.28	5.96
CK (U/L)	174.76±5.39	6.90

V - variability; X - average value; n - number of blood samples.

The total protein concentration did not show major changes compared to the results published on this topic (Tomenendalova et al., 2014; Kuttner and Wiesner, 1987; Gupta et al., 2005).

The total protein was 55.44 ± 5.14 g/l in the summer season and 76.92 ± 2.82 g/l in the winter season (Tables 4 and 5).

Creatinine showed an average values of 99.55 ± 3.57 µmol/L in summer and, 112.00 ± 6.59 µmol/L in winter. Cholesterol is an important parameter for the body, and his changes give us important information about the major disturbances that occur in the body. This parameter was significantly influenced by the season, and it was 1.82 ± 0.14 mmol/L in summer, and 1.95 ± 0.06 mmol/L in winter (Tables 4 and 5).

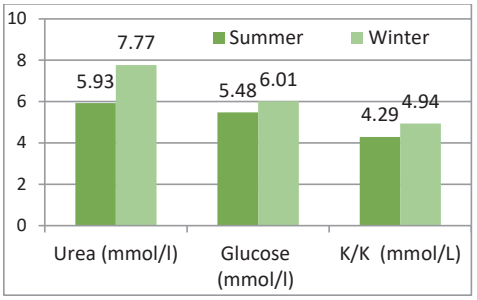
Similar results with our study were reported by Zinkl et al. (1997), Jordana et al. (1998), Alves (2008), Etana et al. (2011), Girardi et al. (2013) and Sgorbini et al. (2013). However, serum biochemical parameters, with the exception of total protein, were significantly affected by the fact that the animals were used in agricultural work (Hanzawa and Watanabe, 2000; Koubkova et al., 2002).

Table 6. Statistical interpretation of the biochemical parameters of the blood under the influence of the season

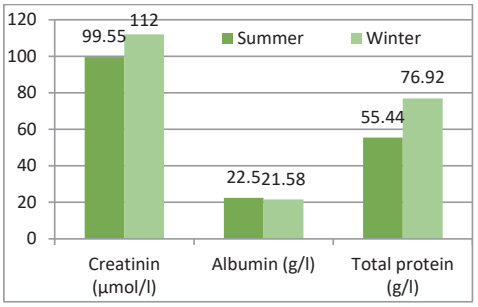
Parameter	Summer	Winter	Significance
Urea (mmol/l)	5.93b	7.76a	***
Total protein (g/l)	55.44b	76.92a	**
Glucose (mmol/l)	5.48	6.01	n.s.
Albumin (g/l)	21.58	22.5	n.s.
Creatinine(µmol/L)	99.55	106.98	n.s.
Cholesterol (mmol/L)	1.81	1.95	n.s.
Potassium(K) (mmol/L)	4.29b	4.94a	*
Triglycerides (mmol/L)	0.44b	0.71a	*
Total calcium (mmol/L)	2.05	2.13	n.s.
Sodium (Na) (mmol/L)	115.48b	135.26a	**
Total bilirubin (mmol/L)	7.81b	8.58a	*
Magnesium (Mg) (mmol/L)	0.67	0.71	n.s.
ALT/ALT (U/L)	12.96b	15.63a	**
AST/AST (U/L)	252.02b	273.48a	**
ALP/ALP (U/L)	214.30b	230.48a	*
GGT/GGT (U/L)	84.1	85.6	n.s.
CK/CK (U/L)	196.00a	174.76b	**

The statistical interpretation of the results regarding the influence of the season on the

biochemical profile in the donkey is shown in Table 6.



a



b

Figure 2 a-b. The average values for the biochemical parameters in the summer and winter season in the donkey

In figure 2 a-b are shown the average values for the biochemical parameters of the blood in the donkey depending on the season.

CONCLUSIONS

The season (summer *vs*. winter) had a significant influence on the hematological and biochemical parameters in the donkey (*Equus asinus*). Most hematological and biochemical parameters had the highest average values in the winter season than summer season.

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REFERENCES

Castanheira, M., Paiva, S.R., Louvandini, H., Landim, A., Fiorvanti, M.C.S., Paludo, G.R., McManus, C. (2010). Multivariate analysis for characteristics of heat tolerance in horses in Brazil. *Trop Anim Health Prod.*, 42, 185-191.

Cywinska, A., Czopowicz, M., Witkowski, L., Gorecka, R., Degorski, A., Guzera, M. (2015). Reference intervals for selected hematological and biochemical variables in Hucul horses. *Pol J Vet Sci.,* 18, 439-445.

Etana, K.M., Jenbere, T.S., Bojia, E., Negussie, H. (2011). Determination of reference hematological and serum-biochemical values for working donkeys of Ethiopia. *Vet. Res.,* 4, 90-94.

Fazio, F., Assenza, A., Tosto, F., Casella, S., Piccione, G., Caola, G. (2011). Training and haematochemical profile in thoroughbreds and standardbreds: a longitudinal study. *Livest Sci.,* 141, 221-226.

Girardi, A.M., Marques, L.C., Toledo, C.Z.P., Barbosa, J.C., Maldonado, W., Jorge, R.L.N., Nogueira, C.A.S. (2013). Biochemical profile of the Pêga donkey (*Equus asniuns*) breed: influence of age and sex. *Comp. Clin. Pathol.*, 23(4), 941-947.

Gupta, A.K., Pal, Y., Tandon, S.N., Dwivedi, S.K. (2005). Haematological and biochemical blood profiles in healthy Indian Spiti horses. *Indian Veterinary Journal,* 82, 604-608.

Hanzawa, K., Watanabe, S. (2000). Changes in osmotic fragility of erythrocytes during exercise in athletic horses. *J Equine Sci.,* 11, 51-61.

Hesta, M., Ottermans, C., Krammer-Lucas, S., Zentek, J., Helweg, P., Buyse, J. (2008). The effect of vitamin C supplementation in healthy dogs on antioxidative capacity and immune parameters. *J Anim Physiol Anim Nutr.,* 93, 26-34.

Jordana, J., Folch, P., Cuenca R. (1998). Clinical biochemical parameters of the endangered Catalonian donkey breed: normal values and the influence of sex, age, and management practices effect. *Res. Vet. Sci*., 64(1), 7-10.

Koubkova, M., Knizkova, I., Kunc, P., Hartlova, H., Flusser, J., Dolezal, O. (2002). Influence of high environmental temperature and evaporative cooling on some physiological, haematological and biochemical parameters in high-yielding dairy cows. *Czech J. Anim. Sci.,* 47, 309-318.

Krumrych, W. (2009). Effect of physical exercise on oxygen metabolism of neutrophils in horses. *Bull Vet Inst Pulawy.,* 53, 97-103.

Kuttner, C., Wiesner, H. (1987). Changes of blood values in Przewalski horses (*Equus przewalski przewalski*) and zebras (*Equus zebra hartmannae*) during chemical immobilization. *Journal of Zoo Animal Medicine*, 18, 144-147.

Laus, F., Spaterna, A., Faillace, V., Paggi, E., Serri, E., Vullo, C., Cerquetella, M., Tesei, B. (2015). Reference for hematological and biochemical parameters of mixed breed donkeys (*Equus asinus*). *Wulfenia Journal,* 22, 294-304.

Lemma, A., Moges, M. (2009). Clinical, haematological and serum biochemical reference values of working

donkeys (*Equus asinus*) owned by transport operators in Addis Ababa, *Ethiopia. Heart.*, 36, 38-43.

Lorena, D., Da Ponte, A., Cozzi, M., Carbone, A., Pomati, M., Nava, I., Cappellini, M.D., Fiorelli, G. (2006). Changes in erythropoiesis iron metabolism and oxidative stress after half-marathon. *Intern Emerg Med.,* 1, 30-34.

Michael, L., Schulkin, J. (2013). Maternal regulation of offspring development in mammals is an ancient adaptation tied to lactation. *Applied & Translational Genomics*, 2(1) 55-63.

Mori, E., Fernandes, W.R., Mirandola, R.M.S., Kubo, G., Ferreira, R.R., Oliveira, J.V., Gacek, F. (2003). Reference values on serum biochemical parameters of Brazilizan donkey (*Equus asinus*) breed. *J. Equine Vet. Sci.,* 23(8), 358-364.

Mori, E., Fernandes, W.R., Mirandola, R.M.S., Kubo, G., Ferreira, R.R., Oliveira, J.V., Gacek, F. (2003). Reference values on serum biochemical parameters of brazilian donkey (*Equus asinus*) breed. *Journal of Equine Veterinary Science,* 23, 358-364.

Mueller, P.J., Jones, M.T., Rawson, R.E., Van Soest, P.J., Hintz, H.F. (1994). Effect of increasing work rate on metabolic responses of the donkeys (*Equus asinus*). *Am. Physio.l Soc.,* 77, 1431-1438.

Olaifa, F., Joseph, O., Ayo, S., Ambali, P., Rekwot, I. (2012). Effect of packing on changes in erythrocyte osmotic fragility and malondialdehyde concentration in donkeys administered with ascorbic acid. *Onderstepoort Journal of Veterinary Res.*, 79(1).

Pandey, N., Kataria, N., Kataria, A.K., Joshi, A. (2012). Ambient stress associated variations in metabolic responses of Marwari goat of arid tracts in India. *J Stress Physiol Biochem.,* 8:120-127.

Piccione, G., Grasso, F., Fazio, F., Giudice, E. (2008). The effect of physical exercise on the daily rhythm of platelet aggregation and body temperature in horses. *Vet J.,* 176, 216-220.

Plotka, E.D., Eagle, T.C., Gaulke, S.J., Tester, J.R., Sniff, D.B. (1988).Hematological and blood chemical characteristics of feral horses from three management areas. *J Wildl Dis.*, 24, 231-239.

Raymond, S.L., Smith, T.K., Swamy, H.V. (2003). Effects of feeding a blend of grains naturally contaminated with Fusarium mycotoxins on feed intake, serum chemistry, and hematology of horses, and the efficacy of a polymeric glucomannan mycotoxin adsorbent. *J Anim Sci.,* 81, 2123-2130.

Rico, A.G., Braun, J.P., Benard, P., Bardies, J., Thouvenot, J.P., Periquet, B. (1978). Blood serum biochemistry of ponies. *Ann Rech Vet.*, 9, 393-399.

Ruiz, G., Rosenmann, M., Cortes, A. (2004). Thermal acclimation and seasonal variations of erythrocyte size in the Andean mouse *Phyllotis xanthopygus rupestres*. *Comp Biochem Physiol.*, 139, 405-409.

Satué, K., Muñoz, A., Blanco, O. (2010). Pregnancy influences the hematological profile of *Carthusian broodmares*. *Pol J Vet Sci.*, 13, 393-394.

Satué, K., Muñoz, A., Montesinos, P. (2011). Seasonal variations in the erythrogram in pregnant *Carthusian mares*. Proceedings of 13th Conference of the European Society of Veterinary Clinical Pathology, Dublin, Ireland, 65-73.

Sgorbini, M., Bonelli, F., Rota, A., Baragli, P., Marchetti, V., Corazza, M. (2013). Hematology and clinical chemistry in Amiata donkey foals from birth to 2 months of Age. *J. Equine Vet. Sci.* 33(1), 35-39.

Sgorbini, M., Bonelli, F., Rota, A., Baragli, P., Marchetti, V., Corazza, M. (2013). Hematology and clinical chemistry in Amiata donkey foals from birth to 2 months of age. *J. Equine Vet. Sci.,* 33(1), 35-39.

Shawaf, T., (2017). Some haematological and serum biochemical parameters in apparently clinically healthy Hassawi donkey. *Alex J Vet Sci.*, 53, 125-130.

Tomenendalova, J.R., Vodicka, I., Uhrikova, J. (2014). Determination of haematological and biochemical parameters of Przewalski horses (*Equus przewalski*) kept by the Prague Zoo. *Veterinarni Medicina*, 59(1), 11-21.

Yakubu, A.S., Chafe, U.M. (2008). Haematological studies of donkeys in sokoto state, Nigeria, Sokoto, *The Journal of Veterinary Science,* 7, 41-45.

Zinkl, J.G., Mae, D., Guzman, M.P., Farver, T.B., Humble, J.A. (1990). Reference ranges and the influence of age and sex on hematologic and serum biochemical values in donkeys (*Equus asinus*). *Am. J. Vet. Res.* 51(3), 40.

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STUDY ON THE NUMBER OF CATTLE AND PRODUCTION OBTAINED IN NEAMȚ COUNTY BETWEEN 2010-2018

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Abstract

This paper aims to present the evolution of cattle numbers and production obtained in Neamț County during between 2010-2018. In carrying out this work, we have used statistical data provided by the Directorate for Agriculture and Rural Development, the County Animal Husbandry Office and the Association of Animal Breeders "Operator A.I.". The analysis was carried out within two forms of property: population households and companies and private associations. The following were found: the population households registered a decrease in the number of cattle (ca. 15%) and in the average milk production (ca. 20%), while the companies and private associations had an increase in the cattle population by 44.4% and in the average milk production by 21%. With regard to meat production, the average weight for slaughter increased from 339 kg/head in 2010 to 451 kg/head in 2018, the share being 33.12%. The study found an improvement of the main indicators (cattle number and production) only in the holdings that have at least 40-50 heads compared to the holdings with 1-2 heads.

Key words: *cattle, indicators, meat, milk, Neamț.*

INTRODUCTION

The beef and dairy cattle raising represents a separate production compartment of zoologic culture that is and will remain in the attention of the specialists due to the importance of this sector for the national economy. By its biological ability to convert feed nutrients into valuable products (milk and meat) for human consumption, cattle contribute to the increase of the living standard. Milk, by its composition, meets the requirements for breeding young animals. Over time, genetic and technological improvement (including nutrition), especially in cattle, have led to an increase in milk production to an extent where it can meet a large part of the food requirements of human population in many countries of the world (Halga, 2005). The present age of human society development is characterized by a demographic explosion with a steadily increasing rate of population growth. This is accompanied by an increase in the demand for food, especially of animal origin, and the data in the F.A.O. (www.fao.org) report for 2018 show that approximately 820 million people (almost 11% of the planet's population) deal with food scarcity, more precisely, suffering from hunger and malnutrition. The world total demand for animal products is covered by approximately 42% of developed countries, 12% of developing countries in Group I and over 46% of those in Group II (Georgescu, 2000). In this context, we present the importance of the growth and exploitation of this species, which owns ca. 65% (UVM) of the total number of domestic animals throughout the world and represents the main source of milk and meat, providing over 95% of milk production, 33% of meat production and approximately 90% of the production of hide used in the light industry (Georgescu and Ujică, 1988), to which other important by-products are added (unconventional energy, organic fertilizers etc.). In view of the increasing demand for milk and meat, this can be achieved by increasing the number of cattle and improving their genetics through science and advanced technologies (Ivancia, 2007).

Based on these considerations and taking into account that, in Neamț County, the raising of cattle has always been more consistent compared to other species, we consider this area to offer sufficient arguments to make a study on the evolution of cattle and their productions obtained between 2010 and 2018.

MATERIALS AND METHODS

In order to analyse the results obtained in the field of cattle breeding in Neamț County, a number of indicators were studied: total number of cattle, head-cattle, dairy cows, average milk production per cow head, average weight at slaughter (Acatincăi, 2004). These results were obtained by performing the Official Production Control - milk production in EM (equivalent maturity) over standard lactation (305 days), at the breed level, the situation of the use of the males, according to the number of artificial seeding and natural mounts, as well as from the point of view of combining and correlating the data with numerous observations from the farms studied.

RESULTS AND DISCUSSIONS

During the period studied, we find that the number of cattle decreased from 88,980 heads in 2010 to 75,630 in 2018 (as seen in Table 1 and Figure 1). This decrease occurred mainly in the cattle population owned by the farmers in the area: the explanation is the reduced cattle population as most of the individual households have 1-2 cows and only 0.3% have more than 5 cows per household (Ujică and Maciuc, 2007) and the low productive levels that the species is not profitable and the household has no interest in it.

However, there is an increase in the number of cattle in private companies and associations by 44.4%, most of them with 40-50 heads and the tendency to reach 80-100 heads. This type of cattle farm has not lost its strictly family character.

The basic technological areas (feeding, watering, milking, disposal of manure, etc.) should be automated and computer monitored, thus creating optimal conditions for breeding and exploitation for 100 cows by one farmer and his family members (Otiman, 2006).

Table 1. The dynamics of the cattle population in Neamț County during the years 2010-2018

No.	**Indicator name/Year of reference**	2010	2012	2014	2016	2018	**2010/2018 % (+;-)**
1.	Total number of heads of which:	88,980	86,690	80,682	77,595	75,630	-15.00
2.	Number of cattle in population households	85,774	83,100	77,593	73,525	70,831	-17.42
3.	Number of cattle in companies and private associations	3,206	3,590	3,589	4,070	4,632	+44.44
4.	Head-cattle	44,231	44,028	45,108	47,731	47,041	+6.35
5.	Number of lactating cows	39,860	40,718	43,570	46,041	4,030	+15.47

Source: Directorate for Agriculture and Rural Development

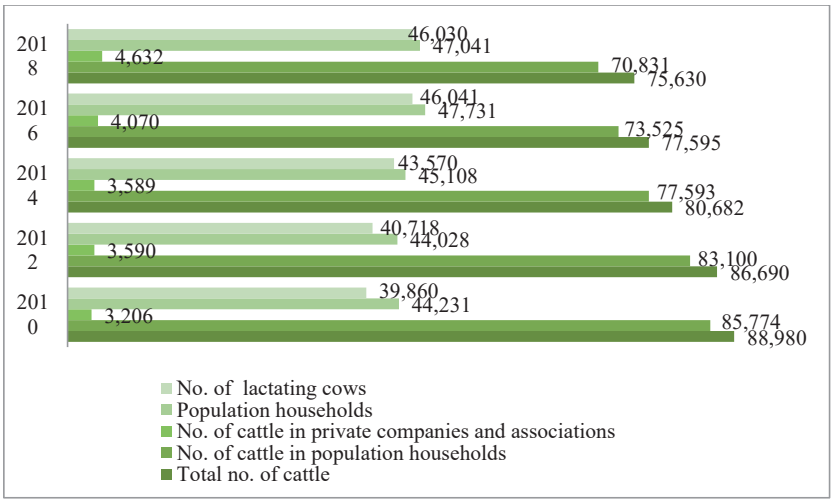

Figure 1. The dynamics of cattle population in Neamț County during the years 2010-2018

Table 2. The dynamics of milk production in dairy cattle in Neamț County during the years 2010-2018

Reference period/Indicator name	Average cattle feed per head (kg)	Average milk production litres per head
2010	39,860	4,113
2012	40,718	3,999
2014	43,570	3,672
2016	46,041	3,041
2018	46,030	3,300

Table 3. Dynamics of beef production in Neamț County during 2010-2018

Reference period/Indicator name	No. of heads slaughtered	Meat production (thousands tons)	Average weight kg per head
2010	32,517	11,012	338
2012	32,944	11,776	357
2014	18,294	7,293	398
2016	15,880	6,668	419
2018	18,697	8,429	451

In Tables 2 and 3 we present the dynamics of the two main productions: milk and meat. We find that the average milk production has decreased continuously from 4,113 litres/head in 2010 to 3,300 litres/head in 2018 in the dairy cattle raised in the households of the population where there is no performance feeding to directly and obviously enhance the level of animal production (Pop, 2006) and where, according to the official control of the performance (COP), the genetic improvement of cattle and reproduction directed mainly by artificial insemination is not applied (Grosu, 2005). If we analyze the data obtained by performing the Official Production Control (Onaciu and Velea, 2000) in the farms studied with a herd of more than 40-50 heads, where cattle of the Brună de Maramureș and the Bălţată cu Negru Românească breeds are raised and exploited and applying modern exploitation technologies (Gemene, 2005), we observed an increase in the average milk production per animal head (as seen in Table 4.)

Table 4. Results obtained in the farms where the O.P.C. was performed

Specification	Average milk production per cow head (litres)		Race code Bull used in A. I.
	2010	2018	
Brună de Maramureș	4,510	5,470	BSW
Bălţată cu Negru Românească	3,750	4,542	HOL

The milk law project aims to regulate the marketing of dairy products, to increase consumer confidence in domestic dairy products and to eliminate falsified products (Coman et al., 2019).

With regard to performance in meat production, the average slaughter weight increased, from 339 kg/head in 2010, to 451 kg/head in 2018, the share being 33.12%, but the increase in meat production in cattle can be obtained not only by increasing the slaughter weight and improving the technological factors of meat production, but also by genetic methods, and crossing with specialized meat breeds is an easy method (Maciuc et al., 2018).

Analyzing the information obtained from the Neamt County Animal Husbandry Office, we can conclude that farmers in Neamț County, who have a small number of animals, have chosen to use in the breeding process of authorized breeding bulls of native breeds. Thus, in the meat production in 2018, 25 bulls were authorized for the natural mount of which a number of 15 bulls were Aberdeen Angus breed, 2 bulls of the Charolaise breed, 1 bull of the Aubrac breed and 7 bulls of the Bălţată Românească breed.

CONCLUSIONS

In Neamț County, there has been a decrease of about 15% in the number of cattle held by the population in the area during the period 2010-2018. This reduction was determined by: the extremely small size of the holdings (1-2 heads); the lack of organized milk collection and its unsatisfying price; mass slaughter of animals; the low level of zoological technical training and information of cattle growers; difficult access to loans in order to obtain financing from European funds for setting up zoological technical farms. However, we found a considerable increase of 44.4% in cattle numbers in farms raising and exploiting more than 40-50 heads, as well as an improvement of milk production; it is unanimously accepted that a larger dimension favours the increase in labour force use as well as in the fixed and working capital with direct and beneficial effect on the economic and social viability of the agricultural holding under commercial agriculture practice conditions. By considering the fact that we are currently importing a significant amount of milk and meat, though we have significant natural and human resources for raising and exploiting cattle in this area of the country in the future, it is necessary to: stimulate and support cattle breeders for the establishment of efficient farms of optimal sizes that can be exploited efficiently and generate profit; the further improvement of the cattle populations, in order to increase the productive potential; extending the biotechnology of artificial insemination by using material from bulls with higher breeding value in the direction of milk and meat production; exploiting the local tradition of raising cattle; increasing the economic power, the level of zoo technical training and information of breeders of this species. The achievement of these objectives implies the formation of a modern breeding sector, in the long term, which, based on the mechanisms specific to the market economy, will enable the full use of the natural and human resources in this area in order to ensure the food security of the population and to strengthen Romania's position in the exchanges of animal products on the world market.

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REFERENCES

Acatincăi, S. (2004). *Productions in bovines.* Second Edition. Timisoara, RO: Eurobit Publishing House.

Alexoiu, A., Roșca, L. (1988). *Practical guide for selection and management of mates in cattle farms.* București, RO: Ceres Publishing House.

Coman, I. T., Vidu, L., Marin, M.P., Ștefan, G., Mărginean, G.E. (2019). Proposals for legislative measures to improve the legal framework on the production and processing of milk, elimination of fake products from the milk market and growth of consumer trust. The milk law project. Scientific Papers, Series D. *Animal Science*, 62(1), 236-241.

Coman, I. T., Vidu, L., Trăistaru, C., Mărginean, G. E, (2019). European legal framework in manufacturing and processing of milk. The milk package. elimination of milk quota. Case study: Germany. Scientific Papers, Series D. *Animal Science*, 62(2),128-135.

Grosu, H., Pascal, A. (2005). *Genetic improvement programs in zootechnics*. Bucuresti, RO: Ceres Publishing House.

Maciuc, V. (2006). *Cattle Breeding Management,* Iasi, RO: Alfa Publishing House.

Maciuc, V., Nacu, G., Zaharia, V, Zota, D. (2003). *Animal Breeding*, Iasi, RO: Alpha Publishing House.

Maciuc, V, Ujica, V, Nistor, I., Dascalu, C. (2003) *Management of dairy cow breeding*, Iasi, RO: Alfa Publishing House.

Maciuc, V., Ujică, V., Nistor, I. (2003). *Practical guide for genetic improvment of cattle for milk production*. Iasi, RO: Alfa Publishing House.

Maciuc, V., Korlyuk, S., Tarasenko, L., Nistor, C. (2018). Research concerning the exploitation technologies and production of bovine meat in Romania's East Cross-Border Area. *Seria Zootehnie*, 70(23).

Marușca, T. (2001). *Elements of gradient and mountain ecology.* Brasov, RO: Transilvania University Publishing House.

Motcă, Gh., Oancea, I., Geamănu, L. (1994). *Romanian Meadows, Typology and Technology,* Bucuresti, RO: Agricultural Technical Publishing.

Onaciu, G., Velea, C. (2000). *Apreciation and control productions in cattle.* Cluj-Napoca, RO: Carti de stiinta Publishing House.

Podar, C., Oroian, I. (2003). *Raising and exploiting dairy cows in population households.* Targu Mures, RO: Tipomar Publishing House.

Pop, I.M., Halga, P., Avarvarei T. (2006). *Animal nutrition and feeding*. Iasi, RO: Tipo Moldova Publishing House.

Rey, R. (1985). *The Mountain Civilization*, Bucuresti, RO: Scientific and Encyclopedic Publishing House.
Statistical Yearbook of Romania, 2010-2018, National Institute of Statistics.
Șut-Gherman, M. (2006). *The effect of cattle breeding on the environment*, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca.
Usturoi, M.G. (2007). *Milk and derivates technology*. Iasi, RO: Alfa Publishing House.
Virginia, Z., Maciuc, V., Nacu, Gh., Zota, D. (2003). *Animal breeding*. Iasi, RO: Alfa Publishing House.
*** Law no.18 / 1991 - Land fund law. Retrieved February 25, 2020, from http://www.cdep.ro/pls/legis/legis_pck.htp_act_text?idt=1622.
***Regulation (EC) no. 73/2009 of the Council of 19 January 2009 establishing common rules for direct aid systems for farmers under the common agricultural policy and establishing certain aid schemes for farmers, amending Regulations (EC) no. 1290/2005, (EC) no. 247/2006, (EC) no. 378/2007 and repealing Regulation (EC) no. 1782/2003. Retrieved February 24, 202, from https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A32009R0073.
*** http://www.madr.ro
*** http://www.insse.ro

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RESEARCH REGARDING THE INFLUENCE OF LACTATION STAGE ON MILK PRODUCTION AT CARPATINA BREED

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Abstract

Research aimed to enlighten the effect of age on milk production obtained from local goat populations. To limit the effect of some external factors on lactogen capacity the formed batches were maintained in permanent stabulation on the whole period of the respective lactation. Biological material belonged to local breed Carpatina, being represented by 7 batches differentiating by age from 2.5 years to 9 years, each of them being constituted by 10 females in lactation, all being subjected to the same experimental treatment. Evaluation of performances for milk production was based on some determinations accepted by experimental technique, using Nica method for lactation period and for period in which females were exclusively milked was applied the AT4 method respecting the technical specifications suggest by International Committee for Animal Recording. The obtained results were statistically processed by statistical analysis using REML (REstricted Maximum Likelihood) algorithm, which offers the obtaining of statistical estimating parameters in normal interval. Analyse of recorded performances with the occasion of applying of productive control highlight that at batch formed by females which have in the second and third lactation the age of four and five years was recorded a mean milk production higher with 14.38% and respectively 32% face to the ones in the first lactation. The obtained results are very important because, in Romania, the sector represented by goat rearing is into an advanced development and modernization stage as well regarding the applied technologies.

Key words*: age, Carpatina goat, fat, milk production, protein.*

INTRODUCTION

Nowadays, in European Union, goat rearing represents, for some countries, a traditional activity which dates since ancient times. Nevertheless, both sheep as well as goats enjoys a real interest, particularly in countries with large areas of pasturelands which couldn't be capitalized by other herbivorous breeds.

The realised research is important because aimed to show the influence of age on production performances, with positive effects on economical efficiency specific for basic productions obtained from goats.

Directly the main goal of the current studies was represented by evaluation of age at which are obtained the highest milk productions at Carpatina goat breed reared in the habitat representing by the North-East area of Romania. Knowledge of this aspect could contribute to the development of the sector, based on application of a management adequate to the development level of the exploitation.

Function of this aspect farmer could establish an age structure which will allow the facilitation in obtaining of superior productions. Also, in the production nucleolus for farm animals, batches are formed by farmer function of its needs and criteria (Margetínová et al., 2003).

One of the criteria could be represented also by age structure which could contribute to obtaining of superior milk productions. In the effectuated research was observed that face to the level recorded at first lactation, at Carpatina breed milk production progressively increase with 15% at second lactation and with around 32% at the third lactation. After this moment the production level decrease and lactation curve on productive life decrease.

So, in conditions in which milk production represent the main reason for goat rearing, in

the structure of flock must dominate the goats which are in the first three lactations.

MATERIALS AND METHODS

Biological material was represented by adult goats from Carpatina breed constituted in batches function of age group, maintained on the whole duration of reproduction and production cycle in permanent stabulation. To study the effect due to age was established a batch of 70 females with age from 2.5 years to 9 years and with a homogenous corporal mass for each batch, all of them being subjected to the same experimental treatment.

The performance evaluation for milk yielded during the lactation submitted to the control was based on the application of successive productive checking, using for the lactation period the Nica method (this process takes into account the fact that 1 kg gain achieved by lambs in the lactating period is obtained with 4.5 kg milk until the age of 60 days), while for the period in which the females were exclusively milked the applied method has been AT4 respecting the technical specifications suggested by the International Committee for Animal Recording.

Under the applied system, the total duration of lactation was 205 days. For the period of lactation the first control was planned at 28 days from the moment of lambing and the second one during the 58th day of lactation. For the milking period after lamb weaning, controls were placed at regular intervals of 28 days.

Individual milk yields have been recorded alternated, during the morning milking and in the evening milking at the next control to, and so on. For each productive checkout was utilised the same experimental treatment and estimation of the average total production of milk was carried out using the Fleischmann method, in according with the model described below.

$$\text{Milk yield [kg]} = L_1.\text{int}_1 + \sum_{i=2an}\left(\frac{L_i + L_{i-1}}{2}.\text{int}_i\right) + L_n.14$$

where:

L_1 = milk yield of the 1st monthly test;

L_i = milk yield of the ith monthly test (i = 1,…, n);

L_n = milk yield of the last test;

int_1 = number of days from kidding to 1st monthly test;

int_i = number of days between monthly tests (i-1) and i (i = 1,...,n);

n = total number of monthly test for a specific animal.

Determination of raw chemical composition of goat milk was realised on samples gathered from total milk quantity obtained at each effectuated quantitative control using “Milko-Scan” and “Ecomilk” respectively, both devices based on spectrophotometry.

The achieved results have been input into the data base, used to run statistical analysis with the algorithm REML (REstricted Maximum Likelihood), which provides the achievements of the statistical parametric estimators within the normal range.

The REML estimator is the maximum likelihood estimate of the parameters which uses only the information not contained in the estimate of the regression vector, and thereby automatically corrects for the degrees of freedom which are lost in estimating the regression vector.

RESULTS AND DISCUSSIONS

The necessity of these types of research have as technical support the continuous development of the sector represented by goats rearing and the breeders interest on Carpatina breed which in comparison with other imported breed, have a very good adaptability to the local pedo-climatic conditions.

Quantitative control of total milk production in relation with lactation numbers represented the main target of the effectuated research. Based on the results was observed that, from quantitative point of view, milk production has some particularities determined by lactation month, age, individuality and other factors.

Also, through statistical processing of data recorded on the basis of applied controls on a certain lactation could be noticed the fact that

the higher milk quantities was obtained from goats which were in the third and respectively in the fourth lactation, belonging to the age groups of five and respectively six years (Table 1 and Figure 1).

In literature are many data which highlight the fact the age and number of products at calving influence the level of milk production at goats (Pascal, 2015). Others information sustain the fact that milk production increase with aging, because while animal's age increase, hormonal state of animal body, metabolic activity, secreting cells and intake of nutrients which are used in milk synthesis increase in intensity (Carnicela et al., 2008; Capuco et al., 2001). Analysis of mean milk production distribution in connection with age show an intense increase in the first three lactations after which the determined mean level has a slower decreasing. So, at batch formed by females which have at the second and third lactation the age of four years and five years was recorded a mean milk production higher with 14.38% and respectively 32% face to the ones which were at the first lactation (Table 1).

Based on the obtained results could be observed that age and respectively number of lactations is strongly connected to lactogen capacity of goats. In others studies effectuated on other breeds is highlighted the fact that those ones produce in the first lactation 65-75%, and in the second around 75-85% from milk production obtained from goats with age between four and six years (Capuco et al., 2001; Mochnacs et al., 1978).

The statistical processing of the obtained data regarding total milk quantity obtained from local goats in relation with age show the fact that the highest difference was of 83,121 kg and was recorded from females which were into the third lactation and respectively into the seventh lactation. All the observed differences were statistically significant for $P<0.5$.

This thing presents a remarkable practical importance because by control technical actions, effectuated in the first lactations, we could obtain quite quick information regarding productive level characteristic to goats, aspect which allow also the application of some earlier and more efficient selection on reproduction biological material.

The realised research confirms the fact that into local goats populations presented in the North-East area of Romania are nucleus and individuals with a superior productive potential. In these conditions, if those populations will be subjected to an intense selection process will be possible the prediction that into a certain not so far away time interval will take place a radical change of the quality of biological material, as well as a remarkable increase of productive performances.

Table 1. The level of milk production in correlation with age and lactation number

Lactation	Age	n	Total milk production (kg)		Difference face to the level recorded at first lactation	
			$\bar{x} \pm s_{\bar{x}}$	V (%)	absolute (kg)	relative (%)
Ist	2.5	10	157,534 ± 0.654	11.9	-	-
IInd	4	10	183,974 ± 0.327	12.7	+ 26,440	+ 14.38
IIIrd	5	10	232,098 ± 0.341	13.5	+ 74,564	+ 32.12
IVth	6	10	218,987 ± 0.401	11.7	+ 61,453	+ 28.06
Vth	7	10	188,896 ± 0.243	12.5	+ 31,362	+ 16.60
VIth	8	10	163,988 ± 0.189	9.2	+ 6,454	+ 3.93
VIIth	9	10	148,977 ± 0.274	9.3	- 8,573	- 5.43

		L7	L6	L5	L4	L3	L2	L1
Tukey Test	L1	+ 8,557	- 6,454	- 31,362	- 61,453	-74,564	- 26,440	
	L2	+34,997	+19,986	+4,922	-35,013	-48,124		
	L3	+83,121	+68,110	+43,202	+13,111			
	L4	+70,010	+54,999	+30,091				
	L5	39,919	24,908					
	L6	+15,011						
	L7							

Significant at the 0.05 level (w = 6,741);
n.s: not significant

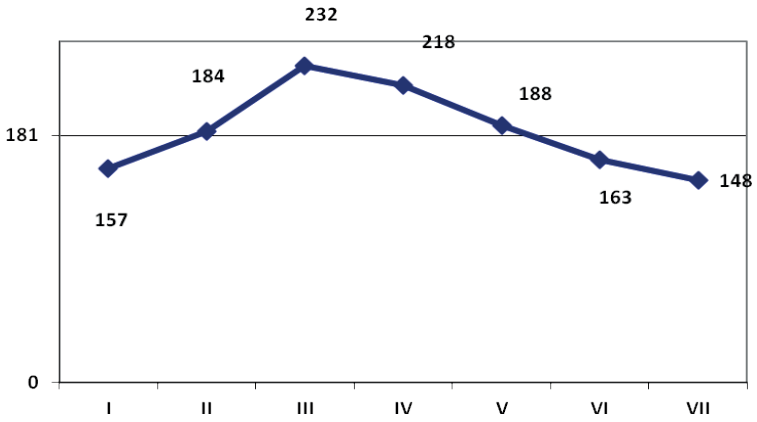

Figure 1. Milk production curve in relation with lactation number and age (kg)

The success could be complete if all those activities will be associated with others which aim to improve maintenance conditions, a foddering in according with nutritional demands specific for each physiological state, conducted rearing of goat youth, pastureland improvement, extension of maintenance in permanent stabulation, optimal dimensioning of age structure etc.

The role and influence of age on productive potential of goats were also studied in other countries but on different breeds (Alderson et al., 1980). All the effectuated research shown the fact that age influence the productive aptitude in the way of decreasing of milk production at the moment in which goats overpass the age of 4-5 years.

In Romania the studies effectuated on Carpatina breed reared in Moldova show that total mean milk production had close values (Zaharia, 2011a).

Evaluation of goat milk quality was realised on samples gathered from total obtained quantity at each of the productive checkouts applied into lactation subjected to control. After gathering were realised samples which were analysed to determine fat and protein content.

Analysis of mean values shows differences of fat and protein content function of lactation's interval in which samples were gathered. So, evolution of fat rate from dry matter had an ascendant dynamic from 3.53% at checkout effectuated in the first 60 days of lactation till 5.22% at the samples gathered at the last checkout effectuated at the beginning of autumn. Practically, on the interval between first and last checkout, the total fat quantity from dry matter increased with more than 30% (Table 2).

Analysing the evolution of basic components which form the milk's dry matter, respectively protein and fat, we can observe that on the interval of the same lactation the recorded levels are different (Figure 2). So, while protein percent is situated almost at the same level at each checkout interval, fat content recorded higher mean values while the lactation is getting to its end.

Table 2. Evolution of fat and protein content in relation with checkout interval (% from DM)

Lactation stage	Fat		Protein	
	$\bar{X} \pm s_{\bar{X}}$	V (%)	$\bar{X} \pm s_{\bar{X}}$	V (%)
Checkout la 50 days of lactation	3.53 ± 0.07	11.13	3.22 ± 0.09	10.31
Checkout la 100 days of lactation	3.80 ± 0.21	14.23	3.31 ± 0.21	11.22
Checkout la 150 days of lactation	4.16 ± 0.11	14.82	3.42 ± 0. 20	12. 28
Checkout la 200 days of lactation	4.25 ± 0.28	15.85	3.47 ± 0, 31	14.33
Checkout la 225 days of lactation	5.22 ± 0.13	13.97	3.61 ± 0.15	13.08
Total period	4.16 ± 0.80	-	4.11 ± 0.20	-

DM - dry matter

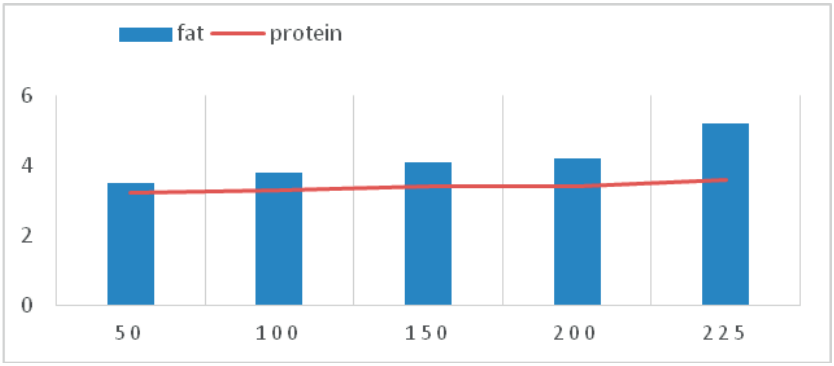

Figure 2. Evolution of fat and protein content in relation with lactation stage

In the case of determination which aimed the appreciation of protein content in relation with lactation phase, analyzing the mean data could be observed the fact that the mean values are lower in comparison with the ones determined for fat, but, also in this case could be noticed a progressive increase of total protein content as we approach to the moment of goats weaning.

Fat represent an important criterion in expressing milk quality because from its total, triacylglycerols constitute the greatest group (almost 98%), including a high number of esterification fatty acids, which are fats with very complex structures. In addition to them, into the fat matter from goat milk could be found other simple fats (diacylglycerols, monoacylglycerols and esters of cholesterol), complex lipids (phospholipids) as well as liposoluble compounds (sterols, esters of cholesterol, hydrocarbures) (Zaharia, 2011b). Fat from milk is presented and the shape of globules, characteristic for ovine and goats being that the majority of those globules (at goat, 65% less than 3 μ) had dimensions smaller than 3.5 μ (Park and Haenlein, 2006).

The lowest protein content was determined on the samples of milk gathered from total milk obtained at first checkout (3.22 ± 0.09% from DM) and the maximum value was of 3.61 ± 0.15% determined on the sample gathered at the last productive checkout. Goat milk contains a higher quantity of non-protein nitrogen and lower rate of nitrogen tied in casein. From this reason, goat milk had a lower efficiency regarding cheese production and weak structure and texture of yogurts, while sheep milk had a very strong coagulation power (Guo et al., 2003; Călin et al., 2015).

The highest value of variability showed the lack of homogeneity for both characters as well as the fact that by selection the rate of the basic milk components which determine the nutritive value to be upgraded to a superior level.

In the realised research could be observed that for fat and protein content weren't recorded significant statistical differences for the considered statistical levels.

Also regarding milk quality in numerous studies is shown that milk gathered from young goats had a better quality because have a higher fat content in comparison with the one gathered from other goats with an older age (Jaafar et al. 2018; Pascal, 2006; Taftă et al., 2006; Gall, 1986; Pearl et al., 1973; Jenness, 1980).

Into an ample study effectuated on goats reared and exploited in the North-East region of Romania it was observed that in the debut months of lactation, when also the milk quantity was greater, fat percent had mean values of around 3.5%, value recorded at the checkout effectuated in May, progressively increasing to the end of lactation up to 5.85%, maximal value which was recorded at the checkout from October.

In the same study is mentioned that not even in the case of milk quantitative production, nor in the case of fat content from milk recorded on the whole checkout period weren't observed significant statistical differences ($P<0.05$) between the studied goat populations (Zaharia, 2011b)

CONCLUSIONS

Statistical processing of data recorded on the basis of applied controls on a certain lactation

show the fact that the higher milk quantities was obtained from goats which were in the third and respectively in the fourth lactation, belonging to the age groups of five and respectively six years.

Study of milk mean production distribution in correlation with age show that at batch formed by females which have in the second and third lactation the age of four and five years were recorded a mean milk production higher with 14.38% and, respectively, 32% face to the ones in first lactation.

The practical importance of realised research highlights the fact that by control technical actions, realised in the first lactations, could be obtained information regarding goats' productive level, aspect which allow application of a more efficient selection on reproductive biological material.

Fat and protein recorded increases of mean values, while lactation is getting to the end showing a direct correlation with milk quantity.

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REFERENCES

Alderson, P., Pollak, E.J. (1980). Age - season adjustment factors for milk and fat of dairy goats. *J. Dairy Science*, 63, 148–151.

Carnicela, D., Dario, M., Consuelo, M., Ayres, C., Laudadio, V. (2008). The effect of diet, parity, year, and number of kids on milk yield and milk composition in Maltese goat. *J. Small Rumin. Researches*, 77, 71–74.

Capuco, A.V., Wood, D.L., Baldwin, R., Mcleod, K., Paape, M.J. (2001). Mammary cell number, proliferation and apoptosis during a bovine lactation: relation to milk production and effect of BST. *J. Dairy Science*, 84, 2177–2187.

Călin, I., Răducuţă, I., Dărăban, S., Vlad, I., Priseceanu, H.I., Pascal, C., Pădeanu, I. (2015). Research on quantitative skills in meat production direction at youth goats from Carpathian breed in relation with the rearing system**.** *Agriculture and Agricultural Science Procedia*, 6, 191–196.

Gall, C. (1996). *Goat breeds of the world*. Technical Centre for Agricultural and Rural Cooperation, Wageningen, The Netherlands, p. 186.

Guo, M., Aston, C., Burchett, S.A., Dyke, C., Fields, S., Rajarao, S.J., Uetz, P., Wang Y., Young, K., Dohlman, H.G. (2003). The yeast G protein alpha subunit Gpa1 transmits a signal through an RNA binding effect on protein Scp160. *Mol. Cell,* 12(2), 517–524.

Jaafar, S.H.S., Hashim, R., Hassan, Z., Arifin, N. (2018). A Comparative study on physicochemical characteristics of raw goat milk collected from different farms in Malaysia. *Trop Life Sci Res*., 29(1), 195–212.

Jenness, R. (1980). Composition and characteristics of goat milk: Review 1968-1979. *J. Dairy Sci*., 63, 1605–1630.

Margetínová, J., Brouček J., Apolen D., Mihina Š. (2003). Relationship between age, milk production and order of goats during automatic milking. *Czech J. Anim. Sci.*, 48(6), 257–264.

Mochnacs, M., Taftă, V., Vintilă, I. (1978). *Genetics and breeding of sheep*. Bucharest, RO: Ceres Publishing House.

Norris, D., Ngambi, J.W., Benyi, K., Mbajiorgu, C.A. (2011). Milk production of three exotic dairy goat genotypes in Limpopo Province, South Africa. *Asian Journal of Animal and Veterinary Advances*, 6, 274–281.

Park, Y.W., Haenlein, G.F.W. (2006). Overview of milks of minor species. In: *Handbook of Milk of Non-Bovine Mammals*. Blackwell Publishers. Ames, Iowa and Oxford, England. p. 3–10.

Pascal, C., Nechifor, I., Cristian, C., Florea, A.M. (2018). The influence of technological factors on the lactogen potential at goats. *Magyar Allatorvosok Lapja*, 140, 432–439.

Pascal, C. (2015). *Treated by raising sheep and goats*. Iaşi, RO: Ion Ionescu de la Brad Publishing House.

Pascal, C., (2012). The main diseases of sheep and goats, specific to the cold season. *International congress of Hungarian Association for Buiatrics, Proceedings* ISBN 978-963-87942-5-3. Budapest, Hungary, 188–198.

Pascal, C. (2006). Researches concerning at the quantitative and qualitative milk production at the indigenous goats growed and exploited in the North-Eastern area of Romania. *IGA World, Proceedings,* Goat in Central and Eastern European Countries: present and future, Constanţa, Romania.

Park, Y.W., Haenlein, G.F.W. (2006). *Goat Milk - Chemistry and Nutrition.* Iowa and Oxford, UK: Blackwell Publishing House, p. 34–58.

Pearl, S.L., Downey, H.F., Lepper, T.L. (1973). Intramammary pressure and mammary blood flow in lactating goats. *J. Dairy Science,* 56, 1319–1323.

Sandu, Gh. (1993). *Engineering in the operation of sheep*. Bucharest, RO: Alutus Publishing House.

Taftă, V., Vintilă, I., Zamfirescu, S. (1997). *Production, improving and reproduction of sheep*. Bucharest, RO: Ceres Publishing House.

Taftă, V., Machidon, N. (2006). Comparative researches regarding the fattening of goats and sheep youth in industrial system. *Proceeding of the International Symposium "Goat Farming in Central and Eastern European Countries: Present and Future*". Constanţa, Romania.

Torres-Vázquez, J.A., Valencia-Posadas, M., Castillo-Juárez, H.M., Hugo H.M. (2009). Genetic and phenotypic parameters of milk yield, milk

composition and age at first kidding in Saanen goats from Mexico, *Livestock Science*, 126 (1-3), 147–153.

Zaharia, N. (2011a). The study of some goat populations raised in the North-East part of Romania. *PhD thesis*, UASVM Iasi.

Zaharia, N., Salamon, R., Pascal, C., Zaharia, Roxana (2011b). Changes in fatty acid composition and cholesterol content of goat colostrums, *Biotechnology in Animal Husbandry*, Belgrade, 27, 1201–1209.

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STUDY REGARDING MORPHOLOGICAL PARAMETERS OF CERTAIN HORSE CATEGORIES OF SHAGYA ARABIAN BREED IN RĂDĂUȚI STUDFARM

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Abstract

This paper aimed to analyze height, heart perimeter and cannon girth of Shagya Arabian mares, stallions and young horses during their growth; Rădăuți studfarm provided data regarding livestock of these three categories of horses in 2000-2015 and it was interesting to study and compare each bloodline of this wonderful breed (Koheilan, Hadban, Dahoman, Shagya, Siglavy-Bagdady, El-Sbaa, Mersuch, Gazal - only for stallions and young horses). Therefore this study reflected that these body dimensions were representative for Shagya Arabian, regarding the whole population: mares had an average height of 159.59 cm, an average heart perimeter of 177.20 cm and an average cannon girth of 18.54 cm; regarding the stallions, the average height was 160.58 cm, the average heart perimeter was 180.61 cm and the average cannon girth was 18.95 cm; young male horses had an average value of height of 134.82 cm, an average thorax perimeter of 146.53 cm and an average cannon girth of 16.69 cm. Data obtained indicated that the studied population is included in breed standard regarding the main body dimensions that reflect their morphological type.

Key words*: body indexes, dimensions, horses, Shagya.*

INTRODUCTION

It's very important for sport and endurance horses to maintain certain body dimensions that reflect the characteristics of their breed and also performances. Therefore, we believe it was necessary to analyze the morphological aspects of different categories of Shagya Arabian horses (mares, stallions and young male horses), reared in Rădăuți studfarm, to establish if the breed followed its standards.

The Shagya Arabian horse breed was founded in 1785, in Bablona - Mezohegyes studfarm (Hungary); in 1809, this appendage of the studfarm became an exclusively Arabian breed rearing place. The eastern mares were mated with the famous stallion named Shagya brought from Syria in 1834 specially for founding this exceptional breed (Schipor G., 2007). In the following period the main purpose of the studfarm was to use various and appreciated sires to improve the Shagya Arabian horse breed (Dulugeac, 2005). The Rădăuți stock was founded in 1792, using the Barberino stallion and also Manachi and Hussein which were Pure Arabian sires; the idea was to create a strong horse breed for the Austrian army so that in 1919 when the studfarm was rebuilt there were used for mating Gazal, Siglavy-Bagdady I, Dahoman XXII and Shagya XV stallions and 31 mares (Manole et al., 2005).

In 1978, the World Arabian Horse Organization (W.A.H.O.) announced that all horses that were reared according to Bablona, Rădăuți and Topolcianky will be acknowledged as Shagya Arabian breed and no longer only a variety of Pure Arabian horse (half-breed).

In 1980 the main morphological parameters of adult Shagya Arabian horses were 151.46 ± 0.26 cm for mares and 152.80 ± 1.21 cm for stallions [8]. The reproduction purpose mentioned in Rădăuți studfarms' registers was to increase the height of mares up to 156 cm, the heart perimeter up to 178 cm and the cannon girth up to 19.5 cm; regarding the morphological parameters of stallions there was specified an increase of height up to 158 cm, the heart perimeter up to 178 cm and the cannon girth up to 20.5 cm.

A study regarding the body dimensions of young horses made by Doliș et al., 2011, reflected that females registered a height of

128.8 ± 1.03 cm and males a height of 135.0 ± 1.52 cm (Dolis et al., 2011).

There was a study made by Manole et al. (2004), regarding morphological parameters of Arabian horses reared in Rădăuți studfarm between 1992-2003 which revealed that the height of each genetic bloodline was different compared to the average height of population: Gazal (+1.58 cm), El-Sbaa (+1.08 cm), Hadban (+0.64 cm), Nedjari (+0.58 cm), Shagya (-1.08 cm), Dahoman (-0.62 cm), Siglavy-Bagdady (-0.45 cm), Koheilan (-0.18 cm). When the thorax perimeter was analyzed there were the following differences Gazal (+3.15 cm), Hadban (+2.89 cm), El-Sbaa (-1.87 cm), Mersuch (-0.99 cm) and regarding the cannon girth there were revealed differences comparing the average values of +0.44 cm for Gazal, +0.31 cm for Hadban, -0.39 cm for El-Sbaa and -0.12 cm for Koheilan (Manole and Radu-Rusu, 2004).

MATERIALS AND METHODS

The studied biological material consisted of three categories of Shagya Arabian horses: mares, stallions and young male horses, reared in 2000-2015, in Rădăuți studfarm. They belonged to Koheilan, Hadban, Dahoman, Shagya, Siglavy-Bagdady, El-Sbaa, Mersuch, Gazal – only for stallions and young horses – genetic bloodlines. We considered interesting in analyzing each bloodline to observe if there were differences between them regarding the height, the heart perimeter and the cannon girth.

Rădăuți studfarm registers revealed that the Shagya Arabian horse breed could achieve the following objectives in improving its characters: mares should increase their height up to 156 cm, the thoracic perimeter up to 178 cm, the cannon girth up to 19.5 cm and stallions – a height of 158 cm, 178 cm for heart perimeter and 20.5 cm for shinbone girth.

RESULTS AND DISCUSSIONS

The results were based on analyzing each genetic bloodline of every category of horses, from Shagya Arabian breed, reared in Rădăuți studfarm. Regarding the mares reared in 2000-2015, it can be noticed in Table 1 that the minimum average value of height was 155.25 cm (Mersuch genetic bloodline) and the maximum was 158.28 cm (El-Sbaa). Also regarding the thorax perimeter, the minimum average value for this category was 174.88 cm (Siglavy-Bagdady) and the maximum was 179.31 cm (Shagya bloodline). The minimum average value for cannon girth was 17.89 cm and the maximum 18.74 cm (Hadban). All data indicated that there was no genetic bloodline that presents minimum and also maximum average values for all studied parameters.

Table 1. The average values of studied parameters of Shagya Arabian mares

Genetic bloodline	Average values of height (cm)	Average values of thorax perimeter (cm)	Average values of cannon girth (cm)
Hadban	156.21	177.86	18.74
Dahoman	157.43	178.97	18.45
Shagya	156.36	179.31	18.68
Siglavy-Bagdady	156.41	174.88	17.89
El-Sbaa	158.28	178.13	18.88
Mersuch	155.25	176.05	18.72
Koheilan	156.24	175.26	18.48

From Figure 1 results that the average value of mares' height oscillates between 155.25 cm (Mersuch) and 158.28 cm (El-Sbaa) revealing that the objective regarding to increase the height up to 156 cm, was in this case set.

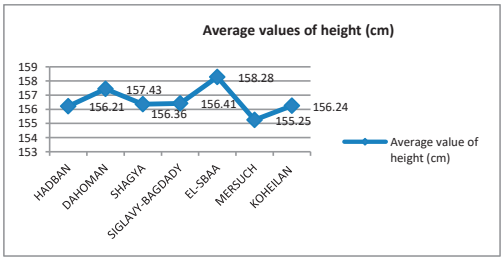

Figure 1. Average values of height of all genetic bloodlines (Shagya Arabian mares)

Figure 2 shows that the average values of thorax perimeter oscillated between 174.88 cm (Siglavy-Bagdady) and 179.31 cm (Shagya) indicating that the objective to increase the thorax perimeter up to 178 cm was set in 57.14% of cases.

Figure 3 reveals that the average values of cannon girth oscillated between 17.89 cm (Siglavy-Bagdady) and 18.88 cm (El-Sbaa) indicating that the objective to increase the shinbone perimeter up to 19 cm in mares isn't

set but is very close in reaching the estimated value (max. of 18.88 cm).

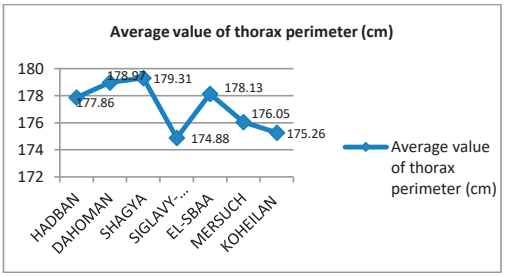

Figure 2. Average values of thorax perimeter of all genetic bloodlines (Shagya Arabian mares)

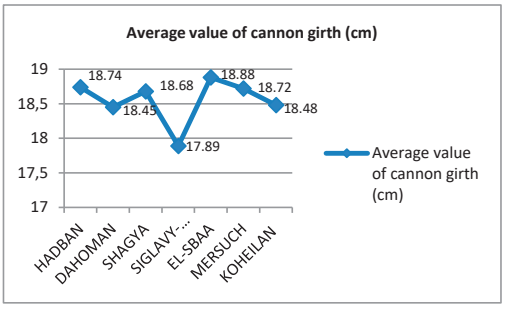

Figure 3. Average values of cannon girth of all genetic bloodlines (Shagya Arabian mares)

Based on average values obtained we calculated the massiveness index, the bone index and the digital-thorax index. The mares' situation is framed in Table 2 and reveals that the average values of massiveness index were situated between 111.80% (Siglavy-Bagdady) and 114.67% (Shagya), the average values of bone index were between 11.43% (Siglavy-Bagdady) and 12.05% (Mersuch), the average values of digital-thorax index were between 10.30% (Dahoman) and 10.63% (Mersuch).

Table 2. The body indexes based on analyzed parameters of Shagya Arabian mares

Genetic bloodline	Massiveness index (thorax perimeter/height, %)	Bone index (cannon girth/height, %)	Digital-thorax index (cannon girth/thorax perimeter, %)
Hadban	113.85	16.46	10.53
Dahoman	113.68	11.71	10.30
Shagya	114.67	11.94	10.41
Siglavy-Bagdady	111.80	11.43	10.22
El-Sbaa	112.54	11.92	10.59
Mersuch	113.39	12.05	10.63
Koheilan	112.17	11.82	10.54

Data obtained indicate that the indexes had similar values between the genetic bloodlines fact that proves that the population is homogenous.

In table 3 is presented the situation of average values of studied parameters of Shagya Arabian stallions.

Data show that all genetic bloodlines have exceeded the objective of reaching the 158 cm height of Shagya Arabian stallions, mentioned in Rădăuți studfarm registers, except the Shagya bloodline which revealed an average value of 157.75 cm for height parameter (however this value is close to the specified objective).

Table 3. The average values of studied parameters of Shagya Arabian stallions

Genetic bloodline	Average values of height (cm)	Average values of thorax perimeter (cm)	Average values of cannon girth (cm)
Dahoman	159.25	178.75	19.12
Hadban	161.33	184.33	19.25
Koheilan	160.14	183.21	19.13
Mersuch	162.75	179.75	19.00
Shagya	157.75	176.50	18.12
Gazal	162.75	184.75	19.25
El-Sbaa	159.00	182.50	19.00
Siglavy-Bagdady	160.50	174.00	18.62

In Figure 4 is revealed the situation of average values of height (cm) of Shagya Arabian stallions, which oscillated between 157.75 cm (Shagya) and 162.75 cm (Mersuch and Gazal).

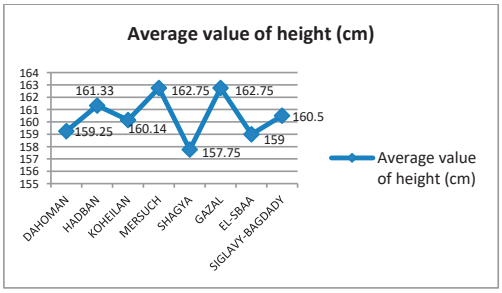

Figure 4. Average values of height of all genetic bloodlines (Shagya Arabian stallions)

Figure 5 shows the situation of average thorax perimeter of stallions, limited between 174.00 cm (Siglavy-Bagdady bloodline) and 184.75 cm (Gazal). The studfarm registers showed that the thorax perimeter of stallions should increase up to 178 cm and data obtained in our study reveal that all genetic bloodlines have

reached and even exceed this limit, except Shagya and Siglavy-Bagdady bloodlines (176.50 cm and, respectively, 174.00 cm).

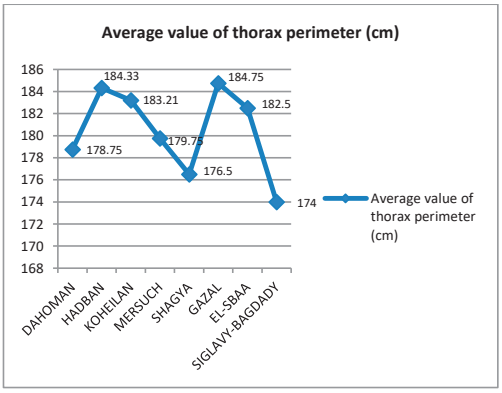

Figure 5. Average values of thorax perimeter of all genetic bloodlines (Shagya Arabian stallions)

The Figure 6 reflects the situation regarding the average values of cannon girth of stallions limited between 19.00 cm (Mersuch and El-Sbaa) and 19.25 cm (Hadban and Gazal); none of the bloodlines have revealed the objective of reaching the 20.5 cm shinbone perimeter mentioned in the studfarm registers.

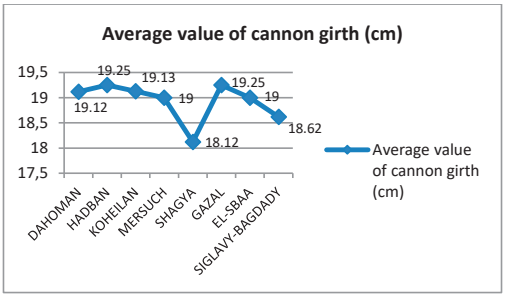

Figure 6. Average values of cannon girth of all genetic bloodlines (Shagya Arabian stallions)

Table 3 shows that the massiveness index had a minimum value of 108.41% (Siglavy-Bagdady) and a maximum value of 114.77% (El-Sbaa). A study regarding this index, revealed an average value of 111.15% for massiveness percent of Shagya Arabian stallions (Prisecaru, 2007). The bone index had limits between 11.48% (Shagya bloodline) and 12.00% (Dahoman) and the digital-thorax index had limits between 10.26% (Shagya) and 10.70% (Siglavy-Bagdady).

Table 3. The body indexes based on analyzed parameters of Shagya Arabian stallions

Genetic bloodline	Massiveness index (thorax perimeter/height, %)	Bone index (cannon girth/height, %)	Digital-thorax index (cannon girth/thorax perimeter, %)
Dahoman	112.24	12.00	10.69
Hadban	114.25	11.93	10.44
Koheilan	114.40	11.94	10.44
Mersuch	110.44	11.67	10.57
Shagya	111.88	11.48	10.26
Gazal	113.51	11.82	10.41
El-Sbaa	114.77	11.94	10.41
Siglavy-Bagdady	108.41	11.60	10.70

Table 4 reveals the situation of studied parameters for Shagya Arabian young male horses, where the average values of height were situated between 130.20 cm (El-Sbaa) and 143.88 cm (Siglavy-Bagdady). The average values of thorax perimeter were situated between 133.50 cm (Dahoman) and 156.77 cm (Siglavy-Bagdady) and the average values of cannon girth had limits between 15.80 cm (El-Sbaa) and 17.25 cm (Gazal). The differences between the genetic bloodlines regarding all parameters are given by the fact that this category is in course of development and it's normal to indicate wide borders.

Table 4. The average values of studied parameters of Shagya Arabian young male horses

Genetic bloodline	Average values of height (cm)	Average values of thorax perimeter (cm)	Average values of cannon girth (cm)
Dahoman	116.75	133.50	16.12
Hadban	138.33	149.50	16.75
Koheilan	135.36	143.27	16.45
Mersuch	138.75	148.75	16.50
Shagya	135.33	149.91	16.95
Gazal	140.00	154.00	17.25
El-Sbaa	130.20	136.6	15.80
Siglavy-Bagdady	143.88	156.77	17.77

Figure 7 shows the average values of height for this category of horses indicating the big difference between the genetic bloodlines (Dahoman 116.75 cm - Siglavy-Bagdady 143.88 cm).

Figure 8 indicates the situation of heart perimeter of young male horses; the minimum average value was registered at Dahoman (133.50 cm) and the maximum at Siglavy-Bagdady (156.77 cm).

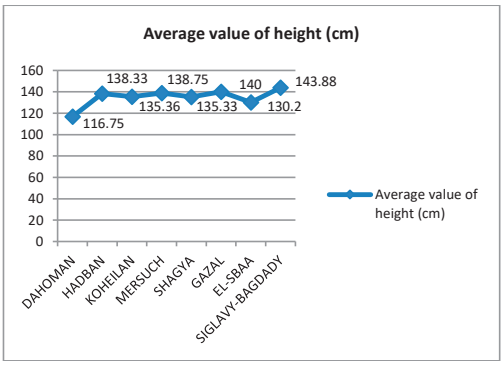

Figure 7. The average values of height for young male horse

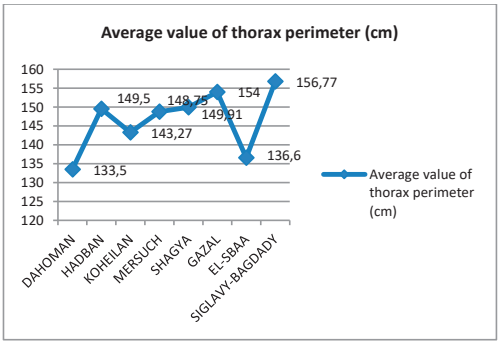

Figure 8. The average values of thorax-perimeter for young male horse

Figure 9 shows that there were big differences between the genetic bloodlines also regarding the cannon girth; the minimum average value was found at El-Sbaa (15.80 cm) and the maximum average value was discovered in Siglavy-Bagdady's case (17.77 cm).

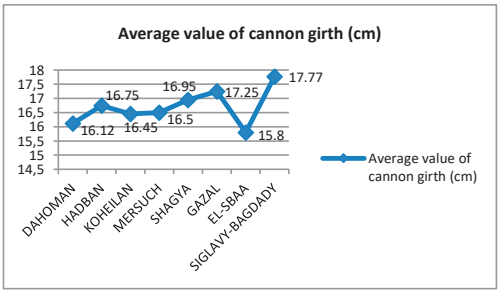

Figure 9. The average values of cannon girth for young male horse

CONCLUSIONS

Regarding the results, we observed that the mares had an average height of 159.59 cm, an average heart perimeter of 177.20 cm and an average cannon girth of 18.54 cm, including the population in breed standard. The average values of massiveness index, bone index and digital-thorax index were close in all bloodline cases.

Regarding the Shagya Arabian stallions, the average height was 160.58 cm, the average heart perimeter was 180.61 cm and the average cannon girth was 18.95 cm; all the indexes were similar to literature specifications and include the Rădăuți population in breed standard.

Studying the young male horses we observed that the average value of height was 134.82 cm, the average thorax perimeter was 146.53 cm and the average cannon girth was 16.69 cm, facts that resemble to literatures' consignments.

All data obtained indicate that the studied population is included in breeds' standard regarding the main body dimensions that reflect their morphological type and that Rădăuți studfarm offers optimum conditions for Shagya Arabian horse rearing.

REFERENCES

Dolis, M. G., Usturoi, M. G., Nagy, P. T., Dolis, L. (2011). Study on the evolution of the body height sizes in young horses, University of Oradea, *Ecotoxicology, Animal science and Food industry technologies*.

Doliș, M., Nagy, P. T., Doliș, L., Nistor, C. (2011). Study regarding body dimensions of Shagya Arabian young females, *Scientific papers, Animal Science Series*, 56.

Dulugeac, I. (2005). *Sport horses*, Iasi, RO: Arena Publishing House.

Manole, I., Radu-Rusu, C. (2004). Contributions to the knowledge of the main morpho-productive traits of the Arabian horses from Rădăuți studfarm, *Scientific papers, Animal Husbandry Series*, 47, 434-441.

Prisecaru, M. (2007): The analysis of corporal indices of stallions from Rădăuți troop. *Animal Science Series*, 59.

Rădăuți studfarm registers.

Schipor, G. (2007). *Rădăuți studfarm under two empires*, Suceava, RO: Cygnus Publishing House.

Velea, C., Târnoveanu, I, Marcu, N., Bud, I. (1980). *Horse breeding*, Cluj Napoca, RO: Dacia Publishing House.

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RESEARCH ON THE QUALITY OF PHYSICAL INDICATORS OF THE TURKEY MEAT OBTAINED FROM THE BIG BUT 6 HYBRID SLAUGHTERED AT DIFFERENT AGE

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Abstract

In the present paper we set out to carry out a study on the quality of the physical indices of the turkey meat, derived from the Big BUT 6 hybrid slaughtered at 16 weeks of age (group L1) and at 18 weeks (group L2) through the following indices: acidity (pH value) achieved 20 minutes after slaughter, 24 hours and 72 hours, colorimetric parameters of the meat, milling (through Warner Bratzler shear forces) and texture, which was achieved by using objective methods such as Texture Profile Analysis (TPA). Regarding the acidity achieved for the four anatomical regions (chest, upper pulp, lower pulp and wings), no statistical differences were observed following the analysis of the data. Regarding the coordinate of the complementary colors red-green (a) the minimums registered were specific to the pectoral muscles, for both L1 (-0.38 ± 0.09) and for L2 (-0.25 ± 0.12), the calculated maxima being responsible for the upper pulp musculature in both experimental groups (5.10 ± 0.15 ÷ 5.52 ± 0.12). The comparative analysis of the average forces for each muscle group observed the superiority of the fragility of the muscle samples collected from the level of the pectoral muscles and the wings.*

Key words: *meat, quality, turkey.*

INTRODUCTION

Obtaining safe and high quality food is a major condition for ensuring public health and commercial success domestically and internationally. The need to identify the origin of ingredients used in the food industry. As well as knowledge of the origins of food is a supreme factor in terms of consumer protection. especially when products are unsafe (Saeger, 2011).

If until recently the poultry industry was under the monopoly of chicken broiler, lately, turkey meat is gaining more and more ground among consumers not only because it is tasty, but also because it has nutritional and sensory properties that makes it an almost ideal product.

The XX century, especially the second half, saw a real growth in the turkey and turkey production industry (Buddiger and Albers, 2009). Until World War II, turkeys were more traditionally raised, with seasonal breeding and natural as well as artificial incubation. After 1945, the turkey industry developed very productively, along with shelters and production per year of slaughter (EFSA, 2004).

The increase in production volume as well as the efficiency of turkeys have contributed to the continuous development of turkey hybrids (Yilmaz et al., 2011). At the same time, an intense development that took place in the breeding area, focused on the reproduction of turkeys with wide chest, with hypertrophy of the chest and leg muscles (EFSA, 2004).

The world market for turkey hybrid producers is under the monopoly of three large British United Turkeys (BUT) companies. Hybrid Turkeys and Nicholas Turkey, each with its own hybrids that have performed differently and achieved different goals.

The choice of the appropriate hybrid by producers is based on the purpose of marketing and the potential of genetic properties to adapt to a type of feed, to have greater resistance to some common diseases and the availability of a

wide range of breeding practices (Roberson et al., 2004).

Obtaining safe and high quality food is a major condition for ensuring public health and commercial success domestically and internationally. The need to identify the origin of ingredients used in the food industry, food as well as knowledge of the origins of food is a supreme factor in terms of consumer protection, especially when products are unsafe (Abeyesinghe et al., 2007).

For these reasons, through this paper we aimed to realize a study on the quality of the physical indices of the turkey path, derived from the hybrid Big BUT 6 slaughtered at the age of 16 weeks (batch L1) and at 18 weeks, (batch L2) in terms of the following indices: acidity (pH value) achieved 20 minutes after slaughter, 24 hours and 72 hours, colorimetric parameters of the meat. Tenderness (via Warner Bratzler shear forces) and texture, which was achieved by using objective methods such as Texture Profile Analysis (TPA).

MATERIALS AND METHODS

As a biological material, the turkey hybrid Big BUT 6 purchased from the supplier Aviagen Turkeys who is developing a genetic selection program, bringing continuous improvements in the development of body weight and health of birds.

The turkey hybrid Big BUT 6 is a massive, fast-growing breed, being mainly used for intensive production. According to the growth guide at the age of 18 weeks, females (Figure 1) belonging to this hybrid reach an average body weight of 12 kg, and males (Figure 2) at the age of 22 weeks reach 22 kg.

Figure 1. Big BUT 6 broiler female

Figure 2. Big BUT 6 broiler female and male

Sampling and preparation of samples

In order to evaluate the quality of the turkey meat through the traceability analysis, it was necessary to harvest the tissue corresponding to the subsequent analyzes. By observing the experimental protocol that requires monitoring the technological conditions of growth, slaughtering operations, as well as the characterization of meat from a physical-chemical, microbiological and sensory point of view, the collection and sampling of samples required the use of muscle tissue, cecum and neck (Figure 3) from the turkeys previously identified and eared.

Figure 3. Gathering of muscular samples from turkey hens carcasses

The results of laboratory tests may be influenced by the correct application of the sampling and preparation procedure.

Working methods used to determine the physical properties of meat

Determination of meat acidity. The measurement of the pH value was carried out in two stages, as follows: 20 minutes after slaughter, using a deep electrode probe, inserted into the housing in the analyzed areas and 24, 72 hours after slaughter, using the glass electrode by immersion. To perform the examinations, the aqueous extract of 10 g of previously minced meat and 100 ml of distilled water is initially prepared. The mixture was allowed to stand for

15-20 minutes during which time it was stirred several times. After this interval the extract was filtered and further examined.

The working method used to determine the color of the meat. Regarding the color of the meat, it was expressed by the coordinates L*, a*, b* in the colorimetric space CIE Lab (AMSA), being corrected by the equation DIN 99, measured by means of the included spectral component (SCI).

The operating principle of the spectrophotometer applies the specifications given in "CIE Colorimetry Second Edition. Publication 15.2 (1986)". From a conceptual point of view, the color of each sample is represented graphically by the point P in Figure 4, with the following significance of the chromatic parameters:

- the brightness (L*) of the color or the psychometric clarity is the color parameter determined by the intensity of the light waves that define it, this being represented by the vertical axis of Figure 4. More light, means light waves of higher intensity, which determines more colors, intense or brighter, the brightness being able to have values between 0 for an opaque black sample and 100 for transparent colorless samples;

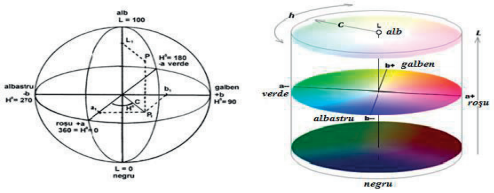

Figure 4. The CIE Lab linear colour space / the colour solid (Source: CIE, 1986)

- the parameter "a*" expresses the color values on the red-green chromatic axis, through which the color stability in time is rendered;
- the parameter "b*" expresses the color values on the yellow-blue color axis;
- hue is the parameter determined by the dominant wavelength in the set of wavelengths that form that color, being defined by the gradation of a color within the visible spectrum. The tint of the color "ho" corresponds to the angle. expressed in sexagesimal degrees. formed by the segment OP_1 and the coordinate "a*", The value of this parameter, theoretically, can vary between 0° and 360°, but for achromatic stimuli it remains undefined. The correlation between the values of the "ho" parameter and the visually perceived colors. inscribed in the $_{a1}Ob_1$ plane of Figure 4 are self-evident: red ÷ 0°. yellow ÷ 90°, green ÷ 180°;

Saturation is the color parameter determined by the color purity, ie by the wavelengths that are combined with the dominant wavelength that defines the color shade, the mathematical definition of chrome and color shade of the analyzed muscle samples being calculated according to the relations:

Color saturation: $\mathbf{C = (a^2 + b^2)^{1/2}}$

The hue (tint) of color: $\mathbf{H= arctg\ (b/a)}$

The appreciation of the color of the meat was made on muscle samples, with a thickness of 1.5-5 cm, these being sectioned perpendicular to the longitudinal axis of the muscles; subsequently, the muscle samples were vacuum packed under polyethylene film and stored by refrigeration at 2-4°C until colorimetric measurements were performed (method adapted from Honikel, 1998; Stevenson et al., 1989). As a method, the actual measurement was performed in three different areas of each muscle sample, at a temperature of 8÷10°C, with the portable spectrophotometer Minolta CM-2600d (Figure 5), being set to view at the standard angle of 10° with a illuminating beam D 65 in the color space CIE Lab.

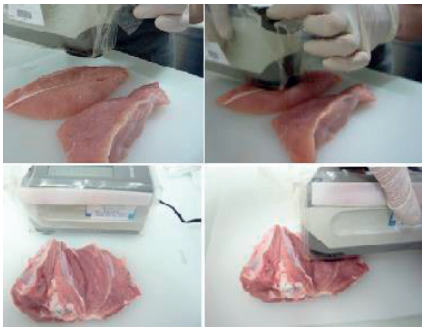

Figure 5. Measurement of muscular samples colour

The working method used to determine the tenderness of the meat by means of Warner Bratzler forces. In order to make this determination, the meat samples were subjected to a heat treatment of boiling on a bain marie for 45 min, at 75°C (in polyethylene bags), then wrapped in aluminium foil, stored for 24 hours at 4°C and sectioned in a cylindrical shape (3

cylinders with a diameter of 1.5 cm and a length of 2 cm) in the direction of the muscle fibers.
The use of a specific blade (60º angle, travel speed 100 mm/min, shear force 1000 N) attached to the TA Plus Lloyd Instruments texturometer aims to determine the forces. The cylindrical muscle samples were sectioned perpendicular to the muscle fibers, the maximum force required to section the sample being the indicator used to describe the tenderness of the meat.
To determine the forces, the device is provided with a rigid flat surface, rectangular in shape, sectioned in the middle and 3 blades in different shapes: one blade in the shape of a square and two in the shape of a "V".
The NEXYGEN Ondio software integrated in the TaPlus Series texturometer allowed the direct calculation of the shear force values according to the cutting-deformation curve, these being expressed in the form of peaks, corresponding to the maximum value recorded (Honikel, 1998). At the same time, the system ensures the operation of the texturometer according to the requirements stated by BS EN ISO 7500: 1999 (Figure 6).

Figure 6. Determination of meat tenderness using Warner Bratzler forces

Working method used to determine meat texture (TPA). In order to analyze the texture of the samples collected from experimental groups L1 and L2, it was necessary to use the Lloyd LFP Plus universal texturometer in order to apply the compressive force on the muscle samples in the form of a cylinder and obtain a final deformation from the initial sample height. This was done with a flat-faced cylinder of Ø = 45 mm which obtained an alternative movement, which mimics the action of the human jaw.
To achieve the texture profile, the meat samples of the experimental batches were previously subjected to a heat treatment of boiling on bain marie. The sectioning of meat samples in cylindrical form with Ø and H of 20 mm was performed at room temperature by pressing the samples with a cylinder, parallel to the direction of the muscle fibers (Figure 7).
In performing the mechanical determination, the Llyod LFP plus dynamometer was used, the meat samples being in the form of cylinders with Ø and H of 20 mm, obtaining the results involving the use of a pressing cylinder with flat faces, with Ø = 45mm. The actual determination involved performing a double compression, with an intermediate pause between compressions of 5 sec. At a speed of 10 mm/min., and a final deformation of 80% of the initial height of the tested meat sample.

Figure 7. Determination of meat texture (TPA)

The analysis of the force-time curve of the TPA instrumental method led to the obtaining of five instrumental parameters (hardness, cohesiveness, gumminess, elasticity and masticability) illustrating a sample of the force-deformation curve and the TPA parameters.
The expression of the results was performed with the help of NEXYGEN Ondio software, integrated in the texturometer, which allowed the recording and direct calculation of the values of each descriptive textural parameter.

RESULTS AND DISCUSSIONS

In the post-slaughter period associated with the prerigor mortis phase, at 20 minutes after slaughter the meat harvested from turkey hybrids from the experimental group L1 recorded average values between 6.23 ± 0.02 (chest) and 6.38 ± 0.02 (lower leg), and for the muscle tissue harvested from turkeys in L2, the representative average values were 6.24 ± 0.01 (chest) and 6.39 ± 0.02 (lower leg). The mean differences obtained for the anatomical portions of the two groups are characterized by the proportion of short-lived white muscle fibers (fast contractions) and reds resistant to prolonged exertion (slow contractions), thus influencing the amount of glycogen and muscle ATP regeneration. In the pectoral muscles the

majority proportion is held by the white fibers, compared to those of the thighs where the red fibers predominate.

By calculating the coefficient of variation, values were obtained below the threshold of 5% (1.01 ÷ 1.47%) corresponding to group L1 and (0.83 ÷ 1.48) L2, which highlights a very good homogeneity of character for all muscle samples during the prerigor mortis phase (Table 1).

At the beginning of the maturation phase, samples collected from muscle tissues representative of turkey and turkey carcasses showed an average pH value between 5.87 ± 0.01 (chest) and 6.08 ± 0.01 (lower leg), recorded on L1 housings. At this time of the biochemical transformations in the meat, the average acidity of the samples taken from the carcases of batch L2 was characterized by the range 5.91 ± 0.01 (chest) ÷ 6.09 ± 0.01(lower leg), the homogeneity being defined by the values of the coefficient of variation below the threshold of 5% (Table 1).

After keeping the muscle samples representative of the two groups for 3 days in refrigeration conditions, average values of acidity were recorded, the minimums obtained were corresponding to the chest muscles of L1 and L2 (6.01 ± 0.01 ÷ 5.99 ± 0.01), and the maximums characteristic of the lower thigh muscles harvested from both groups (6.24 ± 0.01 ÷ 6.25 ± 0.01).

Compared to the literature, the pH values obtained were positively influenced by the stunning of turkeys with CO_2, the birds not being exposed to stress with undesirable effects due to the handling procedure in order to position them on the conveyor line (Bianchi et al., 2006).

Table 1. Estimators and statistical significance of values differences for turkey hen meat acidity

Specification	Time	Analysed bached	n	Calculated statistical indicators				Significance of differences between batch averages (FISHER test)
				$\bar{X} \pm s_{\bar{x}}$	V%	Min.	Max.	
Chest	20 min.	L_1	15	6.23±0.02	1.01	6.12	6.32	$\hat{F}_{0.22} < F_{0.05\%}(4.20)$ → n. s.
		L_2		6.24±0.01	0.83	6.15	6.32	
	24 h	L_1		5.87±0.01	0.48	5.81	5.91	$\hat{F}_{5.92} > F_{0.05\%}(4.20)$ →*
		L_2		5.91±0.01	0.71	5.84	5.98	
	72 h	L_1		6.01±0.01	0.47	5.96	6.06	$\hat{F}_{4.2} < F_{0.05\%}(4.20)$ → n. s.
		L_2		5.99±0.01	0.5	5.94	6.03	
Upper thigh	20 min.	L_1	15	6.35±0.02	1.16	6.23	6.44	$\hat{F}_{0.38} < F_{0.05\%}(4.20)$ → n. s.
		L_2		6.36±0.02	1.31	6.18	6.49	
	24 h	L_1		6.06±0.01	0.37	6.03	6.1	$\hat{F}_{2.58} < F_{0.05\%}(4.20)$ → n. s.
		L_2		6.08±0.01	0.29	6.05	6.11	
	72 h	L_1		6.23±0.01	0.36	6.19	6.26	$\hat{F}_{0.82} < F_{0.05\%}(4.20)$ → n. s.
		L_2		6.22±0.01	0.26	6.2	6.25	
Lower thigh	20 min.	L_1	15	6.38±0.02	1.47	6.23	6.49	$\hat{F}_{0.12} < F_{0.05\%}(4.20)$ → n. s.
		L_2		6.39±0.02	1.48	6.18	6.51	
	24 h	L_1		6.08±0.01	0.32	6.05	6.12	$\hat{F}_{1.46} < F_{0.05\%}(4.20)$ → n. s.
		L_2		6.09±0.01	0.37	6.06	6.13	
	72 h	L_1		6.24±0.01	0.34	6.2	6.27	$\hat{F}_{1.14} < F_{0.05\%}(4.20)$ → n. s.
		L_2		6.25±0.01	0.31	6.22	6.29	
Wings	20 min.	L_1	15	6.28±0.02	1.05	6.12	6.42	$\hat{F}_{0.26} < F_{0.05\%}(4.20)$ → n. s.
		L_2		6.29±0.02	1.01	6.19	6.37	
	24 h	L_1		6.01±0.01	0.56	5.94	5.97	$\hat{F}_{0.91} < F_{0.05\%}(4.20)$ → n. s.
		L_2		6.02±0.01	0.44	5.97	6.05	
	72 h	L_1		6.10±0.01	0.33	6.07	6.14	$\hat{F}_{5.17} < F_{0.05\%}(4.20)$ → n. s.
		L_2		6.11±0.01	0.31	6.08	6.14	

The statistical significance of the differences between the experimental groups L1 and L2 for the specific acidity values during the prerigor mortis and maturation phases corresponding to each muscle group studied showed significant differences for one test (8.33%) of the total of the 12 tests and 91% presenting insignificant differences (Table 1).

The color of turkey meat

The characterization of turkey meat according to age for the muscle samples studied showed average values corresponding to the brightness

of the L1 group, in a range of 44.74 ± 0.72 (upper leg) and 48.44 ± 0.36 (wing) and 44.04 ± 0.3 (lower leg) ÷ 48.35 ± 0.33 (wing) representative of group L2. Between the groups, the wing muscles in the second group were distinguished by the superiority of the brightness compared to the counterparts of the muscles representative of the first group. By calculating the coefficient of variation of the values that describe the brightness of the 4 muscles studied (chest, upper leg, lower leg, wings) specific to each group, the average homogeneity of the character was noted 2.53-6.22 for L1 and 2.63-3.01 specific to lot L2 (Table 2)

Table 2. Estimators for colorimetric parameter values and statistical significance of turkey hen meat

Specification		Analysed bached	n	Calculated statistical indicators				Significance of differences between batch averages (FISHER test)
				$\bar{X} \pm s_{\bar{x}}$	V%	Min.	Max.	
Chest	L*	L_1	15	46.92±0.31	2.53	45.03	49.23	$\hat{F}_{1.41} < F_{0.05\%}(4.20) \rightarrow$ n. s.
		L_2		47.49±0.37	3.01	45.03	50.08	
	a*	L_1		-0.38±0.09	91.74	-0.89	0.15	$\hat{F}_{0.74} < F_{0.05\%}(4.20) \rightarrow$ n. s.
		L_2		-0.25±0.12	191.23	-1.02	0.73	
	b*	L_1		7.60±0.29	14.76	5.39	9.12	$\hat{F}_{1.38} < F_{0.05\%}(4.20) \rightarrow$ n. s.
		L_2		8.06±0.26	12.61	5.32	9.31	
	C	L_1		7.34±0.26	13.51	5.57	9.15	$\hat{F}_{1.45} < F_{0.05\%}(4.20) \rightarrow$ n. s.
		L_2		7.83±0.31	15.35	5.52	9.34	
	h°	L_1		88.37±2.12	9.27	71.21	100.3	$\hat{F}_{0.67} < F_{0.05\%}(4.20) \rightarrow$ n. s.
		L_2		90.49±1.50	6.41	81.25	101.7	
Upper thigh	L*	L_1	15	44.74±0.72	6.22	40.21	48.46	$\hat{F}_{5.09} > F_{0.05\%}(4.20) \rightarrow *$
		L_2		46.55±0.35	2.91	44.24	48.67	
	a*	L_1		5.10±0.15	11.09	4.36	6.49	$\hat{F}_{5.09} > F_{0.05\%}(4.20) \rightarrow *$
		L_2		5.52±0.12	8.10	4.91	6.58	
	b*	L_1		10.77±0.43	15.49	8.33	14.02	$\hat{F}_{1.11} < F_{0.05\%}(4.20) \rightarrow$ n. s.
		L_2		11.31±0.28	9.52	8.23	12.93	
	C	L_1		12.29±0.48	14.98	8.41	14.85	$\hat{F}_{0.054} < F_{0.05\%}(4.20) \rightarrow$ n. s.
		L_2		12.43±0.42	13.06	8.47	14.82	
	h°	L_1		67.94±1.9	10.82	60.02	78.91	$\hat{F}_{5.64} > F_{0.05\%}(4.20) \rightarrow *$
		L_2		70.00±1.72	9.50	54.01	69.09	
Lower thigh	L*	L_1	15	45.40±0.52	4.46	42.95	51.52	$\hat{F}_{5.06} > F_{0.05\%}(4.20) \rightarrow *$
		L_2		44.04±0.3	2.66	42.31	46.95	
	a*	L_1		4.04±0.51	48.60	1.29	7.32	$\hat{F}_{0.002} < F_{0.05\%}(4.20) \rightarrow$ n. s.
		L_2		4.06±0.44	41.74	1.55	7.29	
	b*	L_1		12.23±0.48	15.17	9.73	15.64	$\hat{F}_{0.181} < F_{0.05\%}(4.20) \rightarrow$ n. s.
		L_2		11.98±0.34	10.99	9.56	14.64	
	C	L_1		12.07±0.67	21.62	9.33	17.22	$\hat{F}_{2.88} < F_{0.05\%}(4.20) \rightarrow$ n. s.
		L_2		13.54±0.54	15.37	9.72	17.20	
	h°	L_1		70.50±0.90	4.94	64.79	75.55	$\hat{F}_{7.07} > F_{0.05\%}(4.20) \rightarrow *$
		L_2		66.27±1.31	7.64	56.63	73.31	
Wings	L*	L_1	15	48.44±0.36	2.87	45.95	51.21	$\hat{F}_{0.03} < F_{0.05\%}(4.20) \rightarrow$ n. s.
		L_2		48.35±0.33	2.63	45.95	50.39	
	a*	L_1		1.28±0.40	119.25	-0.37	4.27	$\hat{F}_{1.944} < F_{0.05\%}(4.20) \rightarrow$ n. s.
		L_2		0.61±0.28	177.93	-0.79	2.51	
	b*	L_1		7.40±0.66	34.77	2.45	11.09	$\hat{F}_{0.031} < F_{0.05\%}(4.20) \rightarrow$ n. s.
		L_2		7.24±0.60	32.29	2.46	11.23	
	C	L_1		7.55±0.71	36.47	2.58	11.83	$\hat{F}_{0.41} < F_{0.05\%}(4.20) \rightarrow$ n. s.
		L_2		8.12±0.52	24.65	4.53	11.94	
	h°	L_1		85.62±2.46	11.14	67.45	97.71	$\hat{F}_{0.10} < F_{0.05\%}(4.20) \rightarrow$ n. s.
		L_2		89.27±2.71	11.76	67.72	103.40	

L* = brightness; a* = coordinate of complementary red-green colors; b* = coordinate of complementary yellow-blue colors; C = color saturation; h° = shade of color.

Regarding the coordinate of the complementary colors red-green (a*) the minimums recorded were specific to the pectoral muscles, both for L1 (-0.38 ± 0.09) and for L2 (-0.25 ± 0.12), the

calculated maximums being responsible for the muscles of the upper thigh in both experimental groups (5.10 ± 0.15 ÷ 5.52 ± 0.12). Red-green coordinate variations were associated with the type and proportion of muscle and connective fibers in the muscles, differentiated levels of glycogen stores, the amount of myoglobin, and age at slaughter.

For the yellow-blue color coordinate (b*) the averages obtained on the lower side were specific to the wing muscles in both groups (7.24 ± 0.60 ÷ 7.40 ± 0.66), while the maximums were characterized by the leg muscles, lower (11.98 ± 0.34 ÷ 12.23 ± 0.48).

By calculating the coefficient of variation of the values of the groups L1 (14.76 ÷ 34.77) and L2 (9.52 ÷ 32.29) which describe the yellow-blue coordinate (b*), an average homogeneity of the characters was noticed. As an overview, for the b* coordinate a superiority of the recorded values was found, specific to the muscles taken from the turkey carcasses slaughtered at 18 weeks compared to the one harvested from the turkey carcasses slaughtered at 16 weeks, the oscillation of the values was taken into account, age differences, the proportion of white and red fibers in the muscles, the state of fattening, antesacrification factors.

The degree and intensity of color saturation of the muscles representative of turkeys and turkeys is rendered objectively using the parameter C (chroma). The lower leg muscles taken from birds raised up to 18 weeks had a higher average (13.54 ± 0.54) than the homologous muscles representative of carcasses obtained after slaughtering birds at 16 weeks (12.07 ± 0.67). The lower mean values specific to parameter C for both ages were characteristic of the pectoral muscles (7.34 ± 0.26 ÷ 7.83 ± 0.31).

Following the characterization of the flesh color in terms of the average values recorded by the Hue angle, the muscles of the lower leg harvested from group L2 (66.27 ± 1.31) had a darker shade than the muscles corresponding to group L1 (70.50 ± 0. 90), in the case of the other muscle groups the results were inversely characterized.

The characterization of turkey meat through descriptive coordinates are close to those in the literature (Bihan-Duval et al., 2003) the brightness of the meat as a whole falling within the range 48.6 ± 49.7. The superiority of the values obtained by the literature (3.2 ± 1.4) regarding the a* coordinate for the pectoralis muscle compared to the samples taken in the study is noticeable.

The level of statistical significance of the differences between the values corresponding to the muscle samples taken from the carcasses of turkeys and turkeys (L1 and L2) on colorimetric parameters were noticed significant differences in 5 tests (25%) of the total of 20 performed, the remaining 85% (Table 2).

The tenderness of turkey meat

Primary statistical indicators calculated by means of Warner Bratzler shear forces defining the tenderness of turkey meat reported average values corresponding to the standard error in the range of 0.28 ÷ 0.54, being closely related to a degree of homogeneity of 9.24 ÷ 17.03.

The values of the shear forces were directly proportional to aging, so in the case of birds slaughtered at 16 weeks (L1) values of 10.48 ± 0.41 (chest) were recorded, while in turkeys and turkeys slaughtered at 18 weeks (L2) the values obtained were higher, namely 14.45 ± 0.5 N/cm^2.

Table 3. Estimators and statistical significance of turkey hen meat (Warner Bratzler shear forces)

Specification	Analysed bached	n	Calculated statistical indicators				Significance of differences between batch averages (FISHER test)
			$\bar{X} \pm s_{\bar{x}}$	V%	Min.	Max.	
Chest	L_1	15	10.48±0.41	15.02	7.56	12.59	$\hat{F}_{7.09} > F_{0.05\%}(4.20) \rightarrow *$
	L_2		11.8±0.28	9.24	9.87	13.62	
Upper thigh	L_1	15	12.38±0.54	17.03	9.01	16.42	$\hat{F}_{7.79} > F_{0.01\%}(7.64) \rightarrow **$
	L_2		14.45±0.5	13.45	12.02	18.41	
Lower thigh	L_1	15	12.53±0.5	15.32	10.31	16.41	$\hat{F}_{6.47} > F_{0.05\%}(4.20) \rightarrow *$
	L_2		14.22±0.44	11.97	12.03	17.4	
Wings	L_1	15	10.51±0.31	11.41	8.95	13.85	$\hat{F}_{3.49} < F_{0.05\%}(4.20) \rightarrow$ n. s.
	L_2		11.53±0.45	15.14	8.37	14.48	

These values were in agreement with those obtained by Jukna et al., (2012), (8.72 N/cm^2 or 0.89 kg/cm^2) correlating the obtaining of a product without fragility with the deterioration of collagen by prolonging age and thus obtaining increased resistance.

The comparative analysis of the average forces for each muscle group showed the superiority of the fragility of the muscle samples collected from the pectoral muscles and the wings of the carcasses representative of groups L1 and L2. these being also the lower limits (10.48 ± 0.41 N/cm^2 at L1, respectively 11.53 ± 0.45 N/cm^2 at L2) (Table 3).

By comparing the averages obtained for the muscles representative of the groups slaughtered at different ages. We can say that the muscles from the first group, especially the chest muscles, showed a higher fragility than that obtained in the representative samples of group L2.

The statistical analysis of the existing differences. within the muscle group, between the experimental groups for Warner Bratzler force values showed distinctly significant differences for one (25%) of the 4 tests performed, corresponding to the upper leg, 50% of the tests showed differences significant in the lower chest and thigh, while 25% showed insignificant differences in the wing muscles.

The texture of turkey meat

The results of current research (Table 4) on the hardness of muscle samples representative of turkey meat have shown average values in the range of 15.06 ± 1.31 ÷ 19.07 ± 1.35 N/cm^2 corresponding to group L1 and 18.83 ± 0.91 ÷ 22. 21 ± 1.07 N/cm^2 specific to L2.

The minimum values recorded were characteristic of the pectoral muscles in both groups, and the maximums represented the muscles at the level of the wings (L1) and the lower leg (L2) (tab. 4).

By comparing the average values recorded by each batch, we can see the superiority of the forces that characterize the hardness of batch L2 (22.21 ± 1.07 N/cm^2), compared to the minimum values obtained by L1 (15.06 ± 1.31 N/cm^2). Hardness is associated with decreased muscle mass by reducing the number and size of muscle fiber, being doubled by the accumulation of lipofuscin and increased lipid content. Simultaneously with the reduction of the length of the actin muscle fiber, the extracellular space increases being filled with supporting connective tissue.

By calculating the coefficient of variation of the values that describe the hardness of turkey meat, average values were obtained in the range 19.507 ÷ 34.79% specific to group L1, respectively 12.19 ÷ 20.56% corresponding to group L2, observing the lack of homogeneity of the character studied.

Regarding the texture characteristic represented by cohesiveness, the minimums recorded were specific to the pectoral muscles, both for L1 (0.29 ± 0.01 N/cm^2) and for L2 (0.31 ± 0.01 N/cm^2), the calculated maxima being responsible for the muscles of the lower leg in both experimental groups (0.44 ± 0.04 ÷ 0.51 ± 0.04 N / cm^2).

The averages calculated for the strength of the indicator characterizing the tenderness varied in a lower range 5.22 ± 0.24 N/cm^2, specific to the pectoral muscles corresponding to group L1. and higher 7.26 ± 0.68 N/cm^2 attributed to the muscles of the lower leg indicated to the group L2. The superiority of the values characterizes the slaughtered group at 18 weeks, compared to the slaughtered group at 16 weeks.

The elasticity of turkey meat is indicated by means of the registered forces, so the characteristic interval is defined by minimum average values specific to the pectoral muscles (0.36 ± 0.02 N/cm^2) representative of the L1 group and maximum (65 ± 0.04 N/cm^2) recorded by the muscles of the lower leg in the experimental group L2. Lower mean values specific to elasticity for both ages were characteristic of the pectoral muscles (0.36 ± 0.02 ÷0.42 ± 0.02 N/cm^2). Following the calculation of the coefficient of variation for the values that characterize the elasticity of turkey meat harvested from different anatomical regions specific to both groups (13.51 ÷ 38.15%), the lack of homogeneity of character was noticed (Table 4).

Table 4. Estimators and statistical significance of texture values for turkey hen meat

Specification		Analysed bached	n	Calculated statistical indicators				Significance of differences between batch averages (FISHER test)
				$\bar{X} \pm s_{\bar{x}}$	V%	Min.	Max.	
Chest	D	L_1	15	15.06±1.31	33.702	8.15	22.52	$\hat{F}_{5.56} > F_{0.05\%}(4.20) \rightarrow *$
		L_2		18.83±0.91	18.72	10.67	22.89	
	C	L_1		0.29±0.01	17.870	0.21	0.38	$\hat{F}_{0.91} < F_{0.05\%}(4.20) \rightarrow$ n. s.
		L_2		0.31±0.01	12.419	0.25	0.37	
	G	L_1		5.22±0.24	17.875	3.07	6.33	$\hat{F}_{3.81} < F_{0.05\%}(4.20) \rightarrow$ n. s.
		L_2		5.79±0.16	10.89	4.59	6.79	
	E	L_1		0.36±0.02	21.438	0.26	0.52	$\hat{F}_{5.56} > F_{0.05\%}(4.20) \rightarrow *$
		L_2		0.42±0.02	16.954	0.31	0.53	
	M	L_1		1.98±0.13	25.736	1.06	3.25	$\hat{F}_{5.60} > F_{0.05\%}(4.20) \rightarrow *$
		L_2		2.88±0.15	19.850	1.98	3.77	
Upper thigh	D	L_1	15	16.39±0.83	19.507	10.94	20.93	$\hat{F}_{5.83} > F_{0.05\%}(4.20) \rightarrow *$
		L_2		18.85±0.59	12.19	14.39	21.94	
	C	L_1		0.43±0.03	29.963	0.26	0.72	$\hat{F}_{0.33} < F_{0.05\%}(4.20) \rightarrow$ n. s.
		L_2		0.46±0.03	22.715	0.29	0.74	
	G	L_1		5.77±0.69	46.642	2.16	10.7	$\hat{F}_{0.02} < F_{0.05\%}(4.20) \rightarrow$ n. s.
		L_2		6.19±0.16	37.852	3.24	10.9	
	E	L_1		0.51±0.04	24.206	0.30	0.78	$\hat{F}_{5.31} > F_{0.05\%}(4.20) \rightarrow *$
		L_2		0.62±0.03	19.54	0.32	0.78	
	M	L_1		2.79±0.40	55.238	0.67	5.55	$\hat{F}_{7.91} > F_{0.01\%}(7.64) \rightarrow **$
		L_2		4.10±0.24	22.65	2.87	5.67	
Lower thigh	D	L_1	15	18.12±1.63	34.790	10.06	28.28	$\hat{F}_{4.41} > F_{0.05\%}(4.20) \rightarrow *$
		L_2		22.21±1.07	18.61	12.06	28.23	
	C	L_1		0.44±0.04	38.697	0.24	0.73	$\hat{F}_{1.53} < F_{0.05\%}(4.20) \rightarrow$ n. s.
		L_2		0.51±0.04	28.151	0.32	0.78	
	G	L_1		6.60±0.81	47.737	2.67	11.15	$\hat{F}_{0.38} < F_{0.05\%}(4.20) \rightarrow$ n. s.
		L_2		7.26±0.68	36.526	3.75	11.93	
	E	L_1		0.52±0.05	38.152	0.31	0.92	$\hat{F}_{4.53} > F_{0.05\%}(4.20) \rightarrow *$
		L_2		0.65±0.04	24.5	0.36	0.91	
	M	L_1		2.84±0.42	56.893	0.97	5.54	$\hat{F}_{4.60} > F_{0.05\%}(4.20) \rightarrow *$
		L_2		3.93±0.29	28.596	2.19	5.87	
Wings	D	L_1	15	19.07±1.35	27.385	10.09	29.11	$\hat{F}_{0.44} < F_{0.05\%}(4.20) \rightarrow$ n. s.
		L_2		20.22±1.07	20.566	16.07	29.13	
	C	L_1		0.34±0.02	18.753	0.26	0.50	$\hat{F}_{7.49} > F_{0.05\%}(4.20) \rightarrow *$
		L_2		0.41±0.02	19.594	0.31	0.57	
	G	L_1		6.27±0.60	37.300	2.68	11.33	$\hat{F}_{0.78} < F_{0.05\%}(4.20) \rightarrow$ n. s.
		L_2		7.00±0.55	30.446	3.69	11.95	
	E	L_1		0.44±0.02	21.037	0.31	0.60	$\hat{F}_{7.49} > F_{0.05\%}(4.20) \rightarrow *$
		L_2		0.53±0.02	13.519	0.41	0.66	
	M	L_1		3.19±0.37	44.643	0.83	5.47	$\hat{F}_{4.65} > F_{0.05\%}(4.20) \rightarrow *$
		L_2		4.18±0.27	25.438	2.43	5.91	

D=hardness; **C** = cohesiveness; **G** = tenderness; **E** = elasticity; **M** = chewability.

The force of chewability induced in muscle samples representative of turkey meat was noted by minimum values acquired by the pectoral muscles (1.98 ± 0.13 N/cm^2) in group L1, in discordance with the maximum values reached by the wing muscles in group L2 (4.18 ± 0.27 N/cm^2).

The differences in the values of the forces that characterize the texture of the meat are associated with the age differences between the batches having an influence on the structure of the supporting tissues.

Thus, the connective tissue representative of the intercellular support reduces its content in fundamental substances both in mucopalysis-haride and in fibrous proteins, collagen, elastin and reticular fibers synthesized by fibroplasts.

The maturation process is accompanied by an increase in the density of the hydrated gel, as well as a decrease in the water content of the dry substances. With age, elastin fibers become more rigid and fragment under the influence of continuous stretching, giving rise to pseudoelastine. Young birds are characterized

by a large number of reticular fibers, which tend to be replaced by collagen, a process noted in elastic fibers. Another transformation correlated with aging occurs at the level of enzymatic processes of increasing collagenosis, and functionally to reduce mobility. then a decrease in elasticity. Biochemically, the content of ATP, glycogen and phosphocreatine is reduced, so the elasticity decreases in the absence of ATP.

Compared to the literature, the resulting data are found in accordance with the cited values, so following a study on the correlation between pH value and texture the forces that characterize the hardness were between 16.6 ÷ 22.6 and those specific to cohesiveness ranged from 0.66 to 0.69 (Chan et al., 2011).

Following the statistical significance of the differences between the groups whose slaughter age did not coincide, distinctly significant differences were noted for 5% of 20 total tests, significant differences for 55% and 40% insignificant differences.

CONCLUSIONS

As conclusions we state the following:

✓ statistical significance of the differences between the experimental groups L1 and L2 for the specific acidity values during the *prerigor mortis* and maturation phases corresponding to each muscle group studied showed significant differences for one test (8.33%) of the 12 tests and 91% showing insignificant differences;

✓ through colorimetric characterization of turkey hen meat was observed that those one is influenced in a direct way by muscle type and by the rate of muscular and conjunctive fibres, and also by age at slaughtering;

✓ turkey hen meat luminosity was more intensely observed in the representative musculature of experimental batch slaughtered at 16 weeks in comparison with the luminosity observed at slaughtering of turkey hens at the age of 18 weeks;

✓ pectoral musculature of turkey hen broilers carcasses belonging to batch L1 enlightened lower values for red-greed coordinate (a*) being associated with the lower concentration of haemoglobin from muscles;

✓ by comparing the obtained means for representative musculature for batches slaughtered at different ages we could tell that muscles provided from the first batch, especially breast muscles, presented a superior tenderness to the one obtained for representative samples gathered from batch L2, values of Warner Bratzler shear forces being imposed by slaughtering age and also by higher resistance of conjunctive tissue during aging;

✓ as regarding texture characterization (TPA) for turkey hen meat by hardness, cohesively, elasticity and chew ability, it is observed that representative musculature of carcasses obtained at age of 16 weeks enlightened lower values, influence factors being associated with age differences between batches having also in view the structure of sustained tissues.

REFERENCES

Abeyesinghe, S.M., McKeegan, D.E.F., McLeman, M.A., Lowe, J.C., Demmers, T.G.M., White, R.P., Kranen, R.W., van Bemmel, H., Lankhaar, J.A.C., Wathes, C.M. (2007). Controlled atmosphere stunning of broiler chickens: I. Effects on behaviour, physiology and meat quality in a pilot scale system at a processing plant. *Poult Sci*., 48, 406-423.

Bianchi, M., Petracci, M. Cavani, C. (2006). Gas stunning and quality characteristics of turkey breast meat. *CD of Proceeding of XII European Poultry Conference*, Verona, Italy.

Bihan-Duval, E., Berri, C., Baeza, E., Sante, V., Astruc, T., Remignon, H., Pottier, G., Bentley, J., Beaumont, C., Fernandez, X. (2003). Genetic parameters of meat techonological quality traits in a grand-parental commercial line of turkey. *Genet Sel Evol*., 35, 623-635.

Buddiger, N., G. Albers. (2009). Future trends in turkey breeding. Hybrid. http://www.hybridturkeys.com/Media/PDF_files/Management/ Mng_future_trds_lbs.pdf.

Chan, J.T.Y., Omana, D.A., Betti, M. (2011). Functional and rheological proprieties of proteins in frozen turkey breast meat with different ultimate pH. *Poultry Science*, 90, 1112-1123.

EFSA (European Food Safety Authority). (2004). *The welfare of animals during transport*. Report of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to the welfare of animals during transport, 50-58.

Honikel, K. O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat Science*, 49(4), 447-457.

Jukna, V., Klementavičiūtė, J., Meškinytė, Pečiulaitienė, N., Samborskyte, M., Ambrasūnas, L. (2012). Comparative evaluation of quality and composition of ostrich, turkey and broiler meat. *Biotechonology in Animal Husbandry*, 28(2), 385-392.

Roberso, K.D., Kalbfleisch, J.L., Dransfield, D. (2004) Comparison of growth performance and carcass

component yield of a new strain of tom turkeys to other commercial strains. *International Journal of Poultry Science*, 3, 791-795.

Saeger, S. (2011). Determining mycotoxins and mycotoxigenic fungi in food and feed. *Woodhead Publishing Series in Food Science*. Tehnology and Nutrition, 203.

Stevenson, J. M., Seman, D. L., Weatherall, I. L., Littlejohn R. P. (1989). Evaluation of venison color by an objective method using CIELAB values. *Journal of Food Science*, 54(6), 1661-1662.

Yilmaz, O., Denk, H., Kucuk, M. (2011). Growth performance and mortality in Hybrid Converter Turkeys reared at high altitude region. *Bulgarian Journal of Agricultural Science*, 17(2), 241-245.

*** Colorimetry, Second Edition, Publication CIE No. 15.2. 1986 - Central Bureau of the CIA. A – 1033 Vienna. P. O. Box 169 – Austria, p. 36-38.

*** BS EN ISO 7500:1999, online at: http://www.chescientific.com/ecat/Eng/lloyd.pdf

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RESEARCH ON PERFORMANCES IN THE PRODUCTION OF MILK CARRIED OUT BY THE BĂLĂ ECOTYPE OF THE TSURCANA BREED

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Abstract

The present paper aims to analyze the quantitative performances and aptitudes in the direction of milk production achieved by the Bălă ecotype of the Tsurcana breed. The quantitative analysis was performed on a sample of female sheep from the Bălă ecotype, selected from farms in the northwest region of Romania, respectively the Maramureș and Bistrița-Năsăud areas. In order to carry out the analysis, statistical data were extracted from the genealogical register, following indicators such as total milked milk production, lactation period, suckling period duration, milking period duration. The results of this study highlight that the average milk production calculated for the period 2016-2019 and for the selected sheep herd is 82 kg milk, according to the standard of the Tsurcana breed, using the t-statistic test for the selected sample. Thus, the data show a difference of approximately 12 kg of milk between the average milk production of the selected sheep from the Bălă ecotype and the average milk production of the Tsurcana breed, a difference which is statistically insignificant. Finally, we can see that the Bălă ecotype has a good performance in milk production within the breed standard.

Key words*: evolution, lactation, milk production, Romania, Tsurcana breed.*

INTRODUCTION

Sheep breeding enjoys a great attention, as it is the main occupation among many farmers. The sheep breeds are exploited for the purpose of valuing their production, the small and medium-sized farms being the basic production units (Taftă, 2003). The sheep exploitation directions at national level mainly concern the milk, meat, wool and sheep pelts production, being appreciated for their diversity and special biological value (Pascal, 2007).

In the context of the diversification of sheep production, milk production is a major interest for consumers, who are mainly interested in the consumption of healthy foods with numerous nutritional benefits. Sheep milk is more popular among consumers in the form of dairy products, such as cheese and yogurt, while fresh sheep milk being rarely consumed due to its fat composition (Mohapatra, 2019). Normally, any sheep breed has milk production, but some breeds are specialized in this direction, while others are used for other productions such as meat or wool. (Stanciu, 2014). Worldwide, the total sheep population is 1209 million sheep, of which 21% are dairy sheep, respectively 254 million sheep, with a total milk production of 10.63 million tons. The most popular dairy sheep breeds (with a production higher than 200 liters/lactation) are Awassi, Assaf, East Friesian, Lacaune, Sarda, Chios, Manchega (Buzu, 2017). According to the latest statistical data worldwide, between the countries producing sheep milk, Turkey is the country with the most significant production of sheep milk (1.4 million tons), followed by China (1.2 million tons) and Greece (753 thousand tons). In the top 10 countries on sheep milk production, Romania is ranked 5th, with an amount of 626,145 tons in 2019, followed by Spain, Italy, Sudan, Mali and Somalia (FAO, 2019).

In accordance with the NIS (National Institute of Statistics) statistical data regarding the evolution of the sheep population in Romania, between 2013 and 2019, the total number of sheep increased steadily, from 9.1 million heads in 2013 to 12.9 million heads in 2019, an increase of 41.6% (NIS, 2019).

The evolution of sheep milk production in Romania has seen an upward trend in recent years, from 2013 to 2015, reaching a peak production of 4520 thousand hl in 2015,

following by a decrease to 3744 thousand hl in 2017. NIS data for 2019 show a significant increase in milk production, 3.7% more than the production recorded in 2017. What is more, in 2019 was registered a production of 5924 thousand hl of sheep milk, increasing by more than 53% compared to 3885 thousand hl registered in 2018 (NIS, 2019).

The most important sheep breeds in Romania, with their characteristic productions, are: Tsurcana - mixed production, Merinos - wool, Țigaie - wool and milk, Karakul - sheep pelts. Being a mixed race, the Tsurcana breed is the most widespread on the territory of Romania. According to NAAB (National Agency for Animal Breeding), the most recent statistical data find that the Tsurcana breed occupies a significant proportion in the breeds raised in our country (NAAB, 2019).

Out of the total number of sheep registered in the Official Production Control (OPC), the Tsurcana breed holds over 73% of the sheep livestock (1.7 million heads), followed at distance by the Țigaie breed with 13% of the livestock (323 thousand heads), respectively Merinos 6% (150 thousand heads) and Karakul 2% (21 thousand heads), out of a total of 2.4 million heads in COP (NAAB, 2019).

In the last 40 years, the following ecotypes have been differentiated from the Tsurcana breed: Tsurcana Bălă, Tsurcana Bucălaie, Tsurcana Brează, Tsurcana Oacheșă of Caransebeș and Tsurcana of Brastavățu. Of the six ecotypes, the Tsurcana Bălă ecotype is part of the white variety of the Tsurcana breed, which occupies the widest range, being the most numerous. At the same time, the white variety is appreciated for its greater milk production.

Therefore, in the exposed context, the present paper aims to carry out an analysis regarding the quantitative performances and aptitudes in the direction of milk production achieved by the Bălă ecotype of the Tsurcana breed, in the northwest region of Romania, highlighting the evolution of milk production in 2016-2019 and the lactation periods of the sheep population analyzed.

MATERIALS AND METHODS

The objective of this study is to present the productive characteristics of the Bălă ecotype of the Tsurcana breed (quantitative results regarding total milk production, lactation period, suckling period duration, milking period duration). In this respect, the quantitative analysis was performed on a sample of 2422 females from the Bălă ecotype, selected from farms in the northwest region of Romania, respectively the Maramureș (MM) and Bistrița-Năsăud (BN) areas, farms growing this ecotype (Table 1). There were selected farms in each area, with variable sheep numbers, registered in the Genealogical Register (GR) of the Tsurcana breed administered by the Association of Shepherd Breeders "Păstorul Crișana" Arad (GR Tsurcana, 2020).

Table 1. Sheep sample selected for analysis (heads)

Year	Area	Lactation					Total/year
		I	II	III	IV	V	
2016	MM	80	80	80	80	80	**400**
	BN	80	80	80	80	80	**400**
2017	MM	80	80	80	80	80	**400**
	BN	80	80	80	80	80	**400**
2018	MM	80	80	80	80	80	**400**
	BN	80	80	80	80	80	**400**
2019	MM	80	80	80	80	80	**400**
	BN	80	80	80	80	80	**400**
Total/ lactation		**640**	**640**	**640**	**640**	**640**	**3200**

Area	**MM**	**BN**	**Total**
Total livestock	1600	1600	3200
Unique animals	1360	1062	2422

The principle of sample selection was based on a lactation period analysis. Thus, lots of 80 sheep heads were chosen for 5 lactation periods, during 2016-2019 (Table 1). There were analyzed 1600 animals in each area, of which a total of 1360 unique animals from Maramureș area and 1062 unique animals from Bistrița-Năsăud area.

Analysis of quantitative results regarding quantitative results regarding total milk production, lactation period, suckling period duration, milking period duration was performed in Microsoft Excel, using descriptive statistical methods.

Also, the paper aims to compare the average milk production for the sample selected from the Bălă ecotype, with the average milk production of the Tsurcana sheep population, specified in the breed standard.

In this respect, the *t-statistic* test was applied to the sample, with the help of the SPSS program, formulating the test hypothesis H_0: $\overline{x} = \mu$, which tests whether the difference between the average milk production of the sheep sample ($\overline{x}$) and the average production of Tsurcana breed (μ) is statistically significant.

RESULTS AND DISCUSSIONS

Tsurcana breed standard

In view of the zootechnical appreciation of the animals, the main characteristics of the Tsurcana sheep are included in the standard of the Tsurcana breed (SR 13502/2006), document approved at national level by the Association of Standardization in Romania. This standard refers to pure-bred breeding sheep, the Tsurcana breed, recorded in the Genealogical Register (ASRO, 2006).

The body weight is between 48-65 kg for males (lambs, rams), respectively 38-48 kg for females (lambs, sheep), but can be exceeded. The sheep of this breed have the average age at the first delivery of 24 months.

Mainly, sheep from this breed are exploited for mixed milk-meat-wool production. Average milk production at the Tsurcana breed on the whole lactation is 100-120 kg, and the milked milk production is 60-70 kg, the quantity of milk being determined by weighing, with an accuracy of ± 40 g.

According to the breed standard, the sheep from the Tsurcana breed are divided into three varieties: White, Black, Rațca (assimilated). The Bălă ecotype of the Tsurcana breed is part of the White variety.

It is a sheep population with very long coarse white wool, with an optimal structure between fibers. They are harmonious sheep with a medium body weight and mixed productions. The most typical herds are found in Alba county, in Valea Sebeşului (Săsciori, Sugag, Mărtinie), Valea Pianului (Loman, Purcareţ) and from this area they have spread in large numbers in the area of Bistrița and in the farms in Cluj County, Maramureș, Satu-Mare, Sălaj, Arad, Timiş and the area of Moldova.

Performances regarding the milk production of the Bălă ecotype during 2016-2019

The results of the research study followed the quantification of the productive aptitudes in the direction of milk production of the Bălă ecotype of the Tsurcana breed, being presented the main performance indicators for the analyzed sample.

In order to characterize the productive performances, the representation of the lactation sequence was performed: L I, L II, L III, L IV, L V for the studied herd. In this regard, for each lactation the number of sheep, the age at the delivery, the number of the lactation or age category, the duration of the lactation period, the total milk production, the duration of the milking period, the average daily production were taken into account.

Also, the method used for milk control, the unit of measure for the quantity of milk, the date of lactation, the date of weaning of the lambs, the number of milk tests on sheep (4 controls), for the calculation of milk production on lactation were analyzed.

Milking period duration

The duration of the milking period represents the time interval between weaning the lamb and ending the lactation.

The milking system used is system II - milking after a period of lactation, according to the *Norms of appreciation of sheep and goats of reproduction*, *Order no. 22 of January 20, 2006*.

The official control of milk production was carried out by the A.C.O.C. Bistrița-Năsăud and A.J.C.O.C. "PRO OVIS" Maramureș, accredited by NAAB to evaluate the performances regarding milk production.

Thus, according to ICAR regulations, milk production is evaluated using the official control methods of milk production in sheep (ICAR Guidelines, 2019).

In the analyzed farms, milk production control was achieved by using the control methods AT (one control per day at 30 days, first in the morning and next in the evening) and A4 (two controls, in the morning and the evening of the control day, at interval of 30 days).

The average duration of the milking period calculated for the livestock analyzed from the Maramureș and Bistrița-Năsăud regions is represented in Table 2.

For the 2016-2019 period, the milking period has an average duration of 109 days (L I), 111 days (L II), 109 days (L III), 108 days (L IV) and 110 days (L V), with a total average value of 109 days.

Table 2. Average milking period duration (days)

Year	Lactation					Average/
Area	I	II	III	IV	V	year
2016	**95**	**96**	**96**	**95**	**97**	**96**
BN	85	88	87	87	88	87
MM	104	104	104	104	105	104
2017	**104**	**105**	**101**	**101**	**101**	**102**
BN	103	104	104	102	103	103
MM	104	105	98	100	100	101
2018	**113**	**116**	**115**	**111**	**115**	**114**
BN	102	102	100	102	101	101
MM	125	130	130	119	130	127
2019	**123**	**127**	**123**	**125**	**126**	**125**
BN	127	130	128	126	128	128
MM	119	124	119	124	123	122
Average/ lactation	**109**	**111**	**109**	**108**	**110**	**109**

Suckling period duration

The duration of the suckling period is the period in which the lambs are suckled by the mother sheep. Following the analysis, in Table 3 we can see that the average duration of the suckling period in 2016-2019, for the selected sheep herd, is 71 days.

The duration of the suckling period in the Tsurcana breed is usually 2-3 months. The result found in this analysis is in the literature data (Tafta, 2003).

Table 3. Average suckling period duration (days)

Year	Lactation					Average/
Area	I	II	III	IV	V	year
2016	**78**	**75**	**76**	**79**	**79**	**77**
BN	91	84	87	92	90	89
MM	66	66	66	66	67	66
2017	**60**	**59**	**60**	**68**	**67**	**63**
BN	65	63	67	75	73	69
MM	56	55	54	60	61	57
2018	**73**	**90**	**74**	**70**	**65**	**74**
BN	83	86	54	86	54	73
MM	63	94	94	53	75	76
2019	**66**	**67**	**68**	**64**	**77**	**68**
BN	67	62	70	68	69	67
MM	65	73	66	60	84	70
Average/ lactation	**69**	**73**	**70**	**70**	**72**	**71**

Lactation period

The lactation period represents the sum, in days, of the suckling period and of the milking period. This can be also calculated as the difference, in days, between the delivery date and the end of lactation.

According to our data, an average suckling period duration of 71 days was obtained, respectively 109 days for the average milking period duration. According to the first calculation method, the lactation period represents the sum of the two periods, respectively 180 days. The same result can be observed in Table 4, where we find that, for the period 2016-2019, the sheep population from the Bălă ecotype of the Tsurcana breed from the two areas of interest (MM and BN) has an average lactation period of 180 days.

Table 4. Average lactation period (days)

Year	Lactation					Average/
Area	I	II	III	IV	V	year
2016	**173**	**171**	**172**	**174**	**175**	**173**
BN	176	172	174	179	179	176
MM	170	170	170	170	172	170
2017	**164**	**164**	**161**	**169**	**169**	**165**
BN	169	167	171	178	176	172
MM	159	160	152	160	161	158
2018	**186**	**206**	**189**	**180**	**180**	**188**
BN	185	188	154	188	155	174
MM	187	224	224	172	205	203
2019	**189**	**194**	**192**	**189**	**202**	**193**
BN	194	192	198	194	197	195
MM	184	197	185	184	207	191
Average/ lactation	**178**	**184**	**178**	**178**	**182**	**180**

Reported on each of the five lactations, we have the following results: 178 days for lactation periods I, III and IV, 184 days for

lactation II, and 182 days for lactation V (Figure 1).

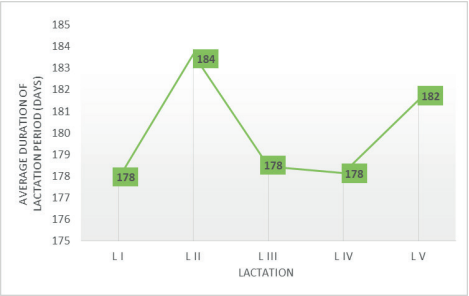

Figure 1. Average lactation period (days)

Total milk production

The total milk production represents milk production from the milking period in the case of lactation after the suckling period. According to the *Norms for the appreciation of sheep and goats for breeding, Order no. 22 of January 20, 2006,* only milk production during milking can be part of the registration of milk on the farm.

Thus, the data extracted from the Genealogical Register represented the basis for calculating the total milk production of the livestock, taking into account the milk after the weaning and the duration of this period which begins with the weaning of the lambs and ends at the end of lactation (Order no. 22, 2006).

Table 5. Average daily milk production (g)

Year / Area	Lactation I	II	III	IV	V	Average/ year
2016	**772**	**742**	**731**	**727**	**701**	**735**
BN	1036	963	957	943	896	959
MM	509	521	504	510	506	510
2017	**710**	**706**	**696**	**724**	**726**	**712**
BN	763	775	724	722	741	745
MM	657	638	668	726	711	680
2018	**803**	**815**	**736**	**803**	**700**	**771**
BN	840	835	687	835	644	768
MM	765	795	786	771	755	774
2019	**828**	**781**	**832**	**774**	**730**	**789**
BN	702	717	710	699	694	704
MM	954	844	954	849	767	874
Average/ lactation	**778**	**761**	**749**	**757**	**714**	**752**

According to the analyzes carried out on 80-head sheep lots, on each of the five lactations, in the period 2016-2019, it turns out that the average daily milk production (obtained from the controls carried out) is 752 g. Regarding the control years, the largest daily registrations for milk production were made in 2019, with an average daily production of 789 g (Table 5). The results from Table 6 show that the average of total milked milk production calculated for the period 2016-2019, for the sheep livestock selected from the two areas in the north-western region of Romania is 82 kg, with relatively close records for each of the five lactations: 84 kg for L I and L II, 81 kg for L III and L IV, respectively 78 kg for L V (Figure 2).

Table 6. Average of total milked milk production (kg)

Year / Area	Lactation I	II	III	IV	V	Average/ year
2016	**70**	**69**	**68**	**67**	**66**	**68**
BN	88	84	83	82	79	83
MM	53	54	52	53	53	53
2017	**73**	**74**	**70**	**73**	**74**	**73**
BN	79	81	75	74	76	77
MM	68	67	66	73	71	69
2018	**90**	**94**	**85**	**88**	**82**	**88**
BN	86	85	68	85	65	78
MM	95	103	102	92	98	98
2019	**101**	**99**	**102**	**97**	**92**	**98**
BN	89	93	91	88	89	90
MM	113	104	114	105	94	106
Average/ lactation	**84**	**84**	**81**	**81**	**78**	**82**

Figure 2. Evolution in 2016-2019 period for the average total milk production (kg)

According to the characteristics presented in the standard of the Tsurcana breed, the total milk production at the Tsurcana breed on the whole lactation is 100-120 kg, and the average of milked milk production is 60-70 kg, with an accuracy of ± 40 g.

In order to make a comparison between the average milk production for the sample selected from the Bălă ecotype, with the average production made by the population of sheep of the Tsurcana breed, specified in the

breed standard, the t-statistical test was applied to the selected sheep sample, which tests whether the difference between the average production of the sheep sample and the average production of the population is statistically significant.

The test involves the formulation of the null hypothesis, meaning the hypothesis that there is no statistically significant difference between the average value of the production for the studied sample and the estimated one at the population level, with a probability of 95% (p = 0.95).

Thus, for a sample size n = 3200 and a significance level $\alpha = 0.05$ (1-p), we verify the hypothesis H_0: $\overline{x} = \mu$, where $\overline{x}$ represents the sample mean, and μ represents the Tsurcana breed mean. The alternative hypothesis is H_1: $\overline{x} \neq \mu$, meaning that the average milk production of the sample is different from the average milk production of the population.

In Table 7 are presented: the average value of the sample, the minimum and maximum limit, the standard deviation and the standard error of the mean.

Knowing the sample data, respectively its size n = 3200, the sample mean $\overline{x}$= 82, the standard deviation s = 17.31, and the population mean μ = 70 (according to the breed standard, the maximum limit of the range 60-70 kg), the t_{calc} statistical value calculation is performed using the calculation method of the t test for a single sample in the SPSS program.

Table 7. Sample descriptive statistics

One-Sample Statistics						
	N	**Mean**	**Minimum**	**Maximum**	**Std. Deviation**	**Std. Error Mean**
Total milk production (kg)	3200	81.82	46.79	199.29	17.31	.306

In order to decide whether hypothesis H_0 is rejected or accepted, it is necessary to compare the calculated value t_{calc} with the critical value t_{crit} (given by the significance level). According to the results presented in Table 8, $t_{calc} < 0.01$, that is $t_{calc} < t_{crit}$.

Therefore, because the value of the calculated statistical parameter of the test does not belong to the critical region, the null hypothesis is accepted. Thus, we can say that there is no statistically significant difference between the average value of the production for the studied sample and the estimated one at the population level, that is the difference of 11.82 kg between the average of the sheep population from the Bălă ecotype and the average of the Tsurcana breed is statistically insignificant.

Table 8. Results of the t-statistic test in SPSS

One-Sample Test						
	Test Value = 70 (μ)					
	t_{crit}	**df (n-1)**	**Sig. (2-tailed) (t_{calc})**	**Mean Difference**	**95% Confidence Interval of the Difference**	
					Lower	Upper
Total milk production (kg)	38.650	3199	.000	11.82	11.22	12.42

CONCLUSIONS

With a percentage of over 73% of the total number of sheep registered in the Official Production Control (OPC), the sheep from the Tsurcana breed represent a point of interest for the sheep farmers in Romania.

The evolution of sheep milk production in Romania has seen an upward trend in recent years, in 2019 being registered a total milk production of 5924 thousand hl of sheep milk, increasing by 53% over 2018.

The objective of this paper was to analyze these performances at the Bălă ecotype, which is part of the White variety of the Tsurcana breed. In this respect, the sheep herds analyzed were relevant to obtain results corresponding to this ecotype, being selected from farms with a majority of sheep from the Bălă ecotype.

The results of the study show that, for the sheep sample from the Bălă ecotype, the average

duration of the suckling period in 2016-2019 is 71 days, the average duration of the milking period is 109 days and the average lactation period is 180 days.

In terms of milk production, the standard of the Tsurcana breed presents two values to which the milk production of the Bălă ecotype is reported, namely: the average of total milk production per lactation which at the Tsurcana breed is 100-120 kg/lactation and the milked milk production which is 60-70 kg.

The data presented show that the average milked milk production calculated for the period 2016-2019, at the sheep herds selected from the two areas in the north-western region of Romania, belonging to the Bălă ecotype, is 82 kg, with relatively close records for each of the five lactations: 84 kg for L I and L II, 81 kg for L III and L IV, respectively 78 kg for L V.

In order to obtain relevant data regarding the quantitative performances in milk production at the Bălă ecotype, it was considered the comparison of the average milk production estimated for the lots of sheep analyzed with the breed standard, respectively an individual average of 82 kg of milk compared to the upper limit of 70 kg established in the breed standard. Performing the statistical test on the sample of 3200 sheep results heads show a mean difference of approximately 12 kg.

From the obtained results we can say that there is no statistically significant difference between the average value of the production estimated for the studied sample and the average production at the breed level.

In conclusion, we can appreciate that the Bălă ecotype has a productive performance in milk production within the breed standard, without major difference.

REFERENCES

ASRO (2006). *The standard of the Tsurcana sheep breed* - SR 13502. Retrieved from Asociation of Standardization in Romania: http://www.asro.ro.

Buzu, I. (2017). Worldwide trends development of sheep breeding. Chișinău, Republic of Moldova: *Scientific Papers, Series D. Animal Science,* LX.

FAO (2019, March 23). FAOSTAT. Retrieved from Food and Agriculture Organization Corporate Statistical Database: http://www.fao.org/faostat/en/#data.

GR Tsurcana (2020). *Genealogical Register of the Tsurcana breed.* Retrieved from Association of Shepherd Breeders "Păstorul Crișana" Arad : http://pastorulcrisana.ro/.

ICAR Guidelines. (2019). Retrieved from International Committee for Animal Breeding: https://www.icar.org.

Mohapatra, A. A. (2019). Sheep milk: A pertinent functional food. *Small Ruminant Research*, 181, 6-11.

NAAB (2019). National Agency for Animal Breeding. Retrieved from NAAB: http://www.anarz.eu

NIS (2019). National Institute of Statistics. Statistical Yearbook of Romania 2019. Retrieved from https://insse.ro/cms/ro/tags/anuarul-statistic-al-Romaniei.

Order no. 22. (2006). NAAB. Retrieved from The National Agency for Animal Breeding: http://www.anarz.eu.

Pascal, C. (2007). *Breeding of sheep and goats.* Iasi: PIM Publishing House.

Stanciu, S. (2014). *Romanian milk market analysis*. SEA - Practical Aplication of Science, 1(2 (4)), 373-380.

Taftă, V. N. (2003). *Sheep breeding in small and medium farms*. Bucharest, RO: Ceres Publishing House.

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STUDY ON THE OPTIMAL SIZE OF BOVINES FARMS ACCORDING TO DIFFERENT FACTORS OF INFLUENCE

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Abstract

The paper aimed to present the optimal size of different types of bovine farms for meat and milk production. The study is based on the statistical data provided by Ministry of Agriculture, Forests and Rural Development, National Institute of Statistics and The Food and Agriculture Organization of the United Nations. The data have been processed into the following indicators: cattle livestock, number of dairy cows, number of bovine farms, type of farm and economic efficiency. During the analysed period, cattle livestock has continuously increased and dairy cows number decreased. As a conclusion, the best performing cow farms in our country are those that have medium size and use modern breeding and exploitation technologies.

Key words*: bovines farms, economic efficiency, meat production, milk production.*

INTRODUCTION

Cattle breeding in our country is influenced by a number of general and specific factors. The general factors are those that depend on: climate change, the legislative framework, the type of technology used on the farms, consumer preferences, food prices etc.

Climate change is obvious and already affects relatively large areas in our country, which leads to problems related to the management of extreme weather events, ensuring drinking water resources and quality feed for cattle.

In terms of consumer preferences in our country, they are generally correlated with the price of products on the market. Beef is not among top preferences of consumers due to the higher price and poor culinary culture. Instead, milk and all its derivatives products are a source of food consumed in large quantities by the population of our country.

The specific factors influencing the individual production of cattle are internal and external. External factors are numerous, among the most important being: technical exploitation factors (feeding level, water supply), organizational exploitation factors (body care, animal movement, proper milking) and microclimate factors (temperature, air humidity, light, atmospheric pressure, air currents and weather) (Georgescu et al., 1995; Ghirilă et al., 2007).

The internal factors that influence milk production are genetic (breed, individual and age) and physiological (reproductive activity, lactation etc.) (Georgescu et al., 1995; Ghirilă et al., 2007).

The principles of production organization on cattle farms are:

1. **Concentration of herds in farms** of optimal dimensions, which allows the introduction of technical progress and advanced modern managerial principles in raising dairy cows, respectively obtaining high performance and profitable economic results.

In our country, after 1989, this principle was cancelled due to the fragmentation, restructuring and privatization of dairy farms. Thus, the process of undersizing of dairy farms was recorded, it reached an incredibly small size of only 1.4 cows/farm.

2. **The intensification of dairy farming** is reflected in the significant increase in production per animal and per unit of built-up area, per unit of agricultural area or per unit of time.

Promoting this principle provides a number of advantages, namely: reducing specific food

consumption, increasing labour productivity, reducing production costs, saving funds and materials for the construction of production facilities. Intensification directly expresses the response of animal production to certain operating conditions, constituting a principle of exploitation that can be applied to any size of farm.

3. **The integration of production** consists in achieving a rational flow of all factors of production, processing and capitalization (land, shelters, animals, production facilities and equipment, harvesting milk production, primary treatment, storage and marketing of dairy products).

In the world, this principle is intensely manifested in production cooperatives, which are well organized both horizontally and vertically. Thus, these cooperatives have the entire material base for achieving high performance in milk production, but at the same time benefit upstream from specialized services for the supply of equipment, seeds, technical assistance etc., and downstream of a well-organized system for efficient takeover and capitalization of milk production.

4. **Diversification and industrialization** of production means that milk obtained on the farm is processed in direct relation to market requirements. In general, it is recommended to have a high degree of milk processing to obtain the most varied dairy products, ensuring stability and efficiency for both the producer and the processor.

5. **Modern marketing** must be developed for the study of the market (knowledge of demand and supply) both in terms of quantity and quality and the establishment of efficient ways of marketing (Oancea, 1997). The development of production in farms of optimal sizes and dimensions is the indispensable organizational framework for increasing production and economic efficiency.

The concept of farm size reflects **the qualitative side of the production organization** and the level of its intensification.

The size of the farm reflects the **quantitative side of the production organization** and is closely related to its size.

The extent to which the investments made multiply the positive effect of the existing natural and biological conditions is reflected in the level of average yields, production costs and profit.

The optimal size from the economic point of view of an enterprise consists in those dimensions of the branches of production (including territorial dimensions), which allow the full and rational use of land, material technical means and labour, achieving a maximum production per unit. surface and high quality, with the lowest possible costs and the highest possible profitability in given economic and natural conditions (Vidu, 2002).

The maximum production capacity of the set of material, human and financial resources reflects the upper limit of the optimal size of the farm, **which ensures the premises for achieving high economic efficiency**.

MATERIALS AND METHODS

This paper is a study of data from national and international databases on the current situation of cattle breeding in the E.U. and our country.

At the national level, the studied data was provided by the National Institute of Statistics (INS) and the Ministry of Agriculture and Rural Development (MADR).

At the international level, the data provided by the Food and Agriculture Organization of the United Nations (FOASTAT) was taken into account. Statistical data processing was performed by classical methods of analysis: average, minimum, maximum and percentage.

RESULTS AND DISCUSSIONS

At the level of the European Union, a great variability of the cattle sector is identified between regions (Western European countries and Eastern countries or Northern and Southern Member States).

Farms in Western Europe are specialized in either milk or meat. Eastern farms are most often small, around 10 ha or less, have mixed specialization (milk-meat or even large crops and cattle). The largest herds of cattle are concentrated in the Benelux and around the Alps, eastern Poland, north-western France and Ireland. The cattle sector is of particular importance in naturally disadvantaged areas, such as mountain ranges or other regions with low production potential.

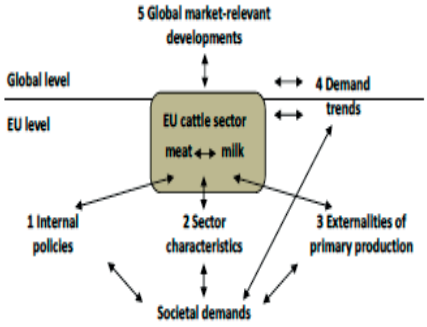

Figure 1. Key points in the cattle sector in the E.U. (Directorate-General For Internal Policies, Policy Department B: "Structural and Cohesion Policies", 2017)

The herds of cattle grown for milk production in our country have evolved sinuously in the last 20 years, from 1,769 thousand heads in 2000, to 1,299 thousand heads in 2010 and increased to 1,389 thousand heads in 2018 (Table 1). Of these, in 2018, only 165 thousand heads were in commercial farms, meaning 11.87% of the total population, the remaining 88.13% are in households.

Table 1. The evolution of cattle herds for milk production in our country (INS, MADR, 2019)

Specification	2000	2005	2010	2015	2018
Total herds (thousand heads)	1,769	1,812	1,299	1,311	1,389
Herds in commercial farms (thousand heads)	-	-	61	107	165

The number of cattle grown for meat production in our country has evolved downwards in the last 20 years, from 3,051 thousand heads in 2000 to 2,093 thousand heads in 2018 (Table 2). Of these, in 2018, only 295 thousand heads were grown in commercial farms, meaning 14.10% of the total national population.

Table 2. The evolution of cattle herds for meat production in our country (INS, MADR, 2019)

Specification	2000	2005	2010	2015	2018
Total herds (thousand heads)	3,051	2,862	2,001	2,092	2,093
Herds in commercial farms (thousand heads)	-	-	118	215	295

Milk production evolved downwards from 48,518 thousand hl in 2000, to 41,598 thousand hl in 2018, and the average milk production per cow head was 3,574 l/head in 2018 (Table 3).

Table 3. The evolution of cow's milk production in our country (INS, MADR, 2019)

Specification	2000	2005	2010	2015	2018
Milk (thousand hl)	48,518	55,334	42,824	42,664	41,598
Average milk production (l/head)	-	3,510	2,595	3,325	3,574

Table 4. The evolution of beef production in our country (INS, MADR, 2019)

Specification	2000	2005	2010	2015	2018
Live meat production (thousand tons)	330	383	205	200	212
Average slaughter weight (kg/head)	-	333	264	335	314

The beef production has decreased from 330 thousand tons in 2000 to 212 thousand tons in 2018, and the average slaughter weight was 333 kg/head in 2005 and reached 314 kg/head in 2018 (Table 4). The decreasing trend of both grown herds for meat and slaughter weight is not favorable for beef production in Romania.

The external market for cow's milk is a dynamic one. According to FAOSTAT in 2000, our country imported 3,894 tons of milk, worth 982 thousand dollars and in 2018 reached 191,625 tons of milk and a value of 94,271 thousand dollars.

The export of cow's milk was only 24 tons in 2000, worth 16 thousand dollars and increased in 2010 after joining the E.U. to 1,092 tons worth 18,449 thousand dollars and reached 42,292 tons in 2018, with a value of 25,906 thousand dollars (Table 5). The upward trend of this product shows us that it is an elementary product, demanded on the domestic market.

Table 5. The evolution of the import and export of cow's milk from our country (FAOSTAT, 2020)

Specification	Import		Export	
	Tons	Thousands of dollars	Tons	Thousands of dollars
2000	3,894	982	24	16
2005	1,941	1,030	890	830
2010	111,334	54,307	1,092	660
2015	141,927	60,760	32,379	18,449
2018	191,625	94,271	42,292	25,906

Table 6. The evolution of the import and export of beef from our country (FAOSTAT, 2020)

Specification	Import		Export	
	Tons	Thousands of dollars	Tons	Thousands of dollars
2000	612	679	25	38
2005	3,305	6,471	151	376
2010	3,171	10,169	867	3,259
2015	5,018	8,036	3,134	9,407
2018	2,800	8,597	10,403	28,492

The external market for beef is smaller. According to FAOSTAT in 2000, our country imported 612 tons of meat, worth 679 thousand dollars and in 2018 reached 2,800 tons of meat worth 8,597 thousand dollars (Table 6).
Beef exports were only 25 tons in 2000, worth 38,000 dollars and reached in 2010 after joining the U.E. to 867 tons worth 3,259 thousand dollars and reached 10,403 tons in 2018, with a value of 28,492 thousand dollars. It is thus observed that the import of beef is lower than the export, especially since in general, high quality meat is imported and meat is exported in the carcass, which does not benefit the Romanian farmer.

Table 7. The evolution of the purchase price of cattle products in our country (INS, MADR, 2019)

Specification	2000	2005	2010	2015	2018
Purchase price of milk (lei/litter)	-	0.64	0.94	1.16	-
Purchase price of beef (lei/kg live)	-	3.30	4.85	6.05	-

The purchase price of beef was 3.30 lei/kg live weight in 2005 and reached 6.05 lei/kg live weight in 2015 (Table 7). As for cow's milk, it was sold at 0.64 lei/litter in 2005 and reached in 2015 at 1.16 lei/litter.
The factors that condition the size of farms are natural, technical, organizational, social and conjunctural factors:

- the area of land cultivated with fodder distributed on the animal's head;
- the existence of urban centres, which should represent markets for farmers;
- the requirements of modern consumption (quality, rhythmicity in supply);
- application of scientific results;
- introduction of modern high productivity equipment;
- automation of heavy work processes;
- the existence of labor resources, the existence of a rhythmicity;
- possibilities for storage and use of manure;
- the existence of other units with the same specialization or with which one can enter into an interconditioning relationship (Vidu, 2002).

The level of the cost per unit of product (per hl of milk) decreases with the increase of the size of the zootechnical unit up to a certain point, where the minimum cost is achieved, after which it increases. The optimal size is considered to correspond to the minimum cost. (Georgescu, 1983).
According to the E.U., the type and size of the farm should be determined on an economic basis, the criterion always remaining positive.
Size classes. There is not a fixed definition of a ”small” or a ”large” farm. There are different classifications available and this information may be aggregated in order to analyse farms of different sizes. An E.U. study used the following categories to differentiate farms by size.
By economic size based on standard output in euro:

- Very small farms: < EUR 2000;
- Small farms: EUR 2000 - < EUR 8000;
- Medium-sized farms: EUR 8000 - < EUR 25000;
- Large farms: EUR 25000 - < EUR 100000;
- Very large farms: ≥ EUR 100000.

By economic size based on standard output in euro, grouped into quintiles:
In order to compare the relative size of agricultural holdings in each country and the economic size of farms, they were divided into several groups:

- the smallest farms, defined as those with the lowest levels of economic output who together cumulatively account for 20% of the total standard output;
- the largest farms, defined as those with the highest levels of economic output who together cumulatively account for 20% of the total standard output.

With this approach, the definition of “small” or “large” farms depends not on a uniform threshold (for the E.U. as a whole), but reflects the distribution in each of the E.U. Member States. Thus, the size of farms can be presented in relation to their standard production in each Member State (https://ec.europa.eu/eurostat/statistics).
By physical size based on utilised agricultural area in hectares:

- Very small farms: < 2 hectares;
- Small farms: 2 hectares - < 20 hectares;
- Medium-sized farms: 20 hectares - < 100 hectares;
- Large farms: ≥ 100 hectares

The categorization is based on the Total Standard Output (TSO) of a farm. This output is calculated as the sum of Standard Output (SO) of each of the farm's activities multiplied by the quantity of the activity's output. The TSO is therefore a monetary value in euros which quantifies the economic size of a farm. It is also very suitable for classifying the specializations of farms because the SOs of the various activities of the farm can be compared to each other as well as to the TSO (Directorate-General For Internal Policies, Policy Department B: "Structural And Cohesion Policies", 2017).

In this way, this methodology allows for the classification of farms according to the pattern shown in Figure 2. For the purpose of this study, the following five principal farming types will be considered:

- Specialist dairying,
- Specialist cattle - rearing and fattening ("specialist fattening"),
- Cattle - dairying, rearing and fattening combined ("dairying and meat"),
- Mixed livestock, mainly grazing livestock ("mixed livestock") and
- Field crops - grazing livestock combined ("crops and cattle").

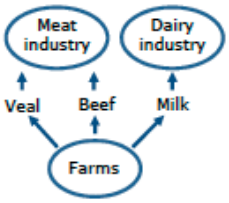

Figure 2. Cattle farming sector in the E.U. (Directorate-General For Internal Policies, Policy Department B: "Structural and Cohesion Policies", 2017)

A 2016 European Commission study shows that most farms specializing in mixed milk and meat production are located in Austria and Romania. While Austrian farms are among the top 50 richest farms in the E.U., with a PPS per capita of 130% compared to the E.U. average, in Romania the regions where these farms are located are very poor, with a PPS per capita of 30-50% of the E.U. average.

An important study conducted in the USA showed that in the period 1970-2006 the number of dairy farms decreased steadily and abruptly from 648,000 in 1970 to 75,000 in 2006. The total number of dairy cows decreased from 12 million in 1970 to 9.1 million in 2006 amid doubling individual milk production and increasing farm production by about 12 times. Also, the average farm size increased from 19 cows in 1970 to 120 cows in 2006. This trend indicates an increasing specialization and an increase in farm size. In the USA, the largest dairy farms have over 15,000 cows, and farms with 1,000-5,000 heads are quite common. (MacDonald, J.M. et al., 2007).

Table 8. The size of cattle farms in our country (heads) (INS, 2019)

Dimension	2010	2013	2016
< 0.1	28,650	23,460	22,282
0.1-0.3	38,659	31,799	27,828
0.3-0.5	29,328	22,978	18,334
0.5-1	80,186	62,036	52,533
1-2	163,764	136,342	106,091
2-5	267,925	236,438	193,775
5-0	86,528	86,765	84,236
10-20	21,026	23,820	25,294
20-30	4,223	4,545	4,773
30-50	3,262	3,250	2,908
50-100	2,382	2,168	1,855
> 100	2,087	1,569	1,228
TOTAL	728,020	635,216	541,137

In our country, cattle farms are of various sizes. The total number of farms decreased by 12.7% in 2013, compared to 2010 and by 14.8% in 2016, compared to 2013. The analysis performed on farm sizes shows a reduction of the number of holdings with a small number of animals and the increase in the number in the segment of 10-20 heads, respectively 20-30 heads. Thus, in the sector of farms with 10-20 heads, the increase in the reference period was by 20.30%, and in the sector of farms with 20-30 heads, the increase was by 13% (Table 8). These increases can be explained by small farm incentive programs.

CONCLUSIONS

The specific factors influencing the individual production of cattle are internal and external. External factors are numerous, among the most important being: technical exploitation factors (feeding level, water supply), organizational exploitation factors (body care, animal movement, proper milking) and microclimate factors (temperature, air humidity, light, atmospheric pressure, air currents and weather). The internal factors that influence

milk production are genetic (breed, individual and age) and physiological (reproductive activity, lactation etc.).
The principles of organizing production on cattle farms refer to: concentration of herds in farms, intensification of dairy farming, integration of production, diversification and industrialization of production and modern marketing.
The number of dairy cows has decreased in our country in recent years, with a production per cow almost constant of about 3,500 l/head. The milk delivery price doubled in the reference period.
The number of beef cows in our country has decreased nationally in recent years, but has increased at the level of commercial farms, and the average weight on delivery is about 300 kg/head live weight with a price of 6.05 lei/kg in 2015.
At European level, the concept of small or large farm is quite flexible, it can be expressed by the economic size of the farm, its physical size or the type of specialization.
In our country, cattle farms are of various sizes. The total number of farms decreased by 12.7% in 2013, compared to 2010 and by 14.8% in 2016, compared to 2013. The analysis performed on farm sizes shows a reduction of the number of holdings with a small number of animals and the increase in the number in the segment of 10-20 heads, respectively 20-30 heads.

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REFERENCES

Georgescu, Gh., Velea, C., Temișan, V., Podar, C. (1995). *Tratat de creşterea bovinelor/Treaty of cattle breeding, vol. III*. Bucharest, RO: Ceres Publishing House.

Ghirilă, A.B., Pece, A., Mureșan, Gh. (2007). Studiul asupra factorilor externi care influențează producția de lapte/Study on external factors influencing milk production. *Agricultura - Știință și Practică/Agriculture - Science and Practice,* 3-4, 65-66.

Ghirilă, A.B., Pece, A., Mureșan, Gh. (2007). Studiul asupra factorilor interni care influențează producția de lapte/Study on the internal factors that influence milk production. *Agricultura - Știință și Practică/Agriculture - Science and Practice,* 3-4, 63-64.

Maciuc, V., Leonte, C., Radu-Rusu, R.M. (2015), *Manual de bune practici în creşterea bovinelor/Handbook of good practices in cattle breeding.* Iași, RO: Alfa Publishing House.

Vidu, Livia (2002). *Cercetări privind exploatarea vacilor de lapte în module de ferme etalon pentru sectorul privat/Research on the exploitation of dairy cows in standard farm modules for the private sector.* Thesis, UASVM Bucharest.

Directorate-General For Internal Policies, Policy Department B: "Structural And Cohesion Policies" (2017): *Research for Agri Committee - the E.U. cattle sector: Challenges and opportunities - milk and meat.*

https://ec.europa.eu/eurostat/statisticsexplained/index.php?title=Small_and_large_farms_in_the_EU_-_statistics_from_the_farm_structure_survey&oldid=406560.

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ONE HEALTH CONCEPT, CONSEQUENCE OF BIO-ECONOMIC AND ECO-ECONOMIC MANAGEMENT APPLICATION IN APICULTURE

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Abstract

One health concept aims to achieve and develop a competent bio-economic and eco-economic beekeeping management, based on scientific principles. It is an activity of national interest, useful for preserving a natural and healthy environment, ecological system and agriculture, in general, intended to ensure natural pollination of honey plants and bee species biological diversity, and in particular for the national genetic background in Romanian bee breed - Apis mellifera carpatica - as autochthonous bee population specific for the Romanian bio-apiculture areas. Concerning the importance of bees in human and humankind, Albert Einstein quoted "If the bee disappeared off the surface of the globe, then man would have only four years of life left". The famous physician referred to bees role as crop pollinators and also in achieving significant agricultural yields, that would not be possible in absence of pollinators, and their lack would have devastating impact on food procurement. The present paper overview one health points for a sustainable management in apiculture.

Key words*: apiculture, bio-economic, eco-economic, management, one health.*

INTRODUCTION

Bio-economic and eco-economic bee management context

Paleontological research confirms that bees appeared on earth long before humans. As was the case everywhere, the first primitive tribes representing the first forms of organization of human society, clustered near the watercourses and forests, where they could procure food by hunting and fishing. By searching food, they discovered in the hollows of trees the sweetness and aroma of honey combs which, at first, they reaped great risks from stings, using water for defence. After the discovery of fire, smoke proved to be a better protector against the aggressiveness of bees. That is how hunting of bee nests began, which lasted for millennia and being still a practice today in some areas of Africa and Asia (FAO).

The honey bee belongs to one of the most advanced groups of *Hymenoptera* insects-order (have two pairs of membrane wings), *Apidae* family, *Apis* genus, *mellifera* or *mellifica* species (ADW). This order shows the social life and organization of individuals into the colony or family, equating as functionality with a macro-organism, which entails division of labour, joint care of the offspring, joint food collection and processing, concentrating the reproductive potential of the organism to a single *queen* and to a few male-*drones*, joint regulation of the social organism warmth. As a consequence of this social life, emerged large population of individuals with maintain functions - *workers*, for accumulation of food supplies, showing remarkable adaptations and morphology adaptation related the digestive, respiratory, muscular, nervous, reproductive, excretory, exceptional performance of the sense organs, on enzymatic and hormonal systems, all resulting in spectacular aspects of behaviour. Given these adaptations and refinements, the colony of the honey bee is considered in zoology as a "superorganism" in which the nutrition, breathing, defence and reproduction functions of have both individual and social level (Hung et al., 2018).

An important role in maintaining an ecological balance in beekeeping and the concept of "*one health*" is the pollination of plants and their biodiversity with the help of bees, having an essential contribution in agriculture (Dietrich et al., 2016), helping to the increase of agricultural yields by up to 35%. Recent studies performed by Food and Agriculture Organization of United Nations (F.A.O.) shows that increasing the density and variety of pollinating insects has a direct impact on crop productivity by helping the development of small farmers to increase their globally production by up to 24%. With a view of development of a bio-economic and eco-economic management at the European Union level (Forstner and Rusu, 2015; Rusu et al., 2020), is important also the number of beekeepers of about 620,000, who practice beekeeping both as a hobby and as a professional activity, and therefore signify-cantly contributing to the society development and economically with about 14.2 billion euros/year to the budget. Throughought a bio-economic and eco-economic approach, the increase in beekeeping economic efficiency is shown by the optimal sizing and exploitation of hives. Also, the honey potential and environmental conditions in Romania can ensure a livestock of more than 2,100,000 bee families by 2025, and with an annual honey production of more than 40,000 tons. Currently, statistical data show a livestock of only 1,650,000 bee families with a production of 25,000 tons of honey. So, from previous information we can easily conclude how great are our country's possibilities in terms of beekeeping development (Popescu, 2017).

The main conditions concurring to achieve a high-performance production in beekeeping:

- holding of optimally sized hives (minimum 50 bee families) equipped with all the inventtory required to achieve a diversified production;
- strong bee families in active state with a maximum number of pickers at the date of honey plants flowering;
- at least 500 m² honey base abundance for producing of nectar and pollen, within an area of maximum 2 km distance from the permanent hearth of the hive;
- hardworking and good organizer beekeeper, and connoisseur of bee breeding technologies and beekeeping production;
- favourable weather conditions for the collection of nectar and pollen by bees.

MATERIALS AND METHODS

The paper is an assessment of the bio-economic and eco-economic status in the apiculture sector in Romania, carried out to point the advances achieved and some relevant aspects to consider
for beekeeping development and management, according to the actual European regulations. M.A.D.R. data were used to perform the present analysis. Also, a forecast for bee sector for our country was made.

RESULTS AND DISCUSSIONS

Strategies and challenges for bio-economic and eco-economic bee management in Romania

Strategy for bio-economic and eco-economic management development of the of Romania's beekeeping in the last 15 years:

Currently, due to the liberalisation of all activities, recognition and consideration for private property in the beekeeping sector too, **essential mutations in the practice of beekeeping and sizing of hives have been produced**, tending to the transition from subsistence to professionalization. Amateur beekeepers with small hives have largely given up this activity, and semi-professional and professional beekeepers have the opportunity to modernize and increase their farms to 50-300 bee families and even more, at some farms level with more than 2,000 hives.

Table 1. Dynamics of Romanian beekeeping in the last 15 years

Year	**U.M.**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2015**	**2018**
Bee families	Thousends	990	1,065	1,150	1,015	1,190	1,250	1,394	1,849
Honey production	tons	12,124	14,579	15,279	14,410	15,678	16,125	27,893	30,875
Average production /hive	kg	12.25	13.69	13.29	16.17	14.02	14.50	20.0	19.25

In terms of beekeeping holdings forms of ownership and structure, the following changes have been made in the last 15 years:

- during 1989 the private sector owned 84.3% of total livestock, and in 2018 was reached 99%;
- regarding the average size of the beekeeping holdings and the production of honey per bee family, in the private sector (majority) the comparative situation is presented according to the data in Table 2.

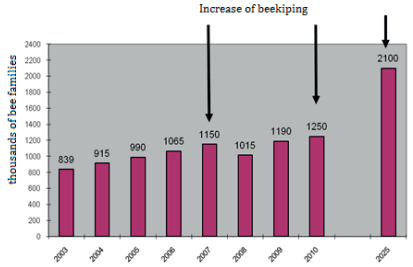

Figure 1. Dynamics of the number of bee families during 2003-2010 and the forecast for 2025

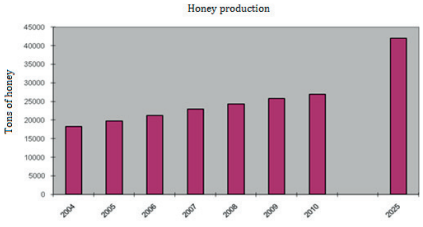

Figure 2. Honey production in the period 2004-2010 and forecast for 2025

Table 2. Comparison between beekeeping farms and honey production on the bee family, in the private sector

Analysed elements	Reference years		Percentage evolution
	1989	2018	
Number of holdings (beekeepers)	98,774	23,161	42.65
Number of bee families	1,196,400	1,848,790	64.7
Number of families per farm	12	79.8	665.0
Honey production kg/bee family	8.5	19.25	226.5

**estimative data, without census*

From the presented data it can be seen a drastic decrease in the number of subsistence farms 3-10 bee families, where honey production is very low, achieved with a high cost price which exceeds selling price, and being intended for self-consumption. There is a substantial increase (90.8%) of the herd on the holding from 10 tons more than 25 bee families, which has led to an increase in the percent of family farm holdings, where the production is achieved at acceptable cost price, intended for direct marketing to consumers and for self-consumption. These types of farms are of interest to their owners, as they contribute to the completion of the family income from the basic activities.

The next step towards the activity of professional beekeepers is the commercial holdings consisting of a herd of at least 50 bee families, equipped with high-performance machinery and installations, in which advanced technologies of growth and exploitation can be applied. **This will increase production and profit per unit of production (bee family). The current data shows honey production per bee family in the reference years (1989 and 2020), main indicator which an increase exceeding 226.8%.**

Currently only 25-30% are included in this commercial holdings category**, and in the next 5-10 years we expect the share of bee families in this category to increase up to (60-70%)**

The eco-economic importance of beekeeping

The role of bees is special and can be easily highlighted, not only by the value of the direct products that man harvests from bee families, but also by the value of the increase of agricultural products that are obtained from cultivated and spontaneous plants, through pollination. Production increases and qualitative increases by pollination by bees shall be assessed to exceed at least 20 times the value of direct bee products obtained from bee families. Every year, honey and other bee products are obtained from each bee family in value equivalent of over 50 kg honey (Champetier et al. 2012).

Bee products have a significant economic value, but also food importance, vitalizing and medicinal energy for humans.

The ecological importance of beekeeping

It shows a crucial role in the sustainable development of rural areas, jobs and provides an important ecosystem service through pollination by improving biodiversity throughout preserving the genetic biodiversity

of plants (Bran et al., 2014). Beekeeping and biodiversity are interdependent. Through pollination, bee colonies provide us with important ecological, economic and social public goods, thus ensuring security, feeding and maintaining biodiversity (Kratschmer et al., 2019).

Figure 3. Bee products

Honey bee. It is obtained from the nectar of flowers or from sweet secretions on plants, being known as an extraordinary source of energy, but also as an excellent product for health due to its content in minerals, enzymes, amino acids, vitamins, and also**:**

•it is found throughout the history of humanity, contributing to the well-being of man for whom it represented and represents a healthy food, but also known for its numerous therapeutic applications.

• depending on the plant sources visited by bees, honey can be mono-flower, poly-flower or manna honey.

The great honey lovers compare, with all conviction, the diversity of honey assortments with that of cheeses and wines, each assortment of honey being unique, its aroma being closely related to the species of flowers "visited" by bees and always varies according to region, altitude and location of the hive. **Sugars, water and plant substances** - that's all honey is composed of, it seems a very simple formula and yet, man is not able to manufacture it - the only thing he can do is take it from bees. Even in the last century, the beekeeper interfered very little in this process, but now, like any modern breeder, seeks to increase the yield and/or quality. But today, as yesterday, honey remains the exclusive product of bee labour**.**

Pollen. It represents the male reproductive element of flowers, which bees collect and mix with their salivary secretions and nectar, they store it in the hive as protein food - in the form of pasture, without which the life of the colony would be impossible. Contains numerous essential elements for life: amino acids, proteins, enzymes, mineral elements and trace elements, B-group vitamins, significant amounts of beta-carotene, vitamins C, D and E, antibiotic-acting substances, hormone-type substances and growth factors. It is consumed as a nutritional supplement in order to strengthen the body and increase its natural resistance to infections. In medicine it has wide applications especially in liver diseases, prostate diseases, psychiatric disorders (Brosi et al., 2017). It is an excellent natural factor in preventing degenerative phenomena **associated with the aging process**.

Propolis. It is a mixture of natural resinous substances, plant and bee waxes, collected from the buds, bark and branches of trees or shrubs, for the purpose of thermal isolation of the nest, polishing the beech cells before the laying of the mats and cleaning the nest. The natural composition of propolis: flavonoids, phenolic compounds, aromatic aldehydes, cumarines, vitamins and mineral elements give the product excellent properties designed to ensure the maintenance of the proper functioning of the body. It has a number of properties: antioxidant, anti-inflammatory, antimicrobial, scarring, local anesthetic, internal remedy (diabetes, cancer, rheumatism, cardiovascular, bronchopulmonary and liver diseases), external remedy (dermatological diseases, burns, wounds, etc.). Its lack of harmfulness has attracted the interest of nutrition and therapeutic specialists, being considered one of the most interesting and promising natural resources of the future.

Royal jelly. It represents glandular secretion of young bees intended to feed the dandruff and for the first phase of brood growth. It is a viscous-looking product of creamy white colour, contains essential elements like: amino acids, proteins, lipids (superior unsaturated fatty acids, phospholipids) and can be considered a true cocktail of vitamins (especially B complex), minerals, substances with antibiotic action, hormonal precursors and

growth factors. It is recommended against fatigue, improvement of physical strength, restoration of physical and mental balance, prevention and treatment of numerous diseases.

Bee venom. It is secreted by specialized glands of worker bees and dandruff and it is used to defend the hive against intruders, and the silks use it to kill and eliminate rivals. Contains a wide variety of substances: biogenic amines, peptides, enzymes, which despite the unpleasant connotation associated with the name "venom" are very important for human body. The effects are known from ancient times, which is why the product has been used in traditional medicine. Recent methods of treatment consist of the application of injections, the application of bee venom therapy in acupuncture centres. To avoid pain associated with conventional bee sting treatment or injections, this is the active component of ointments with local apiphytotherapeutic application. The main conditions in which it proves its effectiveness are rheumatism, neuralgia, multiple sclerosis, but also as a stimulant of the immune system (Marghitas et al. 2010).

Bee wax. It represents a homogeneous and complex blend of organic chemicals that offer well-defined characteristics and which overall determine the extraordinary properties, making it an invaluable, uninhabitable product in a number of areas. Since ancient times it has been used for a wide variety of products: poultices, cheekbones, beauty creams, in the pharmaceutical industry is used in ointments, filming tablets or as glue.

Our country has a long tradition in the field of bee breeding and beekeeping products, beekeeping being a stand-alone occupation according to the historical testimonies existing in this regard (Madas et al., 2020).

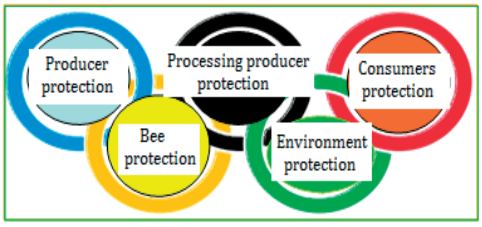

Figure 4. Integrated protection in beekeeping

Environmental protection

Identification and prevention of environmental pollution due to highly developed sense organs bees distinguish as many types of odour as humans perceive. The products resulting from the activity of bees contain the emissions present in the environment within an area of about 3 km around the hive.

The sensitivity of bees is particularly high compared to the main chemical pollutants, industrial and biological noxious substances, radioactive substances, various dusts loaded with heavy metals, toxic gases, noise pollution. Worldwide, specialized FAO organisms have placed bees in fourth place in a classification of environmental pollution sensors.

In order to maintain ecological balance and a healthy living environment, it is necessary to protect the bees. In countries with advanced economies and concerns in environmental quality testing, bees have started to be used in recent years for detection of polluted areas (Matias et al., 2017).

Protection of producers and processors in beekeeping.

Consists in the need to identify and register beehives uniformly at national level, with annual revisions and updates (M.O. no. 119/2011); promoting the establishment of national supervisory systems in close cooperation with beekeepers' associations and the development of harmonised standards at EU level to allow for comparisons; during treatments for prevention and control of diseases and pests in honey plantations, agricultural producers must notify all beekeeper owners about these actions; supporting scientific research on official control of beekeeping production with a view to the creation of elite farms; strengthening relations with beekeepers and beekeeper organisations, compensation granted to beekeepers for losses of bee populations (Bortolotti et al., 2014).

Consumers protection in beekeeping

- establishing clear legal definitions for all bee products, including honey varieties;
- definition of the important parameters for honey quality: proline and sucrose content,

low humidity level, pollen spectrum, honey flavour and sugar content;
- research development in order to find effective methods for detecting honey falsification;
- implementation of EU origin indication systems on labels (PDOs and PGIs) for bee products, by beekeepers and representative organisations;
- measures aimed to increase the consumption of honey and bee products originating in Romania, including by promoting certain types of honey with characteristic properties of certain varieties or geographical areas.

CONCLUSIONS

Beekeeping is the activity that gives practitioners numerous satisfactions. Beekeeping lovers, i.e. beekeepers, are people of all ages and professions, but who have one common point: the love of bees. Beekeeping has both a material side: honey, pollen, pasture, wax, propolis, as well as a spiritual one. There's nothing like the hum of a swarm of bees or a walk through the hive: hives that pulsate with life, bees that come back with full bags of pollen and honey, queens that perpetuate life, drones in constant search of love. What could be more uplifting? What other skill can create so much self-satisfaction? The answer is simple: hives, beekeepers, bees, nature - they are parts of the same whole and that is called beekeeping.

The present approach for defining the conditions for one health concept implement-tation in apiculture in our country is showing bio-economic and eco-economic aspects for an efficient management, adapted to the actual status of bee sector, pointing out new challenges in this area.

The new perspectives compiled in this paper point out both the present requirements and directions for beekeeping, which we recommend to be applied and considered in the apiculture sector.

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REFERENCES

ADW, *Apis melifera*, https://animaldiversity.org/accounts/Apis_mellifera/

Bortolotti, L, Costa, C. (2014). Chemical Communication in the Honey Bee Society. In: Mucignat-Caretta C, editor. *Neurobiology of Chemical Communication*. Boca Raton (FL): CRC Press/Taylor&Francis,Chapter 5. https://www.ncbi.nlm.nih.gov/books/NBK200983/

Bran, M., Ştefan, M., Vidu, L. (2014). Plant biodiversity in South Romania - social and economic sustainability expression, *International Multidisciplinary Scientific GeoConference Surveying Geology and Mining Ecology Management, SGEM*.

Brosi, B. J., Delaplane, K. S., Boots, M., de Roode, J. C. (2017). Ecological and evolutionary approaches to managing honeybee disease. *Nature ecology & evolution*, 1(9), 1250-1262.

Champetier, A., Sumner, D., Wilen, J. (2012). The Bioeconomics of Honey Bees and Pollination. *Environmental and Resource Economics*, 60, 10.1007/s10640-014-9761-4.

Dietrich, T., Villaran Velasco, M.C., Echeverría, P.J., Pop, B., Rusu, A. (2016). *Chapter 1 - Crop and Plant Biomass as Valuable Material for BBB. Alternatives for Valorization of Green Wastes*, Editor(s): Palmiro Poltronieri, Oscar Fernando D'Urso, Biotransformation of Agricultural Waste and By-Products, Elsevier, Pages 1-19, ISBN 9780128036228,https://doi.org/10.1016/B978-0-12-803622-8.00001-X.

FAO, Honey hunting and beekeeping, http://www.fao.org/3/i0842e/i0842e06.pdf.

Forstner, S., Rusu, A. (2015). Development of Personalised food for the nutrition of elderly consumers. 10.3920/978-90-8686-813-1_2.

Hung, K.J., Kingston, J.M., Albrecht, M., Holway, D.A., Kohn, J.R. (2018). The worldwide importance of honey bees as pollinators in natural habitats. *Proc Biol Sci*., 285(1870):20172140.

Kratschmer, S, Pachinger, B, Schwantzer, M, et al. (2019). Response of wild bee diversity, abundance, and functional traits to vineyard inter-row management intensity and landscape diversity across Europe. *Ecol Evol*., 9(7), 4103-4115.

Mădaş, N. M., Mărghitaş, L. A., Dezmirean, D. S., Bonta, V., Bobiş, O., Fauconnier, M.L., Francis, F., Haubruge, E., Nguyen, K. B. (2020). Labeling Regulations and Quality Control of Honey Origin: A Review, *Food Reviews International*, 36 (3), 215-240.

Marghitas, L. A., Dezmirean, D. S., Pocol, C. B., Ilea, M., Bobis, O., Gergen, I. (2010). The Development of a Biochemical Profile of Acacia Honey by Identifying Biochemical Determinants of its Quality.

Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 38(2), 84-90.

Matias, D. M., Leventon, J., Rau, A. L., Borgemeister, C., von Wehrden, H. (2017). A review of ecosystem service benefits from wild bees across social contexts. *Ambio*, 46(4), 456–467.

Popescu, A. (2017). Bee honey production in Romania, 2007-2015 and 2016-2020 forecast, *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development*,17(1), 339-350.

Rusu, A.V., Criste, F.L., Mierliță, D., Socol, C.T., Trif, M. (2020). Formulation of Lipoprotein Microencapsulated Beadlets by Ionic Complexes in Algae-Based Carbohydrates. *Coatings*, 10*,* 302.

Socol, C. T., Criste, F. L., Rusu, A. V., Mihalca, I. (2015). Perspectives on reproductive biotechnologies for FAnGR breeding and conservation, *Bioflux, Porcine Research*, 5(2), 31-38.

Socol, C., Criste, F., Şonea, C., Roşu, I., Rusu, A. V., Şerban, G. C., Ştefan, N., Cosmin, I. P. (2012). Reproductive and molecular tools for animal genetic resources conservation and breeding, *Archiva Zootechnica*, 15(2), 61-68.

Sonea, C. G., Gheorghita, M., Rosu, N., Sonea, A. C., Moise, L., Moise, V. (2012). Study regarding the biodiversity and the conservation of genetic resources on farm animals. *The Annals of -Valahia University of Targoviste - Agriculture*, 7, 63-67.

Sonea, C. G., Gheorghita, M., Rosu, I., Sonea, A. C., Moise, L., Bartolomeu, D. C. (2012). Bio-Beekeeping - a factor in ecosanogenesis, *The Annals of - Valahia University of Targoviste- Agriculture*, 7, 68-70.

Sonea, C. G., Roşu, I., Nistor, C., Dolis, M., Costea, D., Dascălu, C., Stoican, M., Gheorghița, M., Enache, C., Șonea, C., Mirea, C. (2016). Conservation of the biodiversity of domestic animals in Romania in the European context. *Scientific conference with international participation - "Biodiversity in the context of climate change",* Chisinau, 165-168.

TECHNOLOGIES OF THE AGRO FOOD PRODUCTS PROCESSING

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IMPROVING EFFECTIVENESS OF POLYPHOSPHATES ON FOOD QUALITY AND SAFETY IN READY TO EAT MEAT PRODUCTS BY ENCAPSULATION TECHNOLOGY

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Abstract

Protecting consumer health through improving food safety and quality has been an increased focus for both food processors and researchers. Meat and meat product manufacture is vital step for occurrence of microbial contamination in a ready-to-eat (RTE) meat products. Contamination of RTE meat products with pathogenic microorganisms such as Listeria monocytogenes, Escherichia coli, Salmonella spp. and Staphylococcus aureus, or spoilage microorganisms like Pseudomonas spp. can creates life-threatening foodborne illnesses for consumers or causes consumers to avoid meat purchase. In addition, spoilage microorganism contamination in RTE meat products impairs sensory quality, intensifies perishability of these products, and reduces their shelf life. Therefore, foodborne outbreaks cause recalls and negative publicity which can result in a decrease in meat and meat purchases by consumers. Therefore, meat processors and researchers constantly searching for strategies to control potential bio-hazards in RTE meat products. Various food additives are utilized in the product formulations to control the growth of undesirable microorganisms in muscle foods. Polyphosphates (PP) are commonly used in various meat product processing for their beneficial effects such as improved water binding capacity and cooking yield, accelerated curing process, reduced lipid oxidation and improved textural attributes. PP also have the capability of inhibiting the growth of several Gram-negative, Gram-positive bacteria and yeast. Inhibitory effect of PP is associated with pH decrease (acidic PP such as sodium acid pyrophosphate, SPP), formation of complexes with metal ions required for microbial cell division, disruption of microbial cell wall integrity and acceleration of oxidative stress. Inhibitory effect of PP is directly related to their chain length. Longer-chain length PP have superior antimicrobial capability on Gram-positive bacteria compared to shorter-chain length PP. Phosphatase enzymes naturally found in raw meat material have a ability to hydrolyze PP into shorter-chain length PP or orthophosphates. As a result of this reaction, PP may loss some of their antioxidant and antimicrobial properties. Encapsulation is very promising technology for protecting PP from enzymatic hydrolysis caused phosphatases by enrobing PP into capsules. Previous studies demonstrated that encapsulated (e) PP maintained antioxidant capabilities of PP in muscle foods. Therefore, this review study summarizes studies about utilization of ePP to improve antioxidant and antimicrobial properties of PP in meat and meat products.

Key words*: encapsulation, food quality, food safety, meat, polyphosphates.*

INTRODUCTION

The main goal of the meat industry is reducing economic losses and increasing the shelf life and storage stability of meat products by maintaining consumer health. Meat and meat products are highly susceptible to microbiological and chemical deterioration when preservation methods are not used because they are rich in essential nutrients and have high pH and water activity (Jayasena and Jo, 2013). Many methods such as the use of natural or synthetic additives, thermal processes, cooling, freezing, vacuum or modified atmosphere packaging are used in order to ensure safety and to maintain quality in meat products (Gould, 1996). As a result of the use of antimicrobial and antioxidant effective additives in meat industry, it is aimed to protect the products in terms of microbiological and chemical changes. PP are widely used food additives in meat industry due to their antimicrobial and antioxidant properties as well as they provide many beneficial effects such as

improved water binding capacity and cooking yield, accelerated curing process and improved textural attributes (Gadekar et al., 2014).

PP can also be used to control the growth of microorganisms during meat processing. PP are not additives that have antimicrobial effects specifically, but are chemical components that have a suppressive effect on the growth of microorganisms under certain conditions (Moon et al., 2011). Antimicrobial effects of PP occur by changing the chemical structure of the environment by acidification, by binding metal ions such as calcium, magnesium and iron, which must be present in the environment for microbial growth, or as a synergist effect when used with certain antimicrobial additives such as nisin, ethylenediamine tetraacetic acid (EDTA) and nitrite (Maier et al., 1999; Akhtar et al., 2008). Gram-positive bacteria are more affected by PP than Gram-negative bacteria. The inhibitory effect on gram-positive bacteria depends on the chain length of the PP and the preventive effect increases with increases of chain length, and also plays a role in this effect at pH and temperature (Palmeira-de-Oliveira et al., 2011).

Lipid oxidation is a reaction that causes discoloration, formation of toxic compounds, nutrient losses and reduction of shelf life (Falowo et al., 2014). This reaction is affected by many intrinsic and extrinsic factors such as unsaturation of their fatty acids, low molecular weight metal ions, pH, oxidative enzymes, storage temperature, light, oxygen, water activity (Shahidi and Zhong, 2010). PP exhibit their antioxidant effects by binding metal ions which catalyze oxidation reaction (Kılıç et al., 2014). The antioxidative activities of PP depend on the type and concentration of used PP (Kılıç et al., 2016a). It is stated that PP such as sodium tripolyphosphate (STP), sodium acid pyrophosphate (SPP), sodium hexametaphosphate (HMP) and tetrasodium pyrophosphate (TSPP) have antioxidant effects in meat products and they show synergistic effect when used with vacuum or modified atmosphere packaging (Lee et al., 1998; Kılıç et al., 2014; Kılıç et al., 2016b; Kılıç et al., 2018). Lee (1993) indicated that tripolyphosphates form stronger complexes with metal ions (especially iron and copper) than those of pyrophosphates. A similar statement also reported by Sofos (1986). Researcher noted that the best ion-sequestering agents are long chain PP, and the ion-sequestering ability increases with increases of chain length. The powerful antioxidant effect of long chain PP can be reduced by the phosphatase enzymes before cooking (Kılıç et al., 2014). There are many studies in the literature on the antioxidative effects of phosphates. In addition, in recent studies, it is reported that this effect can be increased by using encapsulation technology (Kılıç et al., 2014; Du and Claus, 2015; Kılıç et al., 2016a; 2016b).

Encapsulation is described as a technology to entrap solids, liquids, or gaseous materials within closed capsules that can release their contents at controlled rates under certain conditions (Fang and Bhandari, 2010; Nedovic et al., 2011). Encapsulation technology in food processing contains the coating of tiny particles of food components such as flavors, sweeteners, colorants, acidulants, vitamins and enzymes (Desai and Jin, 2005). This review study is aimed to inform about usage of ePP to improve antioxidant and antimicrobial properties of PP in meat and meat products.

ANTIOXIDATIVE EFFECTIVENESS OF ENCAPSULATED POLYPHOSPHATES

Besides many beneficial effects of PP in food products, there are also numerous studies that exhibit antioxidant effects (Cheng and Ockerman, 2007; Allen and Cornforth, 2009; Kılıç et al., 2014). It has also been reported in recent studies that their effectiveness can be improved with the use of encapsulation technology (Kılıç et al., 2014; Du and Claus, 2015; Kılıç et al., 2016a; 2016b). Sickler et al. (2013a) evaluated that the effects of the use of encapsulated and unencapsulated PP (STP and SPP) in cooked ground beef patties on pH, color and oxidative changes at different storage times. According to study results, researchers stated that the lowest cooking loss and TBARS values were obtained in uSTP usage, and all PP treatments had also lower TBARS values than control samples. Sickler et al. (2013b) evaluated in another study that the impacts of uSTP (0.3% and 0.5%) and eSTP (0.3% and 0.5%), or a combination of these forms (0.3%

uSTP + 0.2% eSTP) and two different end-point cooking temperature (74ºC and 79ºC) on cooked ground turkey at different raw storage (4 h and 24 h) and post-cooked storage (0, 5 and 10 days) times. Researchers reported that eSTP reduced the TBARS by 77% (0.3% eSTP) and 80% (0.5% eSTP) in comparison to the same amount of uSTP. Kılıç et al. (2014) investigated that the effects of encapsulation technology on protecting PP from hydrolysis by phosphatases. For this purpose, researchers tested ePP (STP, HMP and SPP) at two different coating levels (30% and 50%) on lipid oxidation in ground chicken and ground beef during raw and cooked storage. Consequently, researchers noted that encapsulated or unencapsulated forms of STP and SPP were the most effective PP types in delaying lipid oxidation in both meat species. Additionally, they also stated that the coating level had no impact on the lipid oxidation inhibition level (Kılıç et al., 2014). Xie et al. (2014) investigated the impact of hydrolysis of phosphates by phosphatases on cook yield and oxidation. For this purpose, a comparison was carried out between STP, encapsulated STP and Lem-o-Fos® using a grilled beef patty model. Researchers noted that the eSTP demonstrated an enhanced antioxidant effect. Furthermore, the study results showed that the antioxidant effect provided by eSTP was more significant as the storage time increases. The effects of different end-point cooking temperatures (71ºC, 74ºC and 77ºC) on the efficiency of ePP was also investigated in another study conducted by Kılıç et al. (2015). As a result of the study, researchers stated that the application of higher end-point cooking temperature decreased TBARS values in cooked ground beef, whereas increased LPO values in cooked ground beef and chicken. Researchers suggested that using lower end-point cooking temperatures provided more benefits when using ePP (Kılıç et al., 2015). Du and Claus (2015) stated that STP, SPP and HMP are significantly effective in limiting the lipid oxidation in ground turkey, as well as PP degradation due to phosphatases are reduced by encapsulation technology. Kılıç et al. (2016a) studied to determine optimum level of ePP addition (0%, 0.1%, 0.2%, 0.3%, 0.4% and 0.5%) to enhance lipid oxidation inhibition during storage in precooked meat products. Researchers indicated that the antioxidant effect of eSTP or eSPP can be improved with increasing added ePP level in product formulation. In another study, Kılıç et al. (2016b) also investigated effects of the melting release point (60ºC and 68ºC) of the PP from ePP and heating rate (slow and fast cooking) on lipid oxidation inhibition in cooked ground meat. According to the study results, researchers noted that eSTP and eSPP were the better for inhibiting oxidative changes in cooked ground beef and chicken. Furthermore, they stated that the antioxidative effectiveness of these ePP can be improved with a higher melting release point of the encapsulation material. Claus et al. (2016) evaluated effectiveness of post-mortem pH on inhibition of lipid oxidation in raw and cooked ground turkey breasts by ePP. Researchers noted that pH difference between two sets (high, 6.4 to 6.7; low: 5.9 to 6.2) of turkey breasts had a little effect on lipid oxidation inhibition by ePP. Another study (Kılıç et al., 2018) was carried out on hamburger patty production by using the optimum coating rate, melting release point and ePP usage rates determined in previous studies mentioned above. Researchers stated that the antioxidative effectiveness of STP and SPP can be improved with 0.25% ePP usage combined with cohort uPP in patty formulation. This study showed that meat industry should consider adding 0.25% additional ePP to their product formulations in order to achieve more effective inhibiting the lipid oxidation in pre-cooked ready-to-eat meat products (Kılıç et al., 2018).

ANTIMICROBIAL EFFECTS OF ENCAPSULATED POLYPHOSPHATES

PP are additives that do not exhibit specific antimicrobial effects, but exhibit synergistic effects when used with other preventive techniques (Bunkova et al., 2014). PP exhibit potential antimicrobial effects through the following mechanisms: PP may inhibit microbial growth (1) by forming complexes with metal ions which are necessary for cell division, (2) by lowering pH with acidic PP, (3) by disrupting the cell wall integrity (4) by increasing oxidative stress and (5) by causing changes in cell morphology (Maier et al., 1999;

Cheng and Ockerman, 2007; Akhtar et al., 2008; Bunkova et al., 2014). Furthermore, PP reduce the heat resistance of the most bacteria (Luck and Jager, 1997). Many researchers are reported that the inhibitory effect on Gram-positive bacteria depends on PP chain length, and the long chain PP have a better inhibitory effect than shorter-chain PP (Zaika and Kim, 1993; Bunkova et al., 2014). Shorter-chain PP or orthophosphates are released from PP when PP are degraded by phosphatases in the meat system. Therefore, antimicrobial activity of PP may be decreased. Encapsulation is an alternative technology that can be used to protect PP from phosphatases by enrobing PP into capsules (Kılıç et al., 2014). Tenderis et al. (2020) investigated the effects of sodium lactate (SL), eSTP or eSPP forms, and their combinations on *Salmonella typhimurium*, *Escherichia coli* O157:H7 and *Staphylococcus aureus* growth in cooked ground beef during 30 days storage at 4 or 10°C. Researchers indicated that STP or SPP usage in formulation had some inhibitory effect on *S. typhimurium*, *E. coli* O157:H7 and *S. aureus* growth in cooked ground beef during 30 days storage at 4 or 10°C. In addition, researchers indicated that antimicrobial efficiency of PP is not affected by encapsulation, and the usage of PP and SL combinations have synergistic effect on reducing the growth of *S. typhimurium*, *E. coli* O157:H7 and *S. aureus* in cooked ground beef.

CONCLUSIONS

Many studies have been performed in different treatment designs to test the use and effectiveness of ePP. According to the results obtained from these studies, ePP usage can be an effective strategy to control oxidative changes in ready-to-eat meat products. In addition, ePP has not been contributed on antimicrobial activity. However, when PP are used with other antimicrobial agents, PP or ePP can demontrate synergistic effects to inhibit the growth of microorganisms.

REFERENCES

Akhtar, S., Paredes-Sabja, D., Sarker, M. R. (2008). Inhibitory effects of polyphosphates on *Clostridium perfringens* growth, sporulation and spore outgrowth. *Food microbiology*, 25(6), 802-808.

Allen, K., Cornforth, D. (2009). Effect of Chelating Agents and Spice Derived Antioxidants on Myoglobin Oxidation in a Lipid Free Model System. *Journal of food science*, 74(5), C375-C379.

Buňková, L., Pleva, P., Buňka, F., Valášek, P., Kráčmar, S. (2014). Antibacterial effects of commercially available phosphates on selected microorganisms. *Acta universitatis agriculturae et silviculturae mendelianae brunensis*, 56(5), 19-24.

Cheng, J. H., Ockerman, H. W. (2007). Effects of Phosphate, Ascorbic Acid, A Tocopherol and Salt with Nonvacuum Tumbling on Lipid Oxidation And Warmed Over Flavor Of Roast Beef. *Journal of muscle foods*, 18(3), 313-329.

Claus, J., Du, C., Kılıç, B. (2016). Inhibition of lipid oxidation in ground turkey breasts by encapsulated Polyphosphates as influenced by postmortem pH. *Meat Science*, (112), 129.

Desai, K. G. H., Jin Park, H. (2005). Recent developments in microencapsulation of food ingredients. *Drying technology*, 23(7), 1361-1394.

Du, C., Claus, J. R. (2015). Inhibition of lipid oxidation in ground turkey with encapsulated phosphates as affected by meat age, phosphate type, and temperature release point. *Meat Science*, (101), 110.

Falowo, A. B., Fayemi, P. O., Muchenje, V. (2014). Natural antioxidants against lipid–protein oxidative deterioration in meat and meat products: A review. *Food Research International*, 64, 171-181.

Fang, Z., Bhandari, B. (2010). Encapsulation of polyphenols - a review. *Trends in Food Science & Technology*, 21(10), 510-523.

Gadekar, Y. P., Sharma, B. D., Shinde, A. K., Verma, A. K., Mendiratta, S. K. (2014). Effect of natural antioxidants on the quality of cured, restructured goat meat product during refrigerated storage (4±1 C). *Small ruminant research*, 119(1-3), 72-80.

Gould, G. W. (1996). Methods for preservation and extension of shelf life. *International journal of food microbiology*, 33(1), 51-64.

Jayasena, D. D., Jo, C. (2013). Essential oils as potential antimicrobial agents in meat and meat products: A review. *Trends in Food Science & Technology*, 34(2), 96-108.

Kılıç, B., Şimşek, A., Claus, J. R., Atılgan, E. (2014). Encapsulated phosphates reduce lipid oxidation in both ground chicken and ground beef during raw and cooked meat storage with some influence on color, pH, and cooking loss. *Meat Science*, 97(1), 93-103.

Kılıç, B., Şimşek, A., Claus, J. R., Atılgan, E., Aktaş, N. (2015). Effects of different end point cooking temperatures on the efficiency of encapsulated phosphates on lipid oxidation inhibition in ground meat. *Journal of food science*, 80(10), C2161-C2169.

Kılıç, B., Şimşek, A., Claus, J. R., Atılgan, E., Bilecen, D. (2016a). Impact of added encapsulated phosphate level on lipid oxidation inhibition during the storage of cooked ground meat. *Journal of food science*, 81(2), C359-C368.

Kılıç, B., Şimşek, A., Claus, J. R., Atılgan, E. (2016b). Melting release point of encapsulated phosphates and heating rate effects on control of lipid oxidation in

cooked ground meat. *LWT-Food Science and Technology*, 66, 398-405.

Kılıç, B., Şimşek, A., Claus, J. R., Karaca, E., Bilecen, D. (2018). Improving lipid oxidation inhibition in cooked beef hamburger patties during refrigerated storage with encapsulated polyphosphate incorporation. *LWT-Food Science and Technology*, 92, 290-296.

Lee, R. M. L. (1993). Antibacterial mechanism of phosphates in *Staphylococcus aureus*. Doctor of Philosophy, *Department of Food Science and Human Nutrition*, pp. 154.

Lee, B. J., Hendricks, D. G., Cornforth, D. P. (1998). Effect of sodium phytate, sodium pyrophosphate and sodium tripolyphosphate on physico-chemical characteristics of restructured beef. *Meat Science*, 50(3), 273-283.

Maier, S. K., Scherer, S., Loessner, M. J. (1999). Long-chain polyphosphate causes cell lysis and inhibits Bacillus cereus septum formation, which is dependent on divalent cations. *Appl. Environ. Microbiol.,* 65(9), 3942-3949.

Moon, J. H., Park, J. H., Lee, J. Y. (2011). Antibacterial action of polyphosphate on *Porphyromonas gingivalis*. *Antimicrobial agents and chemotherapy*, 55(2), 806-812.

Nedovic, V., Kalusevic, A., Manojlovic, V., Levic, S., Bugarski, B. (2011). An overview of encapsulation technologies for food applications. *Procedia Food Science*, 1, 1806-1815.

Palmeira-de-Oliveira, R., Palmeira-de-Oliveira, A., Gaspar, C., Silvestre, S., Martinez-de-Oliveira, J., Amaral, M. H., & Breitenfeld, L. (2011). Sodium Tripolyphosphate: An excipient with intrinsic *in vitro* anti-*Candida* activity. *International journal of pharmaceutics*, 421(1), 130-134.

Shahidi, F., Zhong, Y. (2010). Lipid oxidation and improving the oxidative stability. *Chemical society reviews*, 39(11), 4067-4079.

Sickler, M. L., Claus, J. R., Marriott, N. G., Eigel, W. N., Wang, H. (2013a). Antioxidative effects of encapsulated sodium tripolyphosphate and encapsulated sodium acid pyrophosphate in ground beef patties cooked immediately after antioxidant incorporation and stored. *Meat science*, 94(3), 285-288.

Sickler, M. L., Claus, J. R., Marriott, N. G., Eigel, W. N., Wang, H. (2013b). Reduction in lipid oxidation by incorporation of encapsulated sodium tripolyphosphate in ground turkey. *Meat science*, 95(2), 376-380.

Tenderis, B., Kılıç, B., Yalçın, H., Şimşek, A. (2020). Impact of sodium lactate, encapsulated or unencapsulated polyphosphates and their combinations on Salmonella Typhimurium, Escherichia coli O157: H7 and Staphylococcus aureus growth in cooked ground beef. *International Journal of Food Microbiology*, 108560.

Xie, J., Yuan, P., Jenkins, R. (2015). Impact of encapsulation of sodium tripolyphosphate on cook yield and oxidative quality of beef patties. *Meat Science*, (101), 116.

Zaika, L. L., Kim, A. H. (1993). Effect of sodium polyphosphates on growth of *Listeria monocytogenes*. *Journal of food protection*, 56(7), 577-580.

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FATTY ACID PROFILE AND QUALITATIVE EVALUATION OF THE FAT FRACTION IN GOAT WHITE BRINED CHEESE ON THE 45TH DAY OF THE RIPENING PROCESS

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Abstract

It has been investigated the fatty acid composition of white brine cheese on the 45th day of the ripening process, produced from goat's milk from three groups of animals: Bulgarian White Dairy (BWD) breed and its crosses with Anglo-Nubian (BWD x AN) and Togenburg (BWD x TG) breeds during the lactation. An assessment has been made of the fatty acid composition in milk fat on the product as a healthy source for human nutrition. MUFAs predominate in the cheese from the crosses of BWD x AN breed- 25.87 g/100 g fat and PUFAs in the cheese from purebred goats- 3.39 g/100 g fat. The biologically important ratio of omega-6/omega-3 in the analyzed batches (2.91-3.09) is kept within the limits of optimal values (up to 5) according to modern notions of rational nutrition. The lipid preventive score is highest in BWD cheese- 61.47 g/100 g cheese, and the AI and TI in BWD x TG cheese - 2.62, 2.75. The analyzed cheeses from three goat groups are defined as products with low content of trans fatty acids and high content of SFA.

Key words: *fatty acids, goat white brined cheese, lipid indices*

INTRODUCTION

Cheese has a long history in the human diet as a source of essential nutrients - proteins, bioactive peptides, amino acids, fatty acids, vitamins and minerals. Goat milk cheese was an important nutritional product and an integral part of a healthy diet, and low levels of lactose make it suitable for use by people with digestive disorders. This demonstrates the importance of cheese as a functional food that, in addition to the presence of physiologically active components, provides nutritional and health benefits (Hasler, 2000).

One of the biggest differences between goat and cow's milk was in terms of the physicochemical structure and composition of milk fat (Park, 2005). Depending on the breed, the milk fat in goat's milk can range from 2.45 to 7.76% (Park, 2005). The fatty acid composition of milk fat in goat's milk is characterized by a significantly higher content of short and medium chain fatty acids (C4:0-C14:0) than cow's milk (Tziboula-Clarke, 2003; Amigo and Fontecha, 2011; Barłowska et al., 2011; Park, 2017).

Short-chain fatty acids in goat's milk account for 15-18% of total fatty acids, compared to 5 - 9% for cows. Goat's milk contains almost twice as much caproic (C6:0), caprylic (C8:0) and capric (C10:0) fatty acids than cows, which has been attributed to differences in the polymerization of acetate from abdominal microflora in goats (Amigo and Fontecha, 2011). The fatty acid profile, especially the content of fatty acids with less than 11 carbon atoms, also plays an important role in the formation and development of the organoleptic characteristics of dairy products, determining their specific taste. The total fatty acid content was high during the summer months (July, August and September). During the early phases of lactation (May and June) and the last phase of lactation (October), the total concentration of fatty acids in cheese is about half that of August. This may be explained by the heat during the summer months, which shows an increase in fat consumption with ration and high levels of lipolysis (Palmquist et al., 1993), leading to an increase in the proportion of long chain fatty acids in milk.

Cheeses obtained from the milk of pasture rearing animals are distinguished by a higher content of unsaturated fatty acids, antioxidants and aromatic constituents and lower cholesterol than those obtained from milk from indoor rearing animals (Rubino and Chilliard, 2003; Chilliard et al., 2005; Luna et al., 2005; Nudda et al., 2005; Cabiddu et al., 2006).

The content of long chain and polyunsaturated fatty acids, as well as that of conjugated linoleic acid and omega-3 fatty acids, was significantly higher and the ratio of omega-6: omega-3 fatty acids was lower in the cheese obtained from goat's milk, received less concentrated feed (Volkmann et al., 2014).

According to EU regulatory measure No 1924/2006, the content of saturated fatty acids and trans fatty acids in solid products should not exceed 1.5 g/100 g fat, where these foods are referred to as foods with low content of saturated fatty acids.

It has been investigated the fatty acid composition of white brine cheese on the 45th day of the ripening process, produced from goat's milk from three groups of animals – Bulgarian White Dairy (BWD) breed and its crosses with Anglo-Nubian (BWD x AN) and Togenburg (BWD x TG) breeds during the lactation. An assessment has been made of the fatty acid composition in milk fat on the product as a healthy source for human nutrition.

MATERIALS AND METHODS

Samples of white brine cheese (3 x 3 pieces) during the lactation period from milk of Bulgarian White Dairy (BWD) breed and its crosses with Anglo-Nubian (BWD x AN) and Togenburg (BWD x TG) for fatty acid composition and evaluation of fatty acid composition and evaluation source have been established. The milk was taken in April, June and September and subjected to technological processing for cheese production. The white brine goat's milk cheeses were examined at day 45 of the ripening process, and the results are presented arithmetically.

Milks from experimental animals reared in one flock were used under the same production conditions at the RIMSA-Troyan base, aged 3 to 5 years (second-fourth lactation), with the indications being in February and the rearing system was pasture-grazing.

Extraction of total lipids was carried out by Roese-Gottlieb method, using diethyl and petroleum ether and subsequent methylation with sodium methylate (CH_3ONa, Merck, Darmstadt) and drying with $NaHSO_4.H_2O$. The fatty acid methyl esters/FAME/ were analyzed using a Shimadzu-2010 gas chromatograph (Kioto, Japan) equipped with a flame ionization detector and an automatic injection system (AOC-2010i). The assay was performed on a capillary column CP 7420 (100 m x 0.25 mm i.d., 0.2 μm film, Varian Inc., Palo Alto, CA). Hydrogen was used as the carrier gas and nitrogen was used as the make-up gas. The four-step furnace mode was programmed – the initial column temperature is 80ºC/min, which was maintained for 15 minutes, then increases by 12ºC/min. to 170ºC and maintained for 20 minutes, followed by a new increase of 4ºC/min. to 186ºC for 19 min. and up to 220ºC with 4ºC/min. until the process is complete.

The qualitative assessment of the fat fraction includes the following indicators: lipid preventive score (LPS), atherogenic (AI) and thrombogenic index (TI) (Ulbricht and Southgate, 1991), the ratio of hyper- and hypocholesterolemic (h/H) fatty acids, trans fatty acids (TFA) and the amount of saturated fatty acids (Regulation (EC) No1924/2006).

LPS=FAT+2xSFA-MUFA-0.5PUFA

AI=12:0+4×14:0+16:0/[ΣMUFAs+PUFAn6+ PUFAn3]

TI=(14:0+16:0+18:0)/[0.5×ΣMUFAs+0.5× PUFAn6+3×PUFAn−3+PUFAn3/PUFAn6]

h/H=(C18:1n-9+C18:1n-7+C18:2n6+C18:3n-3+C18:3n-6+C20:3n-6+C20:4n-6+C20:5n-3+C22:4n-6+C22:5n-3+C22:6n3)/(C14:0+C16:0)

The data were processed using the variation statistics methods using the statistical package of the EXCEL 2013 computer program.

RESULTS AND DISCUSSIONS

The investigated white brined cheeses on the 45th day of the ripening process were characterized by a fat content in BWD from 22.61% to 27.71% during the lactation period, from 23.15% to 29.65% for BWD x TG and from 23.44% to 27.38% for BWD x AN.

It was established that of the saturated fatty acids (Table 1), in all three batches of cheese, palmitic (C-16:0) followed by stearic (C-18:0), capric (C-10:0) and myristic (C-14:0). High results for the butyric acid (C-4:0) were observed with BWD x TG - 3.95 g/100 g fat, and the values for capron (C-6:0), caprylic (C-8:0) and capric acid (C-10:0) predominate in BWD x AN cheese - 3.39; 3.29; 11.03 g/100 g fat.

Table 1. Saturated fatty acids, g/100 g fat (n=3)

	Breed group		
	BWD	**BWD x TG**	**BWD x AN**
Fatty acid	x±Sx	x±Sx	x±Sx
C-4:0	3.88±0.577	3.95±0.719	3.88±0.541
C-6:0	3.29±0.305	3.29±0.395	3.39±0.272
C-7:0	0.01±0.006	0.01±0.006	0.01±0.006
C-8:0	3.10±0.165	3.09±0.261	3.29±0.147
C-9:0	0.03±0.017	0.03±0.015	0.02±0.017
C-10:0	10.30±0.771	10.59±1.018	11.03±0.796
C-11:0	0.04±0.020	0.04±0.021	0.03±0.015
C-12:0	3.41±0.663	3.59±0.947	3.74±0.671
C-13:0	0.05±0.021	0.05±0.023	0.04±0.015
C-14:0	9.85±1.185	9.62±0.983	9.69±1.357
C-15:0	0.57±0.151	0.63±0.181	0.57±0.167
C-16:0	28.16±3.781	28.78±3.641	27.74±3.764
C-17:0	0.54±0.078	0.56±0.081	0.56±0.126
C-18.0	11.35±3.203	11.82±3.357	12.72±4.387
C-20:0	0.24±0.026	0.25±0.040	0.25±0.036
C-21:0	0.06±0.012	0.05±0.015	0.06±0.023
C-22:0	0.06±0.015	0.06±0.015	0.07±0.023
C-23:0	0.02±0.010	0.02±0.015	0.02±0.010
C-24:0	0.02±0.006a*	0.01±0.006	0.03±0.026
C-25:0	0.02±0.012	0.01±0.006	0.01±0
C-26:0	0.02±0.015	0.04±0.025	0.02±0.012

Note: a- BWD/BWD x TG; *P≤0.05

Zucali et al. (2007) received lower results than ours for (C4:0), (C6:0) and (C8:0), respectively - 1.86%, 2.01%, 3.20% for goat cheese from Alpine goats.

The content of lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids, which are associated with an increase in cholesterol levels in the human body, respectively: for (C12:0) from 3.41 g/100 g fat at BWD cheese to 3.74 g/100 g fat at BWD x AN, for (C14:0) from 9.62 g/100 g fat at BWD x TG to 9.85 g/100 g fat at BWD and (C16:0) from 27.74 g/100 g fat at BWD x AN to 28.78 g/100 g fat at BWD x TG, which was close to the results obtained by Popović-Vranješ et al. (2016) in hard goat cheese in Serbia.

White brined cheeses were good source of monounsaturated and polyunsaturated fatty acids. It was found that of the monounsaturated fatty acids in the studied cheeses (Table 2), oleic prevailed (C181c9) in the amounts from 20.34 g/100 g fat at BWD x TG to 21.99 g/ 100 g fat at BWD x AN and vaccenic acid (C18:1t11) from 0.92 g/100 g fat at BWD x TG to 0.96 g/100 g fat at BWD x AN. The content of both acids was highest for BWD x AN (0.96, 21.99 g/100 g fat) and lowest for BWD x TG (0.92, 20.34 g/100 g fat).

Table 2. Monounsaturated fatty acids, g/100 g fat (n = 3)

	Breed group		
	BWD	BWD x TG	BWD x AN
Fatty acid	x±Sx	x±Sx	x±Sx
C-10:1	0.17±0.046	0.15±0.032	0.17±0.046
C-12:1n1	0.02±0.006	0.04±0.035	0.04±0.049
C-14:1n5	0.05±0.012	0.04±0.026	0.03±0.017
C-16:19tr	0.10±0.179	0	0
C-16:1n7	0.33±0.053	0.38±0.056	0.35±0.089
C-17:1n7	0.19±0.042	0.18±0.029	0.19±0.036
C-16:3n4	0.01±0.006	0.01±0.006	0.01±0.006
C-18:1t4	0.01±0	0.01±0	0.01±0
C-18:1t5/6/7	0.16±0.030	0.16±0.040	0.17±0.055
C-18:1t9	0.19±0.036	0.18±0.032	0.18±0.042
C-18:1t10	0.17±0.017	0.18±0.015	0.17±0.046
C-18:1t11	0.93±0.523	0.92±0.587	0.96±0.581
C-18:1c9/C-18:1t12/13/	21.47±2.850	20.34±2.128	21.99±3.139
C-18:1t15/ C-18:1c11	0.40±0.061	0.43±0.092	0.42±0.096
C-18:1c12	0.10±0.025	0.10±0.015	0.10±0.029
C-18:1c13	0.23±0.087	0.24±0.093	0.26±0.139
C-18:1t16	0.02±0.010	0.02±0.006	0.03±0.006
C-18:1c14	0.05±0.020	0.06±0.023	0.06±0.032
C-18:1c15	0.08±0.012	0.09±0.021	0.09±0.021
C-22:1n9	0.03±0.010	0.04±0.015	0.03±0.006

Depending on the type of cheese, polyunsaturated fatty acids ranged from 1.66 to 11.03% (Barac et al., 2016). In our batches of white brined cheese, they were relatively close and varied within a narrow range - from 3.22 (BWD x TG) to 3.39 g/100 g fat (BWD x AN) (Table 3).

Table 3. Polyunsaturated fatty acids, g/100 g fat (n=3)

	Breed group		
	BWD	BWD x TG	BWD x AN
Fatty acid	x±Sx	x±Sx	x±Sx
C-18:2t9,12	0.18±0.012	0.17±0.026	0.17±0.035
C-18:2c9,12/19:0	1.83±0.060	1.70±0.155	1.76±0.245
gC-18:3n6	0.06±0.015	0.06±0.015	0.06±0.006
aC-18:3n3	0.52±0.271	0.50±0.279	0.53±0.291
CLA9c,11t	0.43±0.093	0.39±0.112	0.42±0.110
CLA9c,11c	0.03±0.010	0.03±0.006	0.03±0
CLA9t,11t	0.01±0.006	0.01±0.015	0
C-20:2n6	0.03±0.017	0.04±0.023	0.04±0.021
C-20:4n6	0.03±0.006	0.03±0	0.03±0
C-20:3n3	0.14±0.040	0.17±0.029	0.15±0.044
C-22:2n6	0	0	0.09±0.020
C-22:5n3	0.10±0.026	0.10±0.040	0.10±0.032
C-22:6n3	0.02±0.006	0.02±0.006	0.01±0.006

It should be noted that the values of linoleic (C18:2) and linolenic (C18:3) acids in milk fat depend on animal nutrition, since they were not synthesized in the body and their lack causes a number of biological disorders (Gerchev et al., 2018). The content of g C-18:3n6 in the tested

cheeses was 0.06 g/100 g fat, and the g C-18:3n3 varied slightly - 0.50, 0.53 g/100 g fat.

CLA-containing products have been found to contribute to the reduction of body fat by inhibiting lipogenesis and stimulating lipolysis (Raff et al., 2009).

The established isomers of CLA were at very low concentrations, with higher levels distinguishing the biologically active (CLA9c,11t) - 0.43% for cheese from BWD, 0.39% for BWD x TG and 0.42% for BWD x AN, which coincides with that obtained by Mihailova (2007) - 0.49% CLA content in goat white brined cheese from the Central Balkan mountain.

Arachidonic acid (C20:4n6), which was the other representative besides the linoleic of the omega-6 group, had very low amounts both in the raw milk and in the white brine cheese produced (0.03 g/100 g fat).

Minimum amounts were established in the cheese at day 45 and eicosatrienoic (C20:3n3), eicosapentaenoic (C22:5n3) and docosahexaenoic (C22:6n3) acids.

Whatever of the low percentage of iso and anteiso fatty acids in milk fat, they were of great interest because of their potential role as non-invasive biomarkers of abdominal function, since their variations in milk may reflect changes in bacterial populations caused by nutritional composition rations (Fievez et al., 2012).

The main representative in the studied cheeses was C-17iso and C-17aiso followed by C-15aiso (Table 4).

Table 4. Branched fatty acids, g/100 g fat (n = 3)

	Breed group		
	BWD	**BWD x TG**	**BWD x AN**
Fatty acid	x±Sx	x±Sx	x±Sx
C-13iso	0.03±0.026	0.03±0.021	0.03±0.015
C-15iso	0.20±0.051	0.20±0.040	0.22±0.066
C-15aiso	0.23±0.058	0.28±0.089	0.25±0.060
C:16iso	0.19±0.049	0.21±0.067	0.20±0.050
C-17iso	0.29±0.025	0.29±0.032	0.31±0.032
C-17aiso	0.29±0.044	0.33±0.072	0.31±0.059
C-18iso	0.04±0.012	0.04±0.012	0.04±0.010

Table 5. Fatty acid groups, g/100 g fat (n = 3)

	Breed group		
	BWD	**BWD x TG**	**BWD x AN**
Fatty acid	x±Sx	x±Sx	x±Sx
ΣCLA	0.47±0.081	0.42±0.114	0.46±0.122
Σ C-18:1 trans	1.87±0.594	1.89±0.738	1.92±0.771
Σ C-18:1 cis	21.93±2.872	20.83±2.132	22.50±3.254
Σ SFA	75.02±2.587	76.35±1.883	74.18±4.886
Σ MUFA	25.21±3.034	24.14±2.330	25.87±3.851
Σ PUFA	3.39±0.360	3.23±0.530	3.31±0.592
Σ omega-3	0.79±0.261	0.79±0.283	0.79±0.290
Σ omega-6	2.23±0.036	2.10±0.157	2.17±0.314
Σ omega-6/Σ omega-3	3.09±1.188	2.91±1.102	3.02±1.270
Branched fatty acids	1.29±0.224	1.40±0.298	1.36±0.211
CLA	0.43±0.093	0.39±0.112	0.42±0.110

Total amount of CLA ranged from 0.42 to 0.47 g/100 g in cheese from the individual breed groups (Table 5).

Trans forms range from 1.87 g/100 g fat at BWD to 1.92 g/100 g fat at BWD x AN, and cis forms from 20.83 g/100 g fat at BWD x TG to 22.50 g/100 g fat at BWD x AN, which is lower than that obtained from Mihailova (2007) content of ΣC-18:1 trans forms - 2.59% and higher than that found by the same author content of ΣC - 18:1 cis forms - 17.92% in goat

white brined cheese from the Central Balkan mountain.
SFA is highest for BWD x TG - 76.35 g/100 g fat, MUFA predominates in BWD x AN cheese - 25.87 g/100 g fat, and PUFA in pure breed goat cheese - 3.39 g/100 g fat.
Popovic-Vranjes et al. (2016) found a lower content than what we found for SFA (42%), higher for MUFA (54.4%) and close to ours for PUFA (3.6%) in ripe cheese produced in Serbia.
The ratio of omega-6/omega-3 ranges from 2.91 to 3.09 in the batches of cheese tested and remains within the range recommended by nutritionists to 5.
Volkmann et al. (2014) found a ratio of omega-6/omega-3 lower than ours from 1.3 to 2.2% in cheese after 6 weeks of maturation from milk in two groups of German Alpine goats breed fed rations containing 10 and 40% (on dry matter base) concentrated feed.
The qualitative evaluation of milk fat was based on the following indices - LPS, AI, TI, h/H, TFA and SFA (Table 6).

Table 6. Goat cheese indices

Indices	BWD	BWD x TG	BWD x AN
LPS (g/100 g cheese)	61.47±4.309	56.91±5.929	55.38±5.273
AI	2.52±0.583	2.62±0.548	2.48±0.757
TI	2.60±0.406	2.75±0.352	2.63±0.537
h/H	0.64±0.137	0.60±0.109	0.67±0.166
TFA (g/100 g cheese)	0.51±0.131	0.47±0.146	0.48±0.169
SFA+TFA (g/100 g cheese)	21.18±1.336	19.61±1.857	19.08±1.713

The lipid preventive score is highest with the BWD cheese - 61.47 g/100 g cheese, and the atherogenic and thrombogenic index at the BWD x TG - 2.62, 2.75.
Trans fatty acids vary from 0.47 to 0.51 g/100 g cheese, that is why cheeses produced, can be categorized as low-TFA products under Regulation 1924/2006.

CONCLUSIONS

Based on the lipid indices obtained and the trans fatty acid content, it can be summarized that the white brined cheese on the 45th day of the ripening process was a product of low risk to human health.
The biologically important ratio of omega-6/omega-3 in the studied batches of cheese (2.91-3.09) was kept within the limits of optimal values (up to 5) according to modern notions of rational nutrition.
The studied cheeses are characterized by low cholesterolemic index - under 1.
The lipid preventive score is highest in Bulgarian white dairy breed cheese - 61.47 g/100 g cheese, and the atherogenic and thrombogenic index in Bulgarian white dairy crosses with Togenburg breed cheese - 2.62, 2.75.
The tested cheeses from the three goat groups were defined as a food product with a low content of trans fatty acids from 0.47 to 0.51 g/100 g cheese and a high content of saturated fatty acids - from 19.08 to 21.18 g/100 g cheese according to Regulation 1924/2006.

REFERENCES

Amigo, L., Fontecha, J. (2011). Goat milk. *Encyclopedia of Dairy Science (Second Edition),* (484-493). Spain, Madrid.

Barac, M., Pesic, M., Zilic, S., Smiljanic, M., Stanojevic, S., Vasic, M., Despotovic, S., Vucic, T., Kostic, A. (2016). Protein profiles and total antioxidant capacity of water-soluble and water-insoluble fractions of white brined goat cheese at different stages of ripening. *International Journal of Food Science and Technology*, 51, 1140–1149.

Barłowska, J., Szwajkowska, M., Litwińczuk, Z., Król. J. (2011). Nutritional value and technological suitability of milk from various animal species used for dairy production. *Comprehensive Reviews in Food Science and Food Safety*, 10, 6, 291–302.

Cabiddu, A., Addis, M., Pinna, G., Spada, S., Fiori, M., Sitzia, M., Pirisi, A., Piredda, G., Molle, G. (2006). The inclusion of a daisy plant (*Chrysanthemum coronarium*) in dairy sheep diet. 1 Effect on milk and cheese fatty acid composition with particular

reference to c18:2 cis-9, trans-11. *Life Science Journal*, 101, 57–77.

Chilliard, Y., Rouel, J., Ferlay, A., Bernard, L., Gaborit, P., Raynal- Ljutovac, K., Lauret, A. (2005). Effects of type of forage and lipid supplementation on goat milk fatty acids and sensorial properties of cheeses. *In: Future of the Sheep and Goat Dairy Sectors, Special issue of the International Dairy Federation* No 0501/part 5. *Zaragoza*, pp. 297–304.

Fievez, V., Colman, E., Castro-Montoya, JM., Stefanov, I., Vlaeminck, B. (2012). Milk odd- and branched-chain fatty acids as biomarkers of rumen function-An update. *Animal Feed Science Technology*, 172, 51–65.

Gerchev, G., Naydenova, N., Dimitrova, Ts., Mihailova, G., Markov, N. (2018). Fatty acid composition of milk of Tsigai and Karakachan sheep and meat of their lambs F1 crossbreeds of Awassi. *Journal of Mountain Agriculture on the Balkans,* 21(4), 15–28.

Hasler, C. (2000). The changing face of functional foods. Journal of the American Collage of Nutrition, 19(5), 499–506.

Rubino, R., Chilliard, Y., (2003). Relationship between feeding system and goat milk and cheese quality. *EAAP. In*: 54th *Annual Meeting, Rome*, p. 341.

Luna, P., Fontecha, J., Ju´arez, M., M.A. de la Fuente. (2005). Changes in the milk and cheese fat composition of ewes fed commercial supplements containing linseed with special reference to the CLA content and isomer composition. Lipids. *Journal of the American Oil Chemists' Societ,* 40, 445–453.

Mihailova, G. (2007). Fatty acid profile of dairy products from different agro-ecosystems in Bulgaria and functionality of milk fat. *Doctoral dissertation, Trakia University Stara Zagora, Bulgaria.*

Nudda, A., Mc Guire, M. A., Battcone, G., Pulina, G. (2005). Seasonal variation in conjugated linoleic acid and vaccenic acid in milk fat of sheep and its transfer to cheese and ricotta. *Journal Dairy Science,* 88, 1311–1319.

Palmquist, D. L., Beaulieu, A. D., Barbano, D. M. (1993). Feed and animal factors influencing milk fat composition. *Journal of Dairy Science*, 76. 1753–1771.

Park, Y. W. (2005). Goat Milk: Composition, Characteristics. *In: Encyclopedia of Animal Science, editors Wilson G. Pond, Alan W. Bell, Marcel Dekker* (pp. 474–477).

Park, Y., Jeanjulien, C., Siddique, A. (2017). Factors Affecting Sensory Quality of Goat Milk Cheeses. *A Review. Advances in Dairy Research*, vol.5:3, DOI: 10, 4172/2329-888X,1000185.

Popović- Vranješ, A., Paskaš, S., Krstović, S., Jurakić, Z., Štrbac, L., Grubješic, G. (2016). Quality of hard cheese made from value added organic goat milk. *The Serbian Journal of Agricultural Sciences*, 65, 3-4, 51–56.

Raff, M., Tholstrup, T., Toubro, S., Bruun, J.M., P. Lund, E. M. Straarup, Christensen, R., Sandberg, M.B., Mandrup, S. (2009). Conjugated linoleic acids reduce body fat in healthy postmenopausal women. Journal Nutrition, 139, 1347–1352.

Rubino, R., Chilliard, Y. (2003). Relationship between feeding system and goat milk and cheese quality. *EAAP. In: 54th Annual Meeting, Rome*, p. 341.

Tziboula-Clarke, A. (2003). Goat milk. In: H. Roginski, (Ed.), *Encyclopedia of Dairy Sciences, vol*. 2 (p. 1270). Academic Press, London, England.

Volkmann A., Rahmann, G., Knaus, W. (2014). Fatty acid composition of goat milk produced under different feeding regimens and the impact on Goat Cheese. RAHMANN G & AKSOY U (Eds.), *Proceedings of the 4th ISOFAR Scientific Conference. „Building Organic Bridges", at the Organic World Congress*, (pp. 13-15) Oct., Istanbul, Turkey (eprint ID 24317) 551.

Zucali, M., Bava, L., Penati, C., Rapetti, L. (2007). Effect of raw sunflower seeds on goat milk production in different farming systems. Journal of Animal Science, 6 (suppl. 1), 633–635.

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THE PECULARITIES OF AMINO ACID MIGRATION IN PROTEIN MINERAL CONCENTRATES UNDER THE INFLUENCE OF DIFFERENT pH AND TEMPERATURE VALUES DURING ELECTRO-ACTIVATION OF WHEY

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Abstract

It was investigated the influence of pH and temperature under the degree of isolation of free amino acids in the protein mineral concentrates (PMCs) obtained as a result of processing of whey after the manufacture of the granulated cottage cheese "Grauncior" (company JLC) at membrane electrolyzer EDP-4 at current density j = 10 mA/cm^2 and j = 20 mA/cm^2. The highest degree of extraction of free, especially essential amino acids is recorded in the PMCs during electro-activation at current density j = 20 mA/cm^2, at 10 min of processing, when pH = 11.6 and t = 29.5ºC. The level of migration of each essential amino acid in the PMCs is varying in dependence on the time of electrophysical processing, on the current density, pH and temperature values.

Key words*: amino acids, electrophysical processing, whey.*

INTRODUCTION

Milk and dairy products, including whey (whey products) are attributed to foods needed for human nutrition, as sources of protein and amino acids respectively.

The dairy industry produces ~180 to 190 million tons per year of whey, accounting for more than a half of the total solids present in the original whole milk, including whey proteins (20% of the total protein) and most of the lactose, minerals, and water-soluble vitamins (de Wit, 2001; Chandrapala et al., 2015).

Whey is known to be a rich source of essential amino acids. However, the quality of the nutrients can be affected in the process of processing the whey and obtaining the final products – protein mineral concentrates (PMCs).

The manufacture of protein concentrates requires certain rules in order to maintain a high degree of purity in the protein native form, namely, to exclude thermal denaturation, which, in the case of whey proteins is 55-65ºC (Etzel, 2004), chemical denaturation of proteins and chemical modification of amino acids (Desrosiers and Savoie, 1991; Cheftel, 1977).

One successful technological method is the electrical processing of whey using recovery of the protein mineral concentrate (PMC) and the simultaneous isomerization of lactose into lactulose using the electrochemical activation (ECA) of the liquid. This approach was developed in Chisinau, at the Institute of Applied Physics in the second half of the 1990s (Bologa et al., 1992).

Performance of this method depends on such parameters, as pH and temperature, because these factors determine the solubility and electrostatic protein interactions in the feed (Gonzalez et al., 2008; Luo and Ding, 2011; Rice et al., 2011a; 2011b), as well as current density.

Thus, the present study aimed to establish the peculiarities of amino acid migration in protein mineral concentrates under the influence of different pH and temperature values during electro-activation of whey.

MATERIALS AND METHODS

In the framework of the experiments the electrophysical processing was applied on the whey provided by the "JLC" Joint Stock Company, Chisinau, Republic of Moldova, after the manufacture of the: granulated cottage cheese "Grauncior". The electrophysical processing of whey was performed at the

membrane electrolyzer EDP-4, at j = 10-20 mA/cm^2, in the stationary regime, specially designed for collecting the samples so as to study amino acids (Maximuc et al., 2008). All PMCs were collected every 5 minutes in the cathode cell (CC) (Bologa et al., 2009).

The determination of the content of amino acids in the studied samples was done by the ion-exchange chromatography (Moore et al., 1958) at amino acid analyzer AAA-339M.

The analysis is performed in the standard procedure for the determination of free amino acids using lithium buffer solutions, pH 2.90, 2.95, 3.20, 3.80 and 5.00, with a flow rate of 12.0 ml/hr. On the basis of the qualitative calculation of amino acid content in the liquid studied it is stated that the amount of an amino acid in the sample is proportional to the surface of the pick of the chromatogram. The calculation consists in the fact, that sample and standard mixture of amino acids with the same content is analyzed. The amount of amino acids dosed on the ionic column in the test sample is given by the formula below:

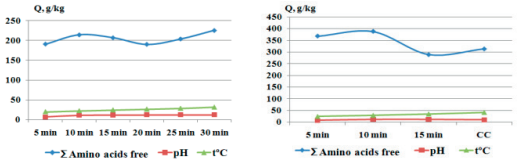
$C_{i(doz.)} = k \cdot n \cdot S_{i(prob.)}/S_{i(st.)} \cdot M_i \cdot 10^{-6}$ (mg),

Where: $C_{i(doz.)}$ - the ionic concentration of amino acids in the volume of the dosed node; n - the amount of the amino acids in the analyzed mixture; $S_{i(prob.)}$ - the tip(pick) surface of the amino acids in analyzed mixture; $S_{i(st.)}$ - the tip (pick) surface of the amino acids in standard mixture; k - correction coefficient considered to be changing the detector sensitivity; M_i - the ionic molecular weight of the amino acid. The automatical analyzer AAA-339M detects ninhydrin positive components within 1-100 nanomoles concentration. The duration of the analysis of the physiological fluids is 3.5 hours.

RESULTS AND DISCUSSIONS

In the frame of investigation, it was studied the influence of pH and temperature on the migration of amino acids in protein minerals concentrates (PMCs) during electro activation of whey at different processing regimes.

The variations of total free amino acid content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior" in the stationary regime of treatment, at current density j = 10 mA/cm^2 and j = 20 mA/cm^2, depending on the variations of pH and temperature, are presented in the Figure 1 (A and B).



A **B**

Figure 1. Variations of total free amino acid content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese "Grauncior" in stationary regime, at current density j = 10 mA/cm^2 **(A)** and j = 20 mA/cm^2 **(B)**, depending on variations of pH and temperature

The content of free amino acids is kept at the same level with increasing pH and temperature during electrophysical processing of whey after the manufacture of the granulated cottage cheese "Grauncior" at current density j = 10 mA/cm^2 (Figure 1 A).

Electrophysical processing of whey at current density j = 20 mA/cm^2, with increasing pH and temperature, more drastically affect the isolation of free amino acids in the PMCs (Figure 1 B).

Thus, during electrophysical processing of whey after the manufacture of the granulated cottage cheese "Grauncior" the greatest amount of free amino acids is extracted at first 5-10 min of processing, and with increasing temperature and pH, the decreasing of their contents is attested.

The variations of the essential amino acid content in the PMCs during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior", in the stationary regime, at current density j = 10 mA/cm^2 and j = 20 mA/cm^2, depending on the variations of pH and temperature, are presented in Figure 2.

Electrophysical processing of whey after the manufacture of the granulated cottage cheese "Grauncior" at current density j = 10 mA/cm^2 revealed that the highest amount of essential amino acids is obtained in the PMCs at 10 min of processing, then the content is kept stable at 15 min and decreases at 20 min of processing. Then, at 25 and 30 min of electrophysical processing, the content of essential amino acids

reach its maximum values in the PMCs when pH and temperature also have higher values (Figure 2 A).

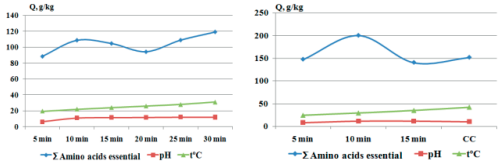


Figure 2. Variations of essential amino acid content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese "Grauncior" in stationary regime, at current density j = 10 mA/cm^2 **(A)** and j = 20 mA/cm^2 **(B)**, depending on variations of pH and temperature

Electrophysical processing of whey after the manufacture of the granulated cottage cheese "Grauncior", at current density j = 20 mA/cm^2, revealed approximately the same variations of the essential amino acid content as at current density j = 10 mA/cm^2: the maximum content is attested at 10 min of electrophysical processsing, then the content decreases (Figure 2 B).

Next is represented the variation of each essential amino acid content in the PMCs during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior" in the stationary regime, at current density j = 10 mA/cm^2 and j = 20 mA/cm^2, depending on the variations of pH and temperature.

The essential amino acids for humans are: threonine (Thr), methionine (Met), valine (Val), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), tryptophan (Trp), lysine (Lys), histidine (His), arginine (Arg). Each essential amino acid plays an important biochemical and functional role in maintaining vital processes in living organism (Reeds, 2000).

The degree of Thr isolation in the PMCs of whey after the manufacture of the granulated cottage cheese "Grauncior" increases during 5-20 min of electrophysical processing with electrolyzer EDP-4 at current density j = 10 mA/cm^2, then decreases at 25 min and slightly increases at 30 min, with a rise of temperature and pH. During electrophysical processing at current density j = 20 mA/cm^2 the maximum degree of isolation of Thr was established at 10 min when pH and temperature were growing (Figure 3).

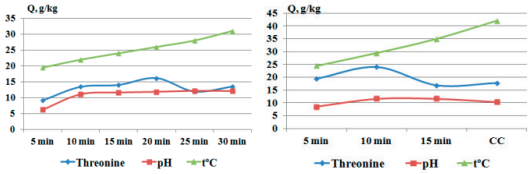


Figure 3. Variations of threonine (Thr) content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese "Grauncior" in stationary regime, at current density j = 10 mA/cm^2 **(A)** and j = 20 mA/cm^2 **(B)** ,depending on variations of pH and temperature

The content of Val extracted in the PMCs of whey after the manufacture of the granulated cottage cheese "Grauncior" increases during 5-15 min of electrophysical processing with electrolyzer EDP-4 at current density j = 10 mA/cm^2, then decreases at 20 min and increases again at 25 and 30 min of electrophysical processing, with the rise of temperature and pH (Figure 4).

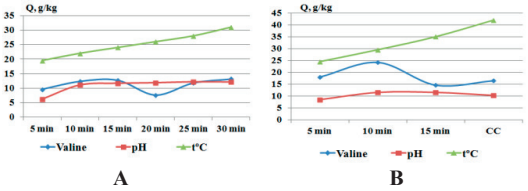


Figure 4. Variations of valine (Val) content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese „Grauncior" in stationary regime, at current density j = 10 mA/cm^2 **(A)** and j = 20 mA/cm^2 **(B)**, depending on variations of pH and temperature

During electrophysical processing at current density j = 20 mA/cm^2 the maximum degree of isolation of Val was established at 10 min, when pH and temperature were higher (Figure 4).

The degree of Met isolation in the PMCs is one of the smallest among essential amino acids extracted in the PMCs during electrophysical processing of the studied whey. But during electrophysical processing of whey after the manufacture of the granulated cottage cheese "Grauncior" with electrolyzer EDP-4 at current density j = 10 mA/cm^2, an increase of Met in PMCs is noted at 10 min of processing, a slight decrease at 15 min and then its content is kept

stable during electrophysical processing. During electrophysical processing at current density j = 20 mA/cm^2, the same variation of Met content in the PMCs is established, with increased values of pH ant temperature (Figure 5).

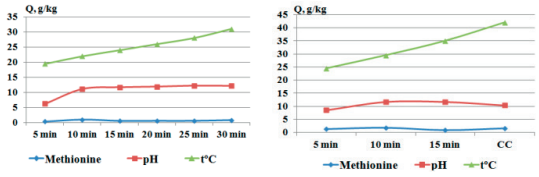


Figure 5. Variations of methionine (Met) content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese "Grauncior", in stationary regime, at current density j = 10 mA/cm^2 **(A)** and j = 20 mA/cm^2 **(B)**, depending on variations of pH and temperature

The content of Ile extracted in the PMCs of whey after the manufacture of the granulated cottage cheese "Grauncior" increases during 5-15 min of electrophysical processing with electrolyzer EDP-4 at current density j = 10 mA/cm^2, then decreases at 20 min and increases again at 25 and 30 min of electrophysical processing, with the rise of temperature and pH. During electrophysical processing at current density j = 20 mA/cm^2, the maximum degree of isolation of Ile was established at 10 min, when pH and temperature were growing (Figure 6).

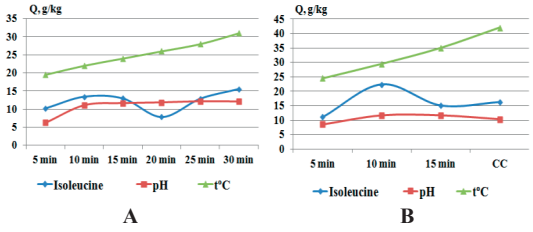


Figure 6. Variations of isoleucine (Ile) content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese "Grauncior", in stationary regime, at current density j = 10 mA/cm^2 **(A)** and j = 20 mA/cm^2 **(B)**, depending on variations of pH and temperature

The content of Leu extracted in the PMCs of whey after the manufacture of the granulated cottage cheese "Grauncior" increases during the entire period of electrophysical processing with electrolyzer EDP-4 at current density j = 10 mA/cm^2, and reaches its maximum content at 30 min of processing, when pH and temperatures have the maximum values. During electrophysical processing at current density j = 20 mA/cm^2 the maximum degree of isolation of Leu was established at 10 min, when pH and temperature are growing (Figure 7).

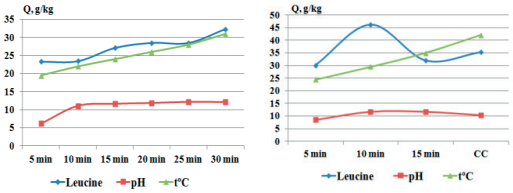


Figure 7. Variations of leucine (Leu) content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese "Grauncior", in stationary regime, at current density j = 10 mA/cm^2 **(A)** and j = 20 mA/cm^2 **(B)**, depending on variations of pH and temperature

The content of the essential amino acid Phe, extracted in the PMCs of whey after the manufacture of the granulated cottage cheese "Grauncior" during electrophysical processing with electrolyzer EDP-4 at current density j = 10 mA/cm^2, is low in comparison with other essential amino acids. A relatively high amount of this amino acid is noted at 5 and 10 min of processing, then its level decreases, although the values of pH and temperatures are growing during the entire electrophysical processing. The maximum degree of isolation of Phe in the PMCs is recorded at 10 min of electrophysical processing, at current density j = 20 mA/cm^2 (Figure 8).

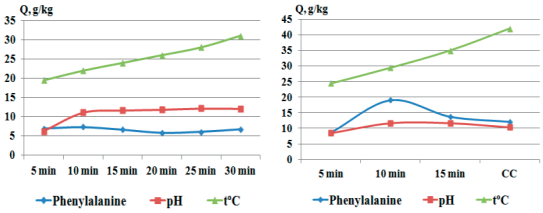


Figure 8. Variations of phenylalanine (Phe) content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese "Grauncior", in stationary regime, at current density j = 10 mA/cm^2 **(A)** and j = 20 mA/cm^2 **(B)**, depending on variations of pH and temperature during electrophysical processing

The degree of Trp isolation in the PMCs increases during electrophysical processing of whey after the manufacture of the granulated cottage cheese "Grauncior" with electrolyzer EDP-4 at current density j = 10 mA/cm^2. During electrophysical processing at current density j = 20 mA/cm^2 a high degree of Trp extraction in the PMCs is established at 10 and 15 min of electrophysical processing, with increased values of pH and temperature (Figure 9).

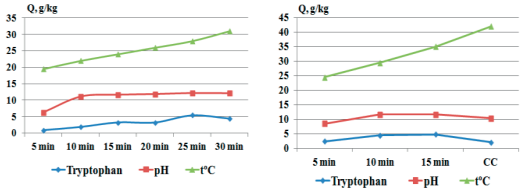


Figure 9. Variations of tryptophan (Trp) content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese "Grauncior", in stationary regime, at current density j = 10 mA/cm^2 **(A)** and j = 20 mA/cm^2 **(B)**, depending on variations of pH and temperature during electrophysical processing

The content of Lys extracted in the PMCs of whey after the manufacture of the granulated cottage cheese "Grauncior" increases during 5-10 min of electrophysical processing with electrolyzer EDP-4 at current density j = 10 mA/cm^2, decreases at 15-20 min and increases again at 25 and 30 min of electrophysical processing, with the rise of temperature and pH. During electrophysical processing at current density j = 20 mA/cm^2, the maximum degree of isolation of Ile was established at 5 min when pH and temperature are growing (Figure 10).

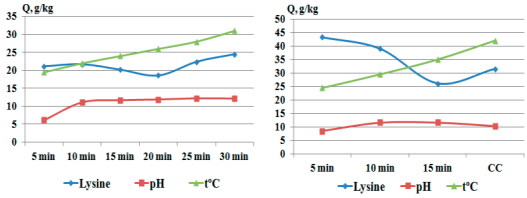


Figure 10. Variations of lysine (Lys) content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese "Grauncior", in stationary regime, at current density j = 10 mA/cm^2 (A) and j = 20 mA/cm^2 (B),depending on variations of pH and temperature

Lys is the limiting amino acid that determines the biological value of many proteins from foodstuff.

The His content in the PMCs of whey after the manufacture of the granulated cottage cheese "Grauncior" increases during 5-10 min of electrophysical processing with electrolyzer EDP-4 at current density j = 10 mA/cm^2, decreases at 15 min and keeps stable at 20-30 min of electrophysical processing, with growth of temperature and pH. During electrophysical processing at current density j = 20 mA/cm^2, the maximum degree of isolation of His is established at 10 min, when pH and temperature are growing (Figure 11).

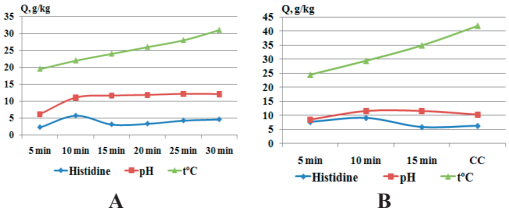


Figure 11. Variations of histidine (His) content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese "Grauncior", in stationary regime, at current density j = 10 mA/cm^2 **(A)** and j = 20 mA/cm^2 **(B)**, depending on variations of pH and temperature

The Arg content in the PMCs of whey after the manufacture of the granulated cottage cheese "Grauncior" increases during 5-10 min of electrophysical processing with electrolyzer EDP-4 at current density j = 10 mA/cm^2, decreases at 15-20 min and then slightly increases at 30 min of electrophysical processing, with the growth of temperature and pH. During electrophysical processing at current density j = 20 mA/cm^2, the maximum degree of isolation of Arg is established at 10 min, when pH and temperature are growing, and the highest content is recorded in the cathode cell (Figure 12).

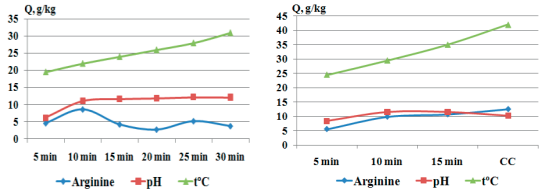


Figure 12. Variations of arginine (Arg) content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese "Grauncior", in stationary regime, at current density j = 10 mA/cm^2 (A) and j = 20 mA/cm^2 (B), depending on variations of pH and temperature

Thus, certain peculiarities of amino acids migration in the concentrates obtained under the influence of different pH and temperature values were determined.

The highest degree of extraction of free, especially essential, amino acids is recorded in the PMCs during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior", at current density j = 20 mA/cm^2, at 10 min of processing, when pH = 11.6 and tºC = 29.5ºC.

The level of migration of each essential amino acid in the PMCs is varying in dependence on the time of electrophysical processing, on the current density, pH and temperature values.

The parameters for the optimal isolation of each essential amino acid in the the PMCs are:

Thr (threonine) - during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior", at current density j = 20 mA/cm^2, at 10 min of processing, pH = 11.6 and tºC = 29.5ºC;

Val (valine) - during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior", at current density j = 20 mA/cm^2, at 10 min of processing, pH = 11.6 and tºC = 29.5ºC;

Met (methionine) - during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the "Grauncior", at current density j = 10 mA/cm^2, at 10 min of processing, pH = 11.8 and tºC = 25ºC;

Ile (isoleucine) - during electrophysical processing with electrolyzer EDP-4 of whey after themanufacture of the granulated cottage cheese "Grauncior", at current density j = 20 mA/cm^2, at 10 min of processing, pH = 11.6 and tºC = 29.5ºC;

Leu (leucine) - during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior", at current density j = 20 mA/cm^2, at 10 min of processing, pH = 11.6 and tºC = 29.5ºC;

Phe (phenyalanine) - during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior", at current density j = 20 mA/cm^2, at 10 min of processing, pH = 11.6 and tºC = 29.5 ºC;

Trp (tryptophan) - during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior", at current density j = 10 mA/cm^2, at 5 min of processing, pH = 12.1 and tºC = 28ºC;

Lysine (Lys) - during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior", at current density j = 20 mA/cm^2, at 5 min of processing, pH = 8.5 and tºC = 24.5 ºC;

His (histidine) - during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior", at current density j = 20 mA/cm^2, at 10 min of processing, pH = 11.6 and tºC = 29.5ºC;

Arg (arginine) - during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior", at current density j = 20 mA/cm^2, at 15 min of processing, H = 11.6 and tºC = 35ºC.

CONCLUSIONS

The peculiarities of amino acids migration in the concentrates obtained under the influence of different pH and temperature values were established.

The highest degree of extraction of total free and essential amino acids, is recorded in the PMCs during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior", at current density j =

20 mA/cm^2, at 10 min of processing, when pH = 11.6 and tºC = 29,5ºC.

The level of migration of each essential amino acid in the PMCs is varying in dependence on time of electrophysical processing, current density, pH value and temperature (tºC), that can be promising investigations in the direction of PMC obtaining with desired amino acids content and spectrum by applying various parameters (regimes) of whey electrophysical processing.

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REFERENCES

Bologa, M., Vrabie, E., Maximuk, E. (2009). A method for whey processing. Patent MD nr. 3793. *BOPI* nr. 1.

Chandrapala, J., Duke, M. C., Gray, S.R., Zisu, B., Weeks M., Palmer, M., Vasiljevic, T. (2015). Properties of acid whey as a function of pH and temperature. *J. Dairy Sci.,* 98, 4352-4363.

Cheftel, J.C. (1977). Chemical and nutritional modification of food proteins due to processing and

Bologa, M.K., Stepurina, T.G., Lupu, E.I., Vrabie, E.G., et al. (1992). *Myasn. Molochn. Prom-st*., 6, 22-23.

storage. *In: Food Proteins. Whitaker JR and SR Tannenbaum (Eds.). Avi. Publishing Co, Westport, Connecticut*, 401-445.

de Wit, J. N. (2001). Lecturer's Handbook on Whey and Whey Products. *Eur. Whey Prod. Assoc., Brussels,* Belgium.

Desrosiers, T., Savoie, L. (1991) Extent of damage to amino acid availability of whey protein heated with sugar. *Journal of Dairy Research*, 58, 431-441.

Etzel, V.R. (2004). The emerging role of dairy proteins and bioactive peptides in nutrition and health. Manufacture and use of dairy protein fractions. *The Journal of Nutrition*, 134(4), 9965-10025.

Gonzalez, M.I., Alvarez, S., Riera, F.A., Alvarez, R. (2008). Lactic acid recovery from whey ultrafiltrate fermentation broths and artificial solutions by NF. *Desalination*, 228, 84-96.

Luo, J., Ding. L. (2011). Influence of pH on treatment of dairy waste water by NF using shear enhanced filtration system. *Desalination,* 278, 150-156.

Maximuk, E., Bologa, M., Confratenco, S., Vrabie, E. (2008). The diaphragm electrolyzer. Patent Md 3496. *BOPI* nr. 2.

Moore, S., Spackman, D.H., Stein W. (1958) Chromatography of amino acids on sulfonated polystyrene resins. *Anal. Chem.,* 30(1), 1185-1190.

Reeds, P.J. (2000). Dispensable and indispensable amino acids for humans. *J. Nutr.* 130(7), 1835S-40S.

Rice, G., Barber, A.R., O'Connor, A.J., Pihlajamaki, A., Nystrom, M., Stevens, G., Kentish, S.E. (2011a). The influence of dairy salts on NF membrane charge. *J. Food Eng.,* 107, 164-172.

Rice, G., Barber, A.R., O'Connor, A.J., Stevens, G.W., Kentish, S.E. (2011b). Rejection of dairy salts by a NF membrane. *Purif. Tech.,* 79, 92-102.

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APPLICATION OF QFD METHODOLOGY (HOUSE OF QUALITY) FOR PRODUCTION OF FRUIT ICE CREAM

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Abstract

Quality Function Development (QFD) is a systematic approach specific to quality management that facilitates product development by ensuring consumer requirements meeting (customer voice), these being taken into account from the design phase, then during the entire technological process, being reflected in the finished product. The purpose of this study was to apply the QFD methodology (House of Quality, HoQ1,) to improve the quality of products in the food industry, taking into account the technological process of obtaining fruit ice cream (the Q product), thus providing a synthetic model. The working method consisted in the participation of a number of 240 ice cream consumers, aged between 20-24 years, who provided the list of consumer requirements, prioritizing and weighting them based on a score from 1 to 5. The following stages were represented by the transposition of consumers' voice in quantifiable technical requirements, their correlation at the "roof" level, establishing the relations between technical measures and the customer's voice by using predefined symbols, establishing the direction of improving the quality of the new product, assessing current competition and determination of target values. Following the analysis, the most important consumer requirements were the amount of fruit added (20.8%), the lack of hazardous additives (20.8%), the creaminess (16.7%) and the lack of ice crystals (16.7%). Thus, in order to meet consumer requirements, the replacement of sugar with maple syrup, artificial stabilizers and emulsifiers with pectin (0.4%) and yolk (0.5%), led to a more nutritious and healthier product, but which will probably have a higher price compared to the products currently available on the market.

Key words*: fruit ice cream, quality function deployment*

INTRODUCTION

Creating new products that meet the desires of consumers is not easy, careful research is needed so that the products made are necessary for consumers. Many companies compete to create new products that can speed up their marketing time. A commonly used method for product management is QFD (Rujito et al., 2020). QFD has been practiced by world-renowned companies since 1966. Its double purpose is to ensure that the true needs of customers are properly developed/implemented throughout the design, construction and delivery of a new product, whether assembled, processed, maintained or even software, and to improve the product development process itself (Akao and Mazur, 2003). QFD is a comprehensive quality system that aims primarily at customer satisfaction.

It focuses on maximizing customer satisfaction, looking for both spoken/explicit and unspoken needs, translating them into actions and models, followed by their communication throughout the organization (Mazur, 2015).

QFD has recently become a widely used quality management tool in product design and development (Van et al., 2018). More recently, the QFD method is used in all stages of manufacturing: planning, design, processing and production of products, and the results of applying QFD in all stages are interdependent. (Dvoryaninova et al., 2020).

QFD facilitates the product development process, ensuring that customer requirements are taken into account throughout the technological process, and then reflecting its voice in the final product. (Zhebo et al., 2019).

QFD is used to receive customer feedback throughout the planning, development, engineering and manufacturing stages for any product (Van et al., 2018). This technique helps organizations to allocate resources and coordinate their skills according to customer needs by reducing costs and the production cycle (Karsak et al., 2015), helping to measure

the impact of organizational learning through innovation (Bhattacharya, 2010; Wasserman, 1993).
QFD is a well-structured inter-functional planning technique, a methodology for continuous product improvement, focusing on multifunctional teams to integrate the voice of consumers in the stages of planning, development, engineering, and manufacturing (Bhattacharya, 2010; Govers, 2001).
The main goal of QFD planning should be to maximize customer satisfaction (Wasserman, 1993; Bhattacharya, 2010).
QFD originated in Japan in the 1970s and has been applied in several fields (Akao, 1990), for product development, concept evaluation, service design and comparative evaluation of competitors. Practically, customer desires for a particular product or service can be represented by a set of intangible marketing requirements (MR). Next, a number of technical attributes (TA) that have an impact on MRs must be determined and performed for product development or service design. Usually, conventional QFD consists of the following four phases (Chan et al., 1999; Lin et al., 2010): the first stage translates the marketing requirements into technical attributes; the second phase translates the technical attributes into the characteristics of the parts; the third phase transposes the characteristics of the part into manufacturing operations, and the fourth phase translates the manufacturing operations into production requirements (Wang and Chen, 2012).
QFD is used for decision-making issues with clear numbers and recently many extended versions of this concept have been proposed.
The main planning tool used in QFD is the Quality House (HoQ). HoQ is a house-shaped matrix that connects the customer's wishes (WHAT?) and how the product will be designed and made to meet the customer's wishes (HOW?) (Rujito et al., 2020; Jones, 2005; Smith, 2009). At the level of the literature, different approaches of the QFD methodology are observed, depending on the vision of the authors. Benner, 2003 argues that the application of QFD in the food industry is more complicated than the current literature suggests. However, QFD proves useful if adaptations are made to the method and the specific characteristics of the food ingredients are taken into account. It has been highlighted that the method has been successfully applied in the field of food development, for example, chocolate bars (Viaene, 1999), filled chocolate (De Pelsmaeker et al., 2015), instant rice noodles (Waisarayutt and Tutiyapak, 2006), pie dough (Pinto and Paiva, 2010), wheat flour (Kristianto et al., 2012), fruit (Miguel et al., 2007), meat (Rosado et al., 2011; Park et al., 2012), mineral water (Moldovan, 2014), olive oil (Sayadi et al., 2017), fish (Dvoryaninova et al., 2020), but also for a number of functional foods (Pinto and Paiva, 2010) and biologically active additives (Ermolaeva et al., 2018).
Since 2015, the QFD methodology has been transposed into the international standard ISO 16355, which includes eight parts structured in several editions, the latest from 2017, and others that are still in progress, making QFD much more credible and practical.
The aim of this study was to apply the QFD (HoQ, Quality House) methodology in the food industry, taking into account the technological process of obtaining fruit ice cream, thus providing a synthetic model of approach.
Fruit ice cream is in fact an ice cream based on dairy products, in which, after freezing the mix, fruits (cherries, raspberries, strawberries etc.) are introduced, pre-sprinkled with powdered sugar and allowed to spread as much as possible (Țibulcă and Jimborean, 2008).

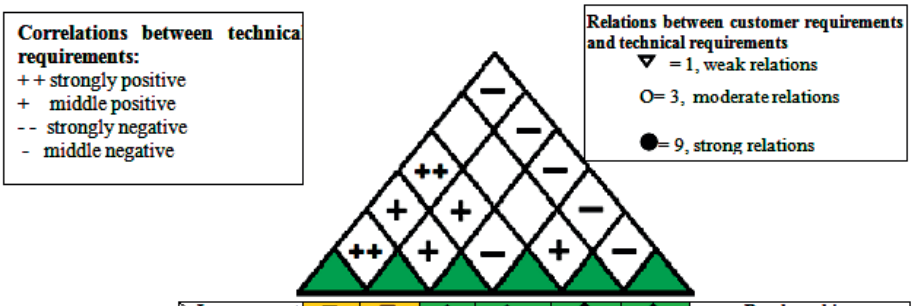
MATERIALS AND METHODS

The methodology consisted in the participation of a number of 240 ice cream consumers, aged between 20-24 years (students from three different Food Engineering specialities, because the QFD methodology recommends the use of multidisciplinary work teams), which provided the list of consumer requirements (Figure 1), prioritizing and weighting them based on a score from 1 to 5. The next steps were to transpose consumers' "voice" into quantifiable technical requirements (design requirements), establishing measurement units of for each requirement, correlating them to the roof to identify possible technological problems, establishing the relationship between technical measures and the customer's voice using pre-defined symbols, establishing the direction to improve the quality of the new

product (which are technical criteria that require a decrease or increase to meet customer requirements), the assessment of current competition (establishing the strengths and weaknesses of the newly designed product, Q) and determining the target values (which need to be improved).

RESULTS AND DISCUSSIONS

Consumer requirements (WHAT?) were represented by elements related to their health concern: the amount of fruit added/high fiber content (20.8%), the lack of hazardous/harmless additives for health (20.8%), components of sensory properties, creaminess/pleasant taste (16.7%), lack of ice crystals (16.7%), availability in different packaging quantities (12.5%) and accessible price (12.5%). To meet these requirements, a new product has been designed, innovative in terms of ingredients traditionally added to ice cream manufacturing technology, replace the sugar with concentrated maple syrup (Figure 1); also the use of artificial additives was avoided and only yolk (0.5%, emulsifier) and pectin (0.4%, stabilizer, gelling agent etc.) were used, along with the realization as far as possible, of this type of ice cream, at production costs as low as possible by using dairy raw materials such as: buttermilk butter, buttermilk powder, whey powder.



Technical requirements / Consumers requirements	Importance 1-5	%	Improvement direction ⇩ % balanced sugars lipids %	⇩ maple syrup, yolk, pectin%	⇧ high fruit pieces %	⇧ protein%, times, T°C, specific stages	⇧ different weights (g), pans, glasses, waffles,	⇧ low cost (lei)	Benchmarking NEW PRODUCT = Q A product 1p	B product 2p	C product 3p	4p	5p
1. Creamy, pleasant taste	4	16.7	●	●	O	●		▽			B	A, C	Q
2. No hazardous ingredients / additives, harmless to health	5	20.8	●	●	O	▽		●		B	C	A	Q
3. With many pieces of fruit / high fiber content	5	20.8	●	●	●	▽		O		B	A	C	Q
4. Without large ice crystals	4	16.7	●	●	●	●	●	O		B	C	A	Q
5. In different packing quantities	3	12.5				▽	●	●	A		B, C	Q	
6. Affordable /cheap price	3	12.5	●	O	▽	O	O	●	Q		A	C	B
	24	100%	788	712	463	392	300	541	=3196				
		%	24.6	22.3	14.5	12.3	9.4	16.9	100%				

Figure 1. HoQ for new fruit ice cream (the Q product)

The "customer's voice" was translated practically into the following technical criteria (HOW?), with related units of measure: balanced amount of carbohydrates/lipids, use of maple syrup instead of sugar, use of yolk (0.5%) and pectin (0.4%) in exchange for artificial emulsifiers and stabilizers, the addition of a higher quantity (over 20%) of whole fruits (raspberry, cherries, sour cherries) or pieces (strawberries, apricots etc.), ensuring a specific amount of protein of dairy origin, observance and monitoring of times and temperatures specific to the stages of the technological flow, ensuring the packaging of ice cream in casseroles, glasses, waffles, briquettes with different weights and selling, as much as possible, at the lowest possible price (by using dairy raw materials such as: buttermilk, powdered buttermilk, whey powder).

The ingredients of the Q product are shown in the production flow chart (Figure 2).

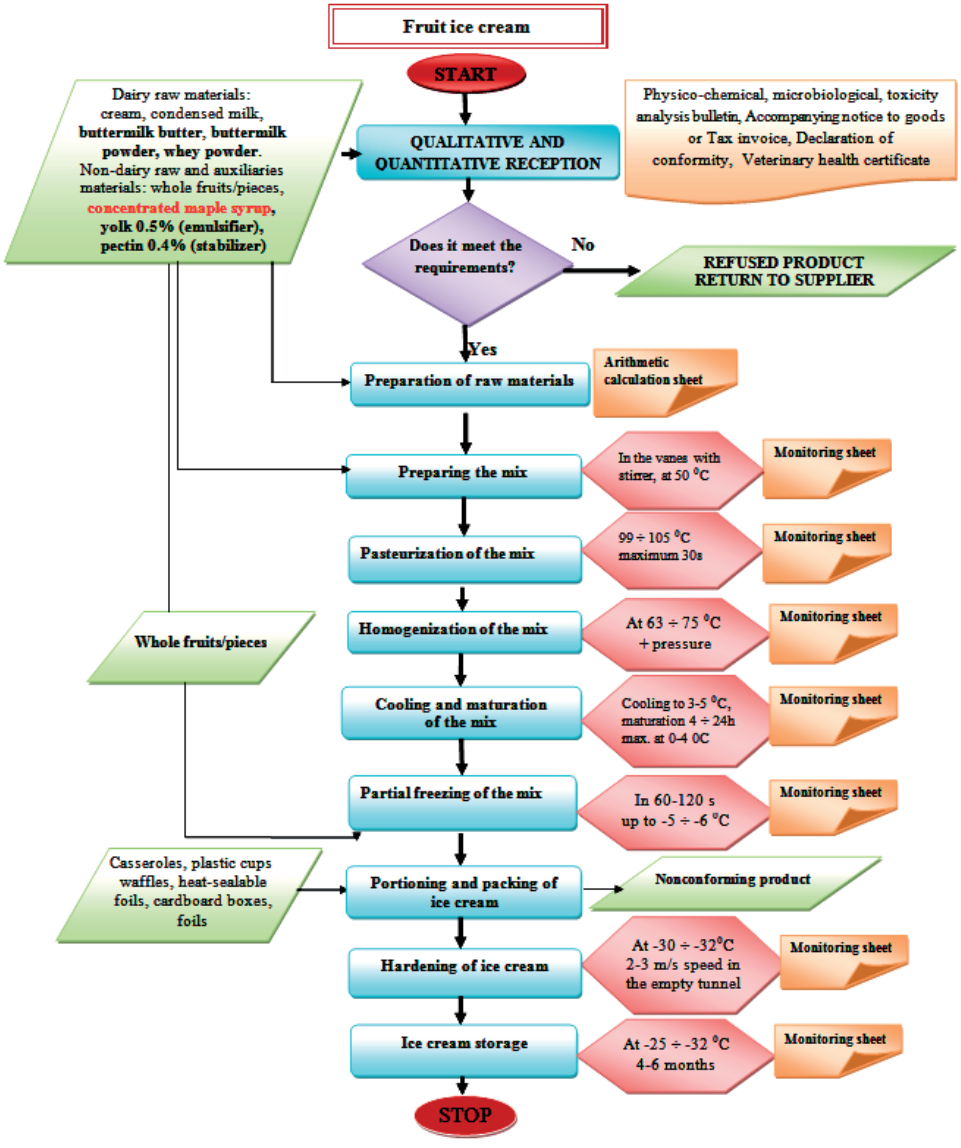


Figure 2. The production process flow chart of new fruit ice cream (adapted, using Usturoi, 2007; Țibulcă, 2008; Banu et al., 2009)

The direction of improving the technical characteristics aims to maintain the balanced level (%) of lipids, carbohydrates (concentrated maple syrup) and to increase the other characteristics.
Predetermined symbols are used to determine the relations between the customer's voice and quantifiable technical measures (WHAT vs. HOW) placed in the cell located at the intersection of each row vs. column (Figure 1.).
At the level of the "foundation" of the HoQ, the higher the values obtained, the more important those characteristics are, because there are strong correlations between them (HOW MUCH); the results from the first house (HoQ 1. product planning) are further used in the following matrices /houses specific to the QFD methodology (HoQ 2. process design, HoQ 3. manufacturing process planning, HoQ 4. production characteristics planning).
The room on the right side of the HoQ is the assessment of current competition (Benchmarking) used to measure the success of the newly designed product that competes with those on the market; thus a scale from 1 to 5 is used for the assessment (1 indicates a requirement that is not met and 5 indicates a requirement that is fully met).
By averaging the numbers in each column, depending on the score obtained, a measure of the degree of customer satisfaction for each product under study is obtained. Following the comparative analysis of the newly designed Q product, with products of three competing companies (*Aloma*, *Amicii* and *Betty Ice* - coded with A, B and C), a good position of product Q was obtained (25 points.), compared to the current competitors (17 to 21 points); the weaknesses being represented by the price of the product and the possibility of packaging in different quantities (imprinted in production cost/selling price).
The strengths of product Q are transposed into the technical criteria represented by the elimination of sugar from the ingredients vs. use of maple syrup, elimination of emulsifiers and artificial stabilizers vs. use of egg yolk adding a large amount of fruit (over 20%). This score reflects a concrete/quantifiable analysis related to customer requirements. The determination of target values is based on the values established by the evaluation of competing products and product Q, establishing strategies to maintain strengths and improve weaknesses.
In order to meet the requirements of consumers throughout the technological process of manufacturing Q fruit ice cream the technological parameters related to each stage must be strictly observed and monitored (Figure 2).

Thus:
1. The use of concentrated maple syrup (12%), pectin (0.4%) and egg yolk (0.5%) ensures compliance with the following consumer requirements: creamy ice cream, without ingredients/additives dangerous/ harmless to health and without large ice crystals;
2. Nutritional sweeteners (concentrated maple syrup) lead to a decrease in the freezing point, to the achievement of the body and texture of the ice cream, more even than fats and proteins (Usturoi, 2007);
3. Pectin (stabilizer) helps to achieve a fine, velvety consistency, avoiding the formation of large ice crystals, evenly distributing the components and maintaining the microcrystalline structure of the product;
4. The preparation of the raw materials is carried out according to an exact arithmetic calculation, and that of the mix, into the hunt, with stirrer with heating system, respecting the order of introduction of the components;
5. The pasteurization of the mix destroys pathogenic bacteria improving technological quality of the product;
6. The homogenization of the mixture is necessary to obtain a stable fat emulsion together with the addition of egg yolk (0.5%);
7. Cooling and maturation of the mix, in addition to preventing development the remaining microorganisms in the mix, has the role of hydrating proteins, solidifying fats, improving the consistency, texture, melting resistance, foaming capacity (aeration) of ice cream;
8. The absence of large ice crystals, fine structure, is ensured by a high freezing speed, using sharp scraping blades during it;
9. Deep hardening or freezing of ice cream should be done quickly to avoid the formation of large ice crystals;

10. Storage of hardened ice cream must be carried out without temperature variations, in order to avoid recrystallization; also the avoidance of recrystallization is ensured by the use of pectin in a concentration of 0.4%.

Unlike competing products, no sugar is used in the manufacturing technology of product Q and neither emulsifiers or stabilizers obtained by chemical synthesis.

Product ingredients currently available on the market are the following:

- water, milk reconstituted from skimmed milk powder, sugar, glucose syrup, vegetable oil, dextrose, sweet whey powder (milk), berries 3.7%, concentrated berries puree and juice 1.33%, modified corn starch, egg yolk powder, stabilizers (guar gum, gum carob, carrageenan, processed Euchema seaweed), emulsifiers (mono and diglycerides of fatty acids), acidifier (citric acid), flavors (product A);
- water, sugar, 15% berries, vegetable fat (coconut oil), whole milk powder, glucose syrup, skimmed milk powder, modified starch from potato, acidifier (citric acid), dextrose, emulsifiers (mono and diglycerides of fatty acids), stabilizers (carob seed gum, guar gum, pectin), flavors, salt (product B);
- reconstituted skim milk, 10% fructose, fiber (inulin) 6%, vegetable fat (coconut oil), sweetener (stevia) 0.04%, emulsifier (mono and diglycerides of fatty acids), stabilizers (guar gum, gum carob, carrageenan), acidifier (citric acid), natural dye (red beet), flavors (product C).

The addition of whole fruits (raspberry, cherries, sour cherries) in Q product avoid the release of specific enzymes and the exposure to ambient air of sectioned parties that influence the loss of vitamin C by oxidation (Murariu et al., 2014) along with adding them after the stage of partial freezing of the mix.

CONCLUSIONS

The application of the QFD method for the manufacture of fruit ice cream makes it possible to transfer consumer requirements into a set of controlled (quantifiable) characteristics and transposes these requirements into technological operations, forming a continuous flow of information; this ensures that all elements of the production system are aimed at meeting consumer requirements.

In the example approached, in order to meet consumer requirements, the replacement of sugar with maple syrup, artificial stabilizers and emulsifiers with pectin (0.4%) and yolk (0.5%), respectively, led to a more nutritious and healthy product, but which will probably have a higher price compared to the products currently available on the market.

REFERENCES

Akao, Y., Mazur, G.H. (2003). The leading edge in QFD: past, present and future. *International Journal of Quality & Reliability Management*, 20(1), 20–35.

Asadabadi, M. R. (2017). A customer based supplier selection process that combines quality function deployment, the analytic network process and a Markov chain. *European Journal of Operational Research*, 263 (3), 1049–1062.

Banu, C. et al. (2009) *The treatise of food industry. Bucharest,* RO: ASAB Publishing House.

Benner, M., Linnemann, A. R., Jongen, W. M. F. and Folstar, P. (2003). Quality function deployment (QFD) - can it be used to develop food products? *Food Quality and Pref.*, 14(4), 327–339.

Bhattacharya, A., Geraghty, J., Young, P. (2010). Supplier selection paradigm: An integrated hierarchical QFD methodology under multiple-criteria environment. *Applied Soft Computing*, (10) 1013–1027.

Chen, K.J., Yeh, T.M., Pai, F.Y., Chen, D.F. (2018). Integrating Refined Kano Model and QFD for Service Quality Improvement in Healthy Fast-Food Chain Restaurants. *Int. J. Environ. Res. Public Health*, 15, 1310; 1–16.

De Pelsmaeker, S., Gellynck, X., Delbaere, C., Declercq, N. and Dewettinck, K. (2015). Consumer driven product development and improvement combined with sensory analysis: A case study for European filled chocolates. *Food Quality and Pref.*, 41, 20–29.

Dinçer H., Yüksel, S., Martínez, L. (2019). Balanced scorecard-based analysis about European energy investment policies: A hybrid hesitant fuzzy decision-making approach with Quality Function Deployment. *Expert Systems with Applications*, 115, 152–171.

Dvoryaninova, O.P., Alekhina, A.V., Kutsova, A.E., Kosenko, I.S., Pegina, A.N. (2020). Using quality function deployment for improving fish preserves technology. *IOP Conf. Series: Earth and Environmental Science*, 422 012048.

Ermolaeva, E.O., Dymova, Y.I., Zhukova, O.V. (2018). Using the house of quality scheme to boost the production of biologically active additives. *International Journal of Civil Engineering and Technology (IJCIET)*, 9(13), 973–992.

Govers, C.P.M. (2001). QFD not just a tool but a way of quality management. *International Journal of Production Economics*, 69(2), 151–159.

Karsak, E.E.; Dursun, M. (2015). An integrated fuzzy MCDM approach for supplier evaluation and selection. *Comput. Ind. Eng*., 82, 82–93.

Kristianto, Y., Ajmal, M. M., Sandhu, M. (2012). Adopting TQM This is to achieve customer satisfaction: A flour milling company case study. *TQM Journal*, 24(1), 29–46.

Mazur, G. (2015). "QFD: Voice of Customer Meets Voice of Process." Journal for Quality and Participation. January 2015.

Miguel, A. C. A., Spoto, M. H. F., Abrahão, C., da Silva, P. P. M. (2007). Consumer profile evaluation by quality function deployment for a pineapple. *Ciência e Agrotecnologia*, 31(2), 563–569.

Moldovan, L. (2013). QFD's work for a new product design in a mineral water company. *7th International Conference Interdisciplinarity in Engineering (Inter-Eng 2013)*, 12, 2014, 462–468.

Murariu, O.C., Murariu F., Veleșcu I. (2014). The effects of processing on the vitamin C in peach compote, preserve and jam destined for the retail. *Journal of Biotechnology-Elsevier*, Lecce, Italia, 185S, S80.

Park, S. H., Ham, S., Lee, M. A. (2012). How to improve the promotion of Korean beef barbecue, bulgogi, for international customers. An application of quality function deployment. *Appetite*, 59(2), 324–332.

Pinto, A. L. D., Paiva, C. L. (2010). Developing a functional ready to bake dough for pies using the Quality Function Deployment (QFD) method. *Ciencia e Tecnologia de Alimentos*, 30, 36–43.

Rosado, J. A. G., Lobato, J. F. P., Echeveste, M., Gerber, A. S., Yang, S., Rossini, K. (2011). Identification of demand in the production of sires using a conceptual model of quality function deployment: A case study. *Revista Brasileira de Zootecnia-Brazilian Jour. of Animal Sci*., 40(1), 210–220.

Rujito, H, Utami, M. M. D., Riskiawan, H. Y., Hermanuadi, D., Retnowati, N. (2020). Product design of kolang kaling grass jelly drink through the application of quality function deployment method (case study in Meru Betiri National Park, Banyuwangi District). *IOP Conf. Ser.: Earth Environ. Sci*., 411 012024, 1–5.

Sayadi, S., Erraach, Y., Parra-Lopez, C. (2017). Translating consumer's olive-oil quality attribute requirements into optimal olive-growing practices. *Brit. Food Jour*., 119(1), 190–214.

Țibulcă, D., Jimborean M.A. (2008). *The technology of obtaining dairy products.* Cluj-Napoca, RO: Risoprint Publishing House.

Usturoi, M.G. (2007) *Milk and dairy products technology*, Iași, RO: ALFA Publishing House.

Van, L. H., Yu, V. F., Dat, L. Q., Dung, C. C., Chou, S. Y., Loc, N. V. (2018). New integrated quality function deployment approach based on interval neutrosophic set for green supplier evaluation and selection. *Sustainability*, 10 (3), 838, 1–13.

Viaene, J., Januszewska, R. (1999). Quality function deployment in the chocolate industry. *Food Quality and Pref.*, 10, 377–385.

Waisarayutt, C., Tutiyapak, O. (2006). Application of quality function in instant rice noodle product development. *Kasetsart Jour.-Natural Sci*., 40, 162–171.

Wang, C.H., Chen, J.N. (2012). Using quality function deployment for collaborative product design and optimal selection of module mix. *Computers & Industrial Engineering*, 63, 1030–1037.

Wasserman, G.S. (1993). On how to prioritize design requirements during the QFD planning process, *IIE Transactions*, 25 (3) 59–65.

Zhebo, A. V., Akeshkov, A. V., Solovev, D. B. (2019). Formation of new segments of the market through quality function deployment of innovative production. *Advances in Economics, Business and Management Research*, 47, 1133–1136.

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CONFORMITY OF RAPI, PEAS AND MAIZE FLOWERS, CONCERNING PESTICIDE RESIDUES FOR ORGANIC BEEKEEPING

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Abstract

The purpose of the present paper was to investigate the degree of conformity of the flowers of some honey crops (rape, peas, maize) regarding pesticide residues, for the practice of organic beekeeping. Scientific research was carried out on the content of pesticide residues in flowers taken from industrial lands, suspected of contamination with pesticides. The results of the research demonstrate that, out of the 62 pesticides tested in the rape flower samples, detectable concentrations of residues were recorded in only 5 pesticides, which constitutes 8.1%. It has been found that rapeseed flowers are heavily polluted with the residues of the Glyphosate pesticide, in a concentration of 0.1772 mg/kg, which exceeds the maximum admissible limits, according to EU and MD standards, by 77.2%. In peas flowers, there were detectable concentrations of residues in only 7 pesticides, which constitutes 11.3%. The peas flowers in the researched industrial land were slightly polluted with the residues of the Glyphosate pesticide, in a concentration of 0.1088 mg/kg, which exceeds the maximum admissible limits, according to EU and MD norms, by 8.8%. In the maize flower samples, of the 62 pesticides tested, residues were detected in concentrations detectable only at 9 pesticides, which constitutes 14.5%. It was found that the maize flowers in the industrial land were heavily polluted with the residues of the neonicotinoid insecticide Thiametoxam at a concentration of 0.0178 mg/kg, which exceeds the maximum admissible limits, according to EU and MD standards, by 78.0%. Glyphosate residues in maize flowers were detected at hazardous (at the limit) concentrations of 0.0962 mg/kg, compared to the maximum admissible limits of 0.1 mg/kg. Therefore, the sites researched with rapeseed, peas and maize plantations are not conformity for organic beekeeping, because the flowers of these crops are polluted with residues of dangerous pesticides, banned by the EU, which can affect the health of bees and inoffensiveness bee products.

Key words*: bees, honey flowers, organic beekeeping, pesticides, residues.*

INTRODUCTION

Beekeeping, in the Republic of Moldova, has a branch of agriculture of particular importance for the national economy, due to the value and quality of the products offered by it, the creation of jobs among the vulnerable sections of the population in the rural areas, as well as for maintaining through pollination the homeostasis and biodiversity of nature ecosystems.

Totally in the country there are approximately 180 thousand families of bees, from which about 4.5-5.0 thousand tons of honey are obtained annually, of which about 4000 tons are exported to different countries, including the European Union.

Other bee products are obtained from bees, which are quite important, such as: wax, pollen, propolis, royal jelly, venom, which are used in various fields of the national economy (food, medicine, pharmaceutical, cosmetics, plastic arts).

One of the most important benefits brought to humans by bees is the additional product obtained from increasing the productivity of the entomophilous plants from the cultivated and spontaneous flora, as a result of their pollination, thus ensuring the perpetuation of nature's biodiversity. By the bees are pollinated about 600 thousand ha of land with agricultural crops, from which an additional 20-30% of harvest in annual value of over 3.6-4.0 billion lei are obtained. Moreover, much of the honey production and other bee products obtained in the country is harvested from the flowers of honeycomb agricultural plants, such as sunflower (*Helianthus annuus*), rapeseed (*Brassica napus* ssp. *oleifera* L.), lavender (*Angustifolia lavandula*), salvia (*Salvia officinalis*) and other related crops (peas, maize) that meet near agricultural fields with basic honey crops.

Regrettably, agricultural producers (cultivators of agricultural crops, agricultural specialists) largely ignore the fact that bees are the main pollinator of entomophilic agricultural crops and can serve as a decisive factor in increasing their harvest, producing organic and organic bee products, ensuring efficiency economic and sustainable development of the respective branches in the country.

The analysis of the situation of the last years demonstrate that, the traditional technology for the growth and exploitation of the bee families does not ensure the production of organic and harmless bee products, of competitive quality, which could be marketed at advantageous prices.

According to a report by MIEPO (Moldavian Investment and Export Promotion Organization) - Organization for Investment Attraction and Export Promotion from Moldova, the purchase price of BIO (organic) honey is currently 90% higher than that of ordinary honey (Report MIEPO, 2016). This is why the beekeepers want to practice the BIO beekeeping system massively, but, not knowing the conditions and the legislation of organic farming, they apply the outdated technologies that they know, activating in the traditional system of beekeeping and, thus, the obtained production is not recognized as BIO, and its harvest decreases significantly.

Therefore, the specific technologies of the bio beekeeping system require a deep study in order to be used.

It is necessary to know the anthropic impact caused by the industrialization and intensification of agriculture, the most commonly encountered polluting factors, their spreading areas and the degree of residual pollution (their concentration in the resources of the environment), which can affect both honey bees as the main entomophilic pollinator, as well as the production obtained from them.

In the European Union, the admissible norms of pesticide residues in plants and plant production are regulated by Regulation (EC) no. 396/2005 of the European Parliament and of the Council of 23 February 2005 on the maximum contents applicable to pesticide residues in/or on foodstuffs and food of animal and plant origin for animals (Regulation (EC) No. 396/2005), as amended and subsequent additions (Regulation (EU) 2017/978; Regulation (EU) 2019/552). Based on this Regulation, national programs for monitoring pesticide residues in plant products have been developed and adopted in EU countries (National Plan, Ro, 2019).

In the Republic of Moldova, also, was elaborated the National Program for the monitoring of pesticide residues and nitrate content in food of plant origin for the years 2015-2020 (National Program, 2015-2020), approved by Government Decision no. 567 of 16.07.2014. In accordance with this Program, the National Agency for Food Safety monitors the situation regarding pesticide residues in plant and animal production in the country. According to a report of this authority, regarding the implementation of the National Program for the monitoring of pesticide residues in foodstuffs of plant origin for 2016, out of the 266 samples taken from 22 products of plant origin, 5 samples were detected (1.9%) in which the pesticide residue content exceeded the LMA, being declared non-compliant with this criterion. Non-compliant samples were contaminated with pesticide residues: Propiconazole, Thiamethoxam, Cipermetrin and others (ANSA Report, 2016).

In Romania, according to a similar report of the Laboratory for the Control of Pesticide Residues in Plants and Vegetable Products in Bucharest (2018), out of the 794 vegetable samples investigated, 7 samples (0.9%) were found to be non-compliant according to the residue content of pesticides: Chlorpyrifos, Chlorothalonil, Dimetoat and Tiametoxam (Report, madr.ro., 2018).

Although in both countries, the percentage of non-compliant samples according to the pesticide residue content in plant products is small, it indirectly explains why, in the apiculture production exported from the Republic of Moldova, they are sometimes detected, by the EU certification and control bodies (Commission Regulation 834/2009; Commission Regulation 271/2010), harmful substances and polluting residues, which leads to the compromise of the competitiveness of the branch and national image internationally (Antonescu et al, 2001; Bogdanov et al, 1999; Buruian, 2011).

Thus, to date, both agricultural producers and beekeepers have not been aware that the uncontrolled use of pesticides in the treatment of agricultural crops and honey bees has an imbalanced impact on the homeostasis of natural ecosystems, with final consequences of risk of human health security.

Therefore, researching the conformity of the honeycomb flora for the practice of organic beekeeping under the conditions of different native anthropogenic ecosystems, revealing the most widespread pesticides that produce residues in the honeycomb flora and bee products, highlighting the polluting sites and the reactive areas clean of residues of the pesticides that can ensure the organic beekeeping, they represent current problems, the solution of which would allow the elaboration of measures of adjustment of the conventional beekeeping to the organic beekeeping in the Republic of Moldova, according to the norms of the EU.

In this context, the purpose of the present paper was to investigate the degree of conformity of the flowers of some honey crops (rape, peas, maize) regarding pesticide residues, for the practice of organic beekeeping.

MATERIALS AND METHODS

Scientific research has been carried out on the content of pesticide residues in the flowers of honey crops in some sites, which have been suspected of contamination with pesticides. Among these crops were investigated: rapeseed (*Brassica napus* ssp. *oleifera* L.), peas (*Pisum sativum*) and maize (*Zea mays*) from industrial land.

The flower samples of the rapeseed crop were taken from the industrial land of an agricultural household in Stefan Voda, which wanted to remain confidential.

Samples of maize flowers (male and female), as well as peas were taken from the industrial land of an agricultural household in Ciadîr-Lunga, which also wanted to remain confidential.

From each site (planting land) mentioned above were taken 5 flower samples of the respective plants. For the sampling of flowers, each agricultural land was virtually divided into 5 equal parts, from each one being taken one sample. In total, 15 samples of biological material of the respective flowers were taken. Each sample of biological material collected weighed from 100 to 150 g. The samples were packaged in plastic bags and transported the same day to the accredited laboratory of the S.C. "Center of Applied Metrology and Certification", in accordance with the sanitary-veterinary norms regarding the methodology of sampling, processing, packing and transport of the samples for laboratory examinations (Sanitary-veterinary norm, 2010).

Each sample of biological material taken was tested for the most dangerous pesticide residues (62 names) most commonly encountered in our country (Table 1).

Table 1. Names of pesticides tested for contents of residues in the samples of flowers taken

No or	Name of the pesticide	No or	Name of the pesticide	No or	Name of the pesticide
1	Acetamipirid	22	Endosulfan	43	Pendimetalin
2	Azoxistribin	23	Epoxiconazol	44	Permetrin
3	Bifentrin	24	Ethion	45	Picoxistrobin
4	Bitertanol	25	Fenvalerat	46	Pirimetanil
5	Boscalid	26	Fenitrotion	47	Pirinicarb
6	Bromuco-nazol	27	Fenixicarb	48	Piridaben
7	Captan	28	Fipronil	49	Pirimitos-metil
8	Chlothianidin	29	Flutriafol	50	Procimid
9	Ciflutrin	30	Folpet	51	Protenotos
10	Cipermetrin	31	Fosalon	52	Prometrin
11	Ciproconazol	32	Haloxifop	53	Propargit
12	Ciprodinil	33	Glifosat	54	Propiconazol
13	Clorotalonil	34	Benzoton	55	Spiroxamină
14	Clorpirifos	35	Imidacloprid	56	Tau-fluvalinat
15	Deltametrin	36	Indoxacarb	57	Tebuconazol
16	Diazinon	37	Iprodion	58	Thiacloprid
17	Diclorvos	38	Kresoxim-metil	59	Tiametoxam
18	Difenoconazol	39	Lambda-Ghalotrin	60	Trifluralin
19	Dimetoat	40	Lufenuron	61	Tiadimeton
20	Dimetomorf	41	Malation	62	Vindozolin
21	Diniconazol	42	Penconazol		

The pesticide residue content was determined in the Laboratory accredited above by gas-chromatography - mass spectrometric (GC-MS) and liquid-chromatographic - mass spectrometric (LC-MS) methods, described by Lazarii I. in Collections of standard methods MS (Lazari, 2000).

The data obtained, regarding the pesticide residue content in the investigated samples, were

compared with the maximum admissible limits (MAL) norms, according to the Sanitary Regulation regarding the maximum permitted residue limits of the plant protection products from/or from food and food of plant origin and animal for animals, approved by the Government Decision of the Republic of Moldova no. 1191 of 23.12.2010 (Sanitary Regulation, 2010), adjusted to EU norms.

As a result of the comparison, conclusions regarding the conformation of the honeycomb flora of the respective sites for the practice of organic beekeeping were made.

The data obtained as a result of the researches were systematized and processed with the help of computerized software "STATISTICA - 12" and appreciated their certainty, according to the variational biometric statistics, according to the methods of Плохинский Н.А. (1989).

RESULTS AND DISCUSSIONS

According to us, honey flowers are the main link in the spread of pesticides in nature in the food chain of honey bees. Without diminishing the importance of air, water and soil, honey flowers occupy the dominant segment in the ecology of bee products, because of these nectar and pollen are collected, as the main predecessors of bee products.

In our previous research (Cebotari et al, 2018; 2019) we studied the content of pesticide residues in forest tree flowers (acacia, linden) and important agricultural crops from different sites with different anthropogenic impact (sunflower flowers from the industrial land and the domestic garden from the village, apple flowers from the industrial orchard and from the domestic garden of the villagers).

At the same time, the situation regarding pesticide residues in the flowers of several entomophilic agricultural crops remains, until now, unknown. Therefore, in this paper we undertook research to identify the degree of conformity of the honeycomb flora from some agricultural sites for organic beekeeping.

Pesticide content in rapeseed flowers (*Brassica napus* ssp. *oleifera* L.). The results of the research showed that out of the 62 pesticides tested, detectable concentrations of residues were recorded in only 5 pesticides in the samples of rapeseed flowers from the industrial land of an agricultural household in Stefan Voda, which constitutes 8.1% (Table 2).

These pesticides include 1 pyrethroid insecticide (Cipermetrin), 3 triazole fungicides (Difenoconazole, Flutriafol, Benzoton) and 1 herbicide (Glyphosate).

Table 2. Pesticide residue content in flowers of rapeseed (N = 5), mg/kg

Name of the pesticide	MAL	M ± m	d (M-MAL)	d, % to MAL
Pyrethroid insecticide				
Cipermetrin	0.2	0.0929±0.0405	-0.1071	-53.6
Triazole fungicides				
Difenoconazol	0.05	0.0398±0.0148	-0.0102	-20.4
Flutriafol	0.2	0.1004±0.0569	-0.0996	-49.8
Benzoton	0.02	0.0190±0.0076	-0.001	-5.0
Herbicides				
Glyphosate	0.1	0.1772±0.0838	+0.0772	+77.2

It was found that the residue concentrations of pyrethroid insecticide and triazole fungicides are insignificant and range from 0.0190 ± 0.0076 mg/kg in Benzoton to 0.1004 ± 0.0569 mg/kg in Flutriafol, those with 5.0-53.6% below the maximum admissible limits (MAL), according to national and European norms.

Quite a different situation (worrying) is the concentration in samples of rapeseed flowers Glyphosate herbicide residues.

Laboratory cross-sectional data show that the concentrations of this hazardous (carcinogenic) herbicide in rapeseed flower samples are quite polluting, accounting for 0.1772 ± 0.0838 mg/kg, which exceeds the maximum admissible limit by 77.2%. This demonstrate that cultivators use this herbicide, prohibited in the EU, in large quantities, uncontrolled by the state authorities empowered to control rape in the respective field.

From the data presented above, we can conclude that rapeseed growers treat plantations with fungicides triazole, insecticide and herbicide more frequently, which can in some cases damage the conformity of the honeycomb flora for its use in organic beekeeping. In the case of

the examined site, the rapeseed is heavily polluted with the Glyphosate pesticide and cannot be used for harvesting for organic beekeeping.

Pesticide content in peas flowers (*Pisum sativum*). The results of the research showed that, out of the 62 pesticides tested, in the samples of peas flowers from the industrial land of an agricultural household in Ceadîr-Lunga (which wanted to remain confidential) were detected detectable concentrations of residues at only 7 pesticides, which constitutes 11.3% (Table 3).

Table 3. Pesticide residue content in peas flowers (N = 5), mg/kg

Name of the pesticide	MAL	M ± m	d (M-MAL)	d, % to MAL
Pyrethroid insecticide				
Cipermetrin	0.2	0.1201±0.0474	-0.0799	-39.9
Dimetilciclo-propan	0.05	0.0417±0.0122	-0.0083	-16.6
Fenoxibenzil	0.05	0.0251±0.0109	-0.0249	-49.8
Triazole fungicides				
Benzanton	0.1	0.0526±0.0312	-0.0474	-47.4
Difenoconazol	0.05	0.0494±0.0183	-0.0006	-1.2
Herbicides				
Glyphosate	0.1	0.1088±0.0435	+0.0088	+8.8
Imazamax	0.02	0.0113±0.0046	-0.0087	-43.5

Among the pesticides detected were 3 pyrethroid insecticides (Cipermetrin, Dimethylcyclopropane, Phenoxybenzyl), 2 triazole fungicides (Benzanton, Difenoconazole) and 2 herbicides (Glyphosate, Imazamax). It should be noted that the concentrations of pyrethroid insecticide residues in peas flowers are quite small, falling in the range from 0.0251 ± 0.0109 mg/kg - to Phenoxybenzyl, up to 0.1201 ± 0.0474 mg/kg - Cypermethrin.

These concentrations are well below the maximum admissible limits - with 16.6-49.8% and do not present any danger for both honey bees, bee products and human health.

The residual amounts of triazole fungicides are also insignificant, falling within the average values of 0.0494 ± 0.0183 mg/kg - for Difenoconazole and 0.0526 ± 0.0312 mg/kg - for Benzanton, being below the level the maximum admissible limits, according to EU norms with 1.2-47.4%. In fact, the residue content of the fungicide Difenoconazole in peas flower samples is at MAL risk level.

Of the residues of the detected herbicides, the concentration of the Imazamax herbicide is quite insignificant, with the value of 0.0113 ± 0.0046 mg/kg being below the maximum admissible limits with 43.5%.

At the same time, the same concern, as in rapeseed flowers, is the residue of the herbicide Glyphosate, which is in the samples of peas flowers 0.1088 ± 0.0435 mg/kg, which exceeds level the maximum admissible limits by 8.8%.

This level of Glyphosate residue concentration shows a slight pollution of peas flowers, according to the classification of pollution levels (Cebotari V. et al, 2016). Therefore, this site, which is part of the flowering peas land, is not conformity for practicing organic beekeeping.

Pesticide content in maize flowers (*Zea mays*). The results of the laboratory analyzes of maize flower samples showed that, out of the 62 pesticides tested, residues in detectable concentrations were recorded in only 9 pesticides, which constitutes 14.5%. Of these pesticides, 2 are pyrethroid insecticides (Cipermetrin, Dimethylcyclopropane), 3 neonicotinoid insecticides (Clothianidin, Imidacloprid, Tiametoxam), 2 triazole fungicides (Benzanton, Difenoconazole) and 2 are herbicides (Glyphosate, Imazamax) (Table 4).

We would like to mention that, in the overwhelming majority of pesticides, the detectable concentrations were quite low and did not exceed the maximum admissible limits, according to EU norms.

Thus, the concentrations of the pyrethroid insecticide residues were 0.1020 ± 0.0437 mg/kg - on Cipermetrin and 0.0446 ± 0.0109 mg/kg - on Dimethylcyclopropane, being below the maximum admissible limits with 49.0 and 10.8% respectively.

Similarly, the residue concentrations of the two neonicotinoid insecticides, from the three investigated, had non-polluting values, respectively 0.0080 ± 0.0025 mg/kg on Clothianidin and 0.0076 ± 0.0023 mg/kg on Imidacloprid, being below the maximum admissible limits with 20.0 and 24.0%, respectively.

Table 4. Pesticide residue content in maize flowers (N = 5), mg/kg

Name of the pesticide	MAL	M ± m	d (M-MAL)	d,% to MAL
Pyrethroid insecticide				
Cipermetrin	0.2	0.1020±0.0437	-0.098	-49.0
Dimetilciclo-propan	0.05	0.0446±0.0109	-0.0054	-10.8
Neonicotinoid insecticides				
Clothianidin	0.01	0.0080±0.0025	-0.002	-20.0
Imidacloprid	0.01	0.0076±0.0023	-0.0024	-24.0
Tiametoxam	0.01	0.0178±0.0088	+0.0078	+78.0
Triazole fungicides				
Benzanton	0.1	0.0492±0.0264	-0.0508	-50.8
Difenoconazol	0.05	0.0368±0.0101	-0.0132	-26.4
Herbicides				
Glyphosate	0.1	0.0962±0.0415	-0.0038	-3.8
Imazamax	0.02	0.0148±0.0054	-0.0052	-26.0

At the same time, one of the three dangerous neonicotinoid insecticides, namely Tiametoxam, left pollutant residues in maize flowers. Thus, the concentration of Tiametoxam residues in the flowers of this plant constituted 0.0178 ± 0.0088 mg/kg, being 78.0% higher compared to national and EU norms. Therefore, the site where the corn plantation is located is heavily polluted with residues of this dangerous pesticide.

Recall that the insecticide Tiametoxam is part of the group of neonicotinoid insecticides, which are applied in maize seed treatment plants before sowing. Being a systemic pesticide, it penetrates from the seed through the strain of the grown plant, reaching up to the leaves, flowers and newly grown corn kernels, presenting a danger for both bees that collect nectar and pollen from flowers, as well as for the health of animals and humans, who consumes corn kernels, honey and pollen from corn harvesting.

The triazole fungicide residues detected in maize flower samples were recorded at insignificant concentrations of 0.0492 ± 0.0264 mg/kg - in Benzanton and 0.0368 ± 0.0101 mg/kg - in Difenoconazole, being much higher small, compared to the maximum admissible limits of the Republic of Moldova and the EU - by 50.8 and 26.4%, respectively.

Regarding herbicide residues, the situation is similar to that recorded in peas flowers, with the exception that the Glyphosate residues were detected at risky (at the limit) concentrations of 0.0962 mg/kg, compared to the maximum admissible limit of 0.1 mg/kg, according to national and EU norms. Imazamax herbicide residues, as well as peas flowers, were recorded in concentrations of 0.0148 ± 0.0054 mg/kg, being below the maximum admissible limits with 26.0%.

CONCLUSIONS

Rapeseed flowers (*Brassica napus* ssp. *oleifera* L.) from the researched industrial land are heavily polluted with the residues of the *Glyphosate* pesticide, in a concentration of 0.1772 ± 0.0838 mg/kg, which exceeds the maximum admissible limits, according to EU and MD standards, with 77.2%.

The peas flowers (*Pisum sativum*) from the researched industrial land are slightly polluted with the residues of the Glyphosate pesticide, in a concentration of 0.1088 ± 0.0435mg/kg, and exceed the maximum admissible limits, according to EU and MD norms, by 8.8%.

The maize flowers (*Zea mays*) in the researched industrial land are heavily polluted with the residues of the neonicotinoid insecticide Tiametoxam in a concentration of 0.0178 ± 0.0088 mg/kg, which exceeds the maximum admissible limits, according to EU and MD standards, by 78.0%.

Glyphosate residues in maize flowers were detected at hazardous (at the limit) concentrations of 0.0962 mg/kg, compared to the maximum admissible limit of 0.1 mg/kg.

The sites surveyed with rapeseed, peas and maize plantations are not conformity for organic beekeeping, because the flowers of these crops are polluted with residues of dangerous pesticides, banned by the EU, which can affect the health of bees and inoffensiveness of the bee products.

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REFERENCES

Antonescu, C., Mateescu, C. (2001). Environmental pollution and its efects on honey quality. *Roman. Biotehnology. Lett*., 6, 371-379.

Bogdanov, S., et al. (1999). Influence of organic acids and components of essential oils on honey taste. *Am. Bee Journal*., 139, 61-63.

Buruian, V. (2011). Tehnici cromatografice pentru determinarea contaminanţilor din mierea de albine/Chromatographic techniques for the determination of contaminants in honey. Autoref. Tezei de dr. în şt. agricole şi med. Veterinară, Cluj-Napoca, 56 p.

Cebotari, V., Buzu, I., Postolachi, O. (2019). Pesticide residues in melliferous flowers from siteswith diverse anthropic impact. *International Scientific Symposium „Modern animal husbandry - food safety and durable development" at the University of Agricultural Sciences and Veterinary Medicine of Iasi. Scientific papers. Animal Science,* 71(24), 123-131.

Cebotari, V., Buzu, I., Gliga, O., Postolachi, O., Granciuc, N. (2018). Content of pesticide residues in the flowers of the acacia and linden trees from the Moldavian Codri area. *Scientific papers. Series D. Animal Science,* LXI(2), 235-242.

Cebotari, V., Gliga, O., Buzu I. (2016). Conţinutul unor metale grele în polenul colectat dealbinele melifere şi legătura acestuia cu factorii mediului ambiant/The content of heavy metals in the pollen collected by honey bees and its connection with environmental factors. *Revista Universităţii de Stat din Moldova „Studia Universitatis Moldaviae", seria „Ştiinţe Reale şi ale Naturii*", Chişinău, 1(91), 32-42.

Commision Regulation (EC) No. 834/2009 implementing Regulation (EC) No.716/2007 of the European Parliament and of the Council on Community statistic son the structure and activity offoreign affiliates, as regards the quality reports. JO al UE, L241, 3-107.

Commision Regulation (EC) No. 271/2010 amending Regulation (CE) nr. 889/2008 laying down detailed rules for the implementation of Council Regulation (EC) No. 834/2007, as regards the organic production logo of the European Union. JO al UE, L84, 19-25.

Government of the Republic of Moldova, Decision no. 567 of 16.07.2014 regarding the approval of the National Program for monitoring pesticide residues and nitrate content in food of plant origin for the years 2015-2020. Monitorul Oficial al Republicii Moldova, 209-216, art. 631.

Lazări, I., et al. (2000). Metode de determinare a reziduurilor pesticidelor în produsele alimentare, furajere şi mediul înconjurător/Methods for the determination of pesticide residues in food, feed and the environment, Chişinău, MD: Chişinău Publishing House, vol. I-II.

Sanitary-veterinary rule regarding the taking of official samples from live animals and animal products. Hotărârea Guvernului Republicii Moldova/Decision of the Government of the Republic of Moldova nr. 782/01.09.2010.

National plan for monitoring pesticide residues in fruit, vegetables and cereals (from domestic production), in accordance with Regulation (EC) No 882/2004 396/2005, for the year 2019. www.madr.ro /docs /fitosanitar/2019/plan.

National Program for monitoring pesticide residues and nitrate content in plant foods for the years 2015-2020. www.justice.gov.md./NPNAL/ PHG- program-reziduuri-pesticide.

Presentation of the MIEPO Honey Export Study to France". Saxofone ConferenceRoom. http://miepo.md/.

Laboratory Report for the Control of Pesticide Residues in Plants and Plant Products. www.madr.ro/docs/fitosanitar/raport-monitorizare-reziduuri.

Report on the implementation of the National Program for monitoring pesticide residues and nitrate content in plant foods for 2016. http://ansa.gov.md/uploads/files/Ordinele%20ANSA/Rapoarte/Raport%20realizarea%20HG%.

Sanitary regulation on the maximum permitted residue limits of phytosanitary products from or on food and feed of plant and animal origin, approved by Government Decision of the Republic of Moldova nr. 1191/23.12.2010.

Regulation (EC) no 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32005R0396&from=RO

Commission Regulation (EU) 2019/1792 of 17 October 2019 amending Annexes II, III and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for amitrole, fipronil, flupyrsulfuron-methyl, imazosulfuron, isoproturon, orthosulfamuron and triasulfuron in or on certain products. https://lege5.ro/Gratuit/gmzdmnzrha2a/ regulamentul-nr-552-2019-de-modificare-a-anexelor-ii-si-iii-la-regulamentul-ce-nr-396-2005.

Commission Regulation (EU) 2017/978 of 9 June 2017 amending Annexes II, III and V to Regulation (EC) No. Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue limits for fluopyram; hexaclorcyclohexane (HCH), alpha isomer; hexachlorocyclohexane (HCH), beta isomer; hexachlorocyclohexane (HCH), sum of isomers, except for the gamma isomer; lindane [hexachlorocyclohexane (HCH), gamma isomer]; nicotine and profenophos from or on certain products, https://eur-lex.europa.eu/legal-content/ RO/TXT/?uri.

Плохинский Н.А. (1989). *Руководство по биометрии для зоотехников*. Изд. «Колос», Москва, 256 с.

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STUDIES ON THE FACTORS WHICH INFLUENCE THE CHEMICAL COMPOSITION OF MEAT FROM THE CHICKEN BROILER

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Abstract

In view of the importance that current consumers attach to the quality of the purchased foods, the paper presents the results regarding how the age of slaughter and the type of biological material influence the chemical composition of the meat in the chicken broiler. In this regard, three hen-meat hybrids (one industrial type: Ross-308 and two slow-growing: Hubbard and HB Color) were studied under identical conditions according to the principles of slow growth and slaughtered at age different (63 and 81 days respectively). Chemical analyzes were performed on samples taken from the muscles of the pulp and chest, in equal proportions. The data obtained showed an increase of 0.90% in the proportion of dry matter of meat in specimens slaughtered at 81 days, but also of lipids (by 0.49%) and proteins (by 0.32%). Of the hybrids tested, Hubbard achieved the highest increases in dry matter (1.06%) and protein (0.44%) and lowest increases in lipids (only 0.46%). The conclusion of the study was that, under slow growth conditions, the Hubbard hybrid slaughtered at 81 days of age offers meat with superior chemical characteristics.

Key words*: broiler, chemical composition, meat, slow growing.*

INTRODUCTION

In the consumer market there has always been a high and constant demand for poultry meat (Magdelaine et al, 2008), characterized by a higher biological value given the presence of a large number of essential, but also non-essential amino acids, high content in essential unsaturated fatty acids, especially omega 3 fatty acids and lack of trans fats responsible for the onset of heart disease (Fletcher, 2002).

This state of affairs required the modification of the technologies of bird breeding, from the semi-intensive, to the intensive and then to the industrial growth (Usturoi, 2008), but also by improving the performances of the biological material used so that in the last decades it has tripled the weight of the chicken at 6 weeks, increased by 1.7 times the yield at slaughter, and the feed conversion rate was reduced by half (Dal Bosco et al., 2014).

The continuous decrease of the slaughter age and the improvement of the feed conversion rate (Custură et al., 2011) have led to the economic return of the chicken broiler growth, so the production of poultry meat on a superintensive basis has become a common practice worldwide (Radu and Popescu-Micloşanu, 2012).

Although poultry meat obtained in the industrial system quantitatively satisfies the existing demands (Vukasovic, 2014; Preisinger, 2005), its quality is increasingly questioned, due to its high water content, its less obvious taste, its exaggerated fragility of muscle fibers. etc (Hall and Sandilands, 2007; Husak et al., 2008).

In this context, a distinct segment of consumers has emerged (Hamon, 2010) which requires meat from poultry with low development and late slaughter, fed with cereal mixtures and raised on small farms and with access to the environment (Castellini et al., 2008; Wang et al., 2009; Almasi et al., 2015; Stadig et al., 2016; Popova et al., 2017).

These social factors have led to the emergence of diversified technologies for raising broilers, among which "Label Rouge" technology, ecological technology (Castellini et al., 2002; Dong-Hun et al., 2009; Castellini et al., 2016), different variants of "Certified chickens" with specific provisions for obtaining (Tudorache et al., 2011).

Starting from the aforementioned considerations, it was considered appropriate to

conduct a study regarding the degree of influence of the hybrid and the age of slaughter on the chemical composition of the obtained meat, under the conditions of the slow growth application.

MATERIALS AND METHODS

In order to achieve the proposed purpose, chicken hybrids for meat were studied in which the principles of slow growth were applied, in halls with controlled environment and with access to the external environment; all chicks had the same breeding conditions and were fed the same types of fodder combined.

The experimental factors were represented by the biological material used (three hens hybrids, of which one industrial type: Ross-308 and two slow-growing: Hubbard and HB Color) and the age at which they were sacrificed (63 days and, respectively, 81 days). After slaughtering the chickens from the two series of experience, the meat samples were taken from the muscles of the pulp and chest, from which a common sample (equal proportions of the two types of muscles) was performed, on which the analyzes related to the chemical composition of the meat. The determinations were carried out in accordance with national standards and aimed at:

- water content - by drying method (SR ISO 1442: 2010), which involves exposing a meat sample to a heat source up to constant weight; weight loss, calculated as a percentage, represents the water content;
- the content in the total dry substance - was calculated by difference, according to the relation: DM = 100-water;
- total protein content - by the Kjeldahl method (SR ISO 937: 2007), based on the following principle: the product subjected to analysis in the presence of sulfuric acid and a catalyst, is decomposed by heat into the constituent elements: C, H, O, P, Fe. Following the breakdown of proteins and other nitrogen compounds, ammonium ions are released, which is combined with sulfuric acid to form ammonium bisulphate. Ammonium bisulphate from mineralized by strong alkalization releases ammonia, which is distilled and captured in an acid solution. Knowing the amount of acid needed to neutralize the distilled ammonia, the amount of nitrogen in the sample is calculated;
- the total fat content - was determined by the Soxhlet method (SR ISO 1444: 2008), in an extractor for quantitative separation of fatty substances from a mixture using an organic solvent; after removal of the solvent, it is weighed and expressed as a percentage;
- the content in mineral substances - represents the residue obtained after calcining the sample at 525 ± 25°C, up to a constant weight (SR ISO 936: 2009);
- meat caloricity - was calculated by calculation, according to the Weende scheme: EB (kcal/kg) = 5.7 kcal x g protein + 9.5 kcal x g lipids x 4.2 kcal x g UES).

RESULTS AND DISCUSSIONS

1. **Chemical composition of meat in chicken broiler slaughtered at 63 days of age.** The determination of the water content of the meat studied revealed that the lowest value was in the Hubbard chicks (69.02 ± 12.29%), followed by that of the HB Color chickens (70.91 ± 12.34%) and Ross-308 chicken meat with the highest water content (71.69 ± 12.79%); from a statistical point of view, significant differences were identified between groups Lexp-1 and Lexp-2, and between groups Lc-1 and Lexp-1 distinctly significant differences.

For the dry matter content, the same types of statistical differences were found, respectively significant between Lexp-1 and Lexp-2 and distinctly significant between Lc-1 and Lexp-1, against values of 30.98 ± 6.63% in Hubbard meat, 29.09 ± 6.56% in HB Color and only 28.31 ± 6.53% in Ross-308 chicken meat (Table 1).

Protein content ranged between 19.56 ± 4.22% (Ross-308 meat) and 20.64 ± 4.31% (Hubbard meat), with statistically significant differences between Lc-1 and Lexp groups. -1 and respectively, between lots Lexp-1 and Lexp-2.

Lipid levels were lower in Ross-308 meat (6.63 ± 1.44%) and higher in Hubbard (7.32 ± 1.51%) and HB Color (7.64 ± 1.48%)), so that between the control group and the experimental groups significant statistical differences were identified.

The content in mineral substances ranged from 1.09 ± 0.10% (Ross-308 meat) to 1.36 ± 0.11%

(Hubbard meat), and that in unaccounted extractive substances between 0.65 ± 0, 02% (HB Color) and 1.66 ± 0.11% (Hubbard); if for statistical substances no statistical differences were reported between groups, for SEN significant statistical differences between experimental groups (Lexp-1 and Lexp-2) were identified.

Caloricity of meat correlated with its content in protein and lipids, being at levels of only 178.81 ± 22.6 kcal/100 g in the case of Ross-308 chickens, of 194.16 ± 22.27 kcal/100 g at Hubbard and 187.49 ± 22.81 kcal/100 g at HB Color; Between the Lc-1 and Lexp-1 groups, distinctly significant statistical differences were identified, and in the comparisons of Lc-1 vs. Lexp-2 and Lexp-1, respectively. Lexp-2 only significant differences.

All the analyzed characteristics showed a good homogeneity, except for the fat content, where the calculated values for the coefficient of variation (V% = 12.59-15.06) show a medium variability (Table 1).

Table 1. Meat chemical composition of hens hybrid slaughtered at 63 days

Parameters	Lots	Statistical estimators (n = 10)			
		$\bar{X} \pm s_{\bar{x}}$	V %	Min.	Max.
Water (%)	Lc-1 (Ross-308)	71.69±12.79	7.58	68.16	73.40
	Lexp-1 (Hubbard)	69.02±12.29	6.62	67.59	71.40
	Lexp-2 (HB Color)	70.91±12.34	5.09	69.45	71.48
	Meaning of differences	Lc-1 vs Lexp-1: Fα0.001 (15.38)> $\hat{F}$ (14.39)> Fα0.01 (8.29) at 1; 18 GL **(**)** Lc-1 vs Lexp-2: $\hat{F}$ (0.92) < Fα0.05 (4.41) at 1;18 GL **(NS)** Lexp-1 vs Lexp-2: Fα0.01 (8.29) > $\hat{F}$ (7.02) > Fα0.05 (4.41) at 1; 18 GL **(*)**			
Dry matter (%)	Lc-1 (Ross-308)	28.31±6.53	7.89	26.90	31.50
	Lexp-1 (Hubbard)	30.98±6.63	6.65	29.00	33.50
	Lexp-2 (HB Color)	29.09±6.56	5.23	28.50	32.00
	Meaning of differences	Lc-1 vs Lexp-1: Fα0.001 (15.38)> $\hat{F}$ (14.81)> Fα0.01 (8.29) at 1; 18 GL **(**)** Lc-1 vs Lexp-2: $\hat{F}$ (2.92) < Fα0.05 (4.41) at 1;18 GL **(NS)** Lexp-1 vs Lexp-2: Fα0.01 (8.29) > $\hat{F}$ (6.91) > Fα0.05 (4.41) at 1; 18 GL **(*)**			
Protein (%)	Lc-1 (Ross-308)	19.56±4.22	6.44	18.40	20.67
	Lexp-1 (Hubbard)	20.64±4.31	7.50	18.90	21.62
	Lexp-2(HB Color)	19.68±4.29	6.24	18.17	20.65
	Meaning of differences	Lc-1 vs Lexp-1: Fα0.01 (8.29) > $\hat{F}$ (5.17) > Fα0.05 (4.41) at 1; 18 GL **(*)** Lc-1 vs Lexp-2: $\hat{F}$ (0.52) < Fα0.05 (4.41) at 1;18 GL **(NS)** Lexp-1 vs Lexp-2: Fα0.01 (8.29) > $\hat{F}$ (4.94) > Fα0.05 (4.41) at 1; 18 GL **(*)**			
Lipids (%)	Lc-1 (Ross-308)	6.63±1.44	12.59	4.26	9.67
	Lexp-1 (Hubbard)	7.32±1.51	15.06	5.11	11.20
	Lexp-2(HB Color)	7.64±1.48	14.34	6.03	10.70
	Meaning of differences	Lc-1 vs Lexp-1: Fα0.01 (8.29) > $\hat{F}$ (5.02) > Fα0.05 (4.41) at 1; 18 GL **(*)** Lc-1 vs Lexp-2: Fα0.01 (8.29) > $\hat{F}$ (7.98) > Fα0.05 (4.41) at 1; 18 GL **(*)** Lexp-1 vs Lexp-2: $\hat{F}$ (3.24) < Fα0.05 (4.41) at 1;18 GL **(NS)**			
Ash (%)	Lc-1 (Ross-308)	1.09±0.10	2.98	1.07	1.15
	Lexp-1 (Hubbard)	1.36±0.11	8.24	1.21	1.80
	Lexp-2(HB Color)	1.11±0.09	9.60	1.00	1.78
	Meaning of differences	Lc-1 vs Lexp-1: $\hat{F}$ (1.11) < Fα0.05 (4.41) at 1; 18 GL **(NS)** Lc-1 vs Lexp-2: $\hat{F}$ (0.14) < Fα0.05 (4.41) at 1; 18 GL **(NS)** Lexp-1 vs Lexp-2: $\hat{F}$ (1.09) < Fα0.05 (4.41) at 1; 18 GL **(NS)**			
UES (%) (unclaimed extractive substances)	Lc-1 (Ross-308)	1.03±0.10	9.90	0.06	1.67
	Lexp-1 (Hubbard)	1.66±0.11	7.06	0.94	2.01
	Lexp-2(HB Color)	0.65±0.02	6.52	0.29	0.94
	Meaning of differences	Lc-1 vs Lexp-1: $\hat{F}$ (3.11) < Fα0.05 (4.41) at 1; 18 GL **(NS)** Lc-1 vs Lexp-2: $\hat{F}$ (1.14) < Fα0.05 (4.41) at 1; 18 GL **(NS)** Lexp-1 vs Lexp-2: Fα0.01 (8.29) > $\hat{F}$ (4.59) > Fα0.05 (4.41) at 1; 18 GL **(*)**			
Caloric value (kcal/100 g)	Lc-1 (Ross-308)	178.81±22.64	3.58	175.0	180.1
	Lexp-1 (Hubbard)	194.16±22.27	9.92	177.0	213.2
	Lexp-2(HB Color)	187.49±2281	342	1845	1914
	Meaning of differences	Lc-1 vs Lexp-1: Fα0.001 (15.38)> $\hat{F}$ (14.93)> Fα0.01 (8.29) at 1; 18 GL **(**)** Lc-1 vs Lexp-2: Fα0.01 (8.29) > $\hat{F}$ (7.75) > Fα0.05 (4.41) at 1; 18 GL **(*)** Lexp-1 vs Lexp-2: Fα0.01 (8.29) > $\hat{F}$ (6.84) > Fα0.05 (4.41) at 1; 18 GL **(*)**			

2. The chemical composition of the meat in the chicken broiler slaughtered at the age of 81 days. Following the determinations made, a 10.98%) and in Hubbard (67.96 ± 10.57%), hence the significant differences between the two experimental and respective groups, distinctly significant between Lc-2 and Lexp-3. Correspondingly, Ross-308 meat had a lower dry content (28.97 ± 7.02%), compared to HB Color (30.07 ± 7.42%) and Hubbard (32.04 ± 7.73%); The same statistical differences were maintained between groups, is significant between Lexp-3 and Lexp-4 groups and distinctly significant between Lc-2 and Lexp-3. higher value of the water content was obtained in the meat of Ross-308 chickens (71.03 ± 11.73%) and lower in HB Color (69.93 ± From the protein point of view, the meat obtained from the Ross-308 hybrid had the lowest content, of only 19.74 ± 3.97%, followed by that of the HB Color hybrid with 20.01 ± 4.07% and Hubbard with 21.08 ± 4.29% protein. Differences with statistical significance between the Lexp-3 group and the Lc-2 and the Lexp-4 groups respectively (Table 2) were obtained from the statistical analysis.

Table 2. Meat chemical composition of hens hybrid slaughtered at 81 days

Parameters	Lots	Statistical estimators (n = 10)			
		$\bar{X} \pm s_{\bar{x}}$	V %	Min	Max
Water (%)	Lc-2 (Ross-308)	71.03±11.73	5.86	68.6	72.7
	Lexp-3 (Hubbard)	67.96±10.57	4.94	66.7	69.6
	Lexp-4 (HB Color)	69.93±10.98	2.48	68.4	70.1
	Meaning of differences	Lc-2 vs Lexp-3: Fα0.001 (15.38)> $\hat{F}$ (13.94)> Fα0.01 (8.29) at 1; 18 GL **(**)** Lc-2 vs Lexp-4: $\hat{F}$ (1.11) < Fα0.05 (4.41) at 1;18 GL **(NS)** Lexp-3 vs Lexp-4: Fα0.01 (8.29) > $\hat{F}$ (7.56) > Fα0.05 (4.41) at 1; 18 GL **(*)**			
Dry matter (%)	Lc-2 (Ross-308)	28.97±7.02	7.65	25.6	33.3
	Lexp-3 (Hubbard)	32.04±7.73	8.15	26.1	34.7
	Lexp-4 (HB Color)	30.07±7.42	6.25	27.4	31.5
	Meaning of differences	Lc-2 vs Lexp-3: Fα0.001 (15.38)> $\hat{F}$ (13.87)> Fα0.01 (8.29) at 1; 18 GL **(**)** Lc-2 vs Lexp-4: $\hat{F}$ (1.09) < Fα0.05 (4.41) at 1;18 GL **(NS)** Lexp-3 vs Lexp-4: Fα0.01 (8.29) > $\hat{F}$ (7.13) > Fα0.05 (4.41) at 1; 18 GL **(*)**			
Protein (%)	Lc-2 (Ross-308)	19.74±3.97	6.90	17.1	23.0
	Lexp-3 (Hubbard)	21.08±4.29	7.99	18.6	24.1
	Lexp-4 (HB Color)	20.01±4.07	7.81	18.8	23.5
	Meaning of differences	Lc-2 vs Lexp-3: Fα0.01 (8.29) > $\hat{F}$ (6.68) > Fα0.05 (4.41) at 1; 18 GL **(*)** Lc-2 vs Lexp-4: $\hat{F}$ (0.61) < Fα0.05 (4.41) at 1;18 GL **(NS)** Lexp-3 vs Lexp-4: Fα0.01 (8.29) > $\hat{F}$ (4.73) > Fα0.05 (4.41) at 1; 18 GL **(*)**			
Lipids (%)	Lc-2 (Ross-308)	7.17±1.90	8.45	5.4	10.0
	Lexp-3 (Hubbard)	7.78±1.97	7.01	6.0	9.8
	Lexp-4 (HB Color)	8.12±2.04	8.28	6.7	11.0
	Meaning of differences	Lc-2 vs Lexp-3: Fα0.01 (8.29) > $\hat{F}$ (6.53) > Fα0.05 (4.41) at 1; 18 GL **(*)** Lc-2 vs Lexp-4: Fα0.01 (8.29) > $\hat{F}$ (6.87) > Fα0.05 (4.41) at 1; 18 GL **(*)** Lexp-3 vs Lexp-4: $\hat{F}$ (1.02) < Fα0.05 (4.41) at 1;18 GL **(NS)**			
Ash (%)	Lc-2 (Ross-308)	1.06±0.08	5.59	0.8	1.5
	Lexp-3 (Hubbard)	1.30±0.19	9.10	1.1	2.1
	Lexp-4 (HB Color)	1.12±0.12	6.05	0.9	1.7
	Meaning of differences	Lc-2 vs Lexp-3: $\hat{F}$ (0.98) < Fα0.05 (4.41) at 1; 18 GL **(NS)** Lc-2 vs Lexp-4: $\hat{F}$ (0.09) < Fα0.05 (4.41) at 1; 18 GL **(NS)** Lexp-3 vs Lexp-4: $\hat{F}$ (0.57) < Fα0.05 (4.41) at 1; 18 GL **(NS)**			
UES (%) (unclaimed extractive substances)	Lc-2 (Ross-308)	1.00±0.13	7.99	0.7	3.1
	Lexp-3 (Hubbard)	1.88±0.11	6.46	0.7	2.9
	Lexp-4 (HB Color)	0.82±0.06	6.39	0.3	1.8
	Meaning of differences	Lc-2 vs Lexp-3: $\hat{F}$ (1.11) < Fα0.05 (4.41) at 1;18 GL **(NS)** Lc-2 vs Lexp-4: Fα0.01 (8.29) > $\hat{F}$ (5.15) > Fα0.05 (4.41) at 1;18 GL **(*)** Lexp-3 vs Lexp-4: Fα0.01 (8.29) > $\hat{F}$ (5.01) > Fα0.05 (4.41) at 1;18 GL **(*)**			
Caloric value (kcal/100 g)	Lc-2 (Ross-308)	184.84±22.58	7.91	173.5	192.1
	Lexp-3 (Hubbard)	201.97±23.61	9.36	197.7	211.0
	Lexp-4 (HB Color)	194.64±23.90	8.99	182.5	205.8
	Meaning of differences	Lc-2 vs Lexp-3: Fα0.001 (15.38)> $\hat{F}$ (14.98)> Fα0.01 (8.29) at 1; 18 GL **(**)** Lc-2 vs Lexp-4: Fα0.01 (8.29) > $\hat{F}$ (7.59) > Fα0.05 (4.41) at 1; 18 GL **(*)** Lexp-3 vs Lexp-4: Fα0.01 (8.29) > $\hat{F}$ (6.88) > Fα0.05 (4.41) at 1; 18 GL **(*)**			

CONCLUSIONS

From the data regarding the water content of the meat it resulted that it reduced, on average, by 0.90% by increasing the age of slaughter of the birds, but in a greater proportion to the hybrids created for the slow growth (by 1.06% at Hubbard and 0.98% at HB Color) and lower at the Ross-308 industrial hybrid (0.66%).

The slaughter of birds at an older age also influenced the meat content in protein (higher by 0.32%) and lipids (higher by 0.49%), but with differences printed by the biological material tested; thus, at Ross-308 the lowest quantitative increase in protein (by 0.18%) and the highest for lipids (by 0.54%), while in the Hubbard chickens the situation was reversed in the sense that they had the highest increase in protein content (0.44%) and the lowest for lipids (0.46%).

Meat composition in mineral substances was within normal limits, with values slightly higher at Hubbard (1.30-1.36%) and lower at Ross-308 (1.06-1.09%), which is valid for non-protein nitrogenous substances for which levels of 0.65-1.88% were found.

The conclusion of our study was that the chicken broiler subjected to slow growth must be slaughtered at 81 days, an age that allows obtaining a higher meat in the aspect of chemical composition; Of the hybrids tested, Hubbard provided the best chemical parameters of the meat and, together with the superior production results, qualifies it for slow growth.

REFERENCES

Almasi, A., Andrassyne, B.G., Milisits, G., Kustosne, P.O., Suto, Z. (2015). Effects of different rearing systems on muscle and meat quality traits of slow- and medium-growing male chickens. *Brit. Poultry Sci*., 56, 320–324.

Castellini, C., Mugnai, C., Dal Bosco, A. (2002). Meat quality of three chicken genotypes reared according to the organic system. *Italian journal of food science*, 14 (4), 401–412.

Castellini, C., Berri, C., Le Bihan-Duval, E., Martino, G. (2008). Qualitative attributes and consumer perception of organic and free-range poultry meat. World's *Poultry Science Journal*, 64, 500–512.

Castellini, C., Mugnai, C., Moscati, L., Mattioli, S., Amato, M., Mancinelli, A., Dal Bosco, A. (2016). Adaptation to organic rearing system of eight different chicken genotypes: behaviour, welfare and performance. *Italian journal of animal science*, 15(1), 37–46.

Custură, I., Van, I., Tudorache, M., Popescu-Micloşanu, E., Pârvuleţ, C. (2011). Researches about influence of feed paramethers on slaughtering performances in free-range chickens. *Scientific papers. Series D. Animal science*, LIV, 289–293.

Dal Bosco, A., Mugnai, C., Amato, M., Piottoli, L., Cartoni, A., Castellini, C. (2014). Effect of slaughtering age in different commercial chicken genotypes reared according to the organic system: 1. Welfare, carcass and meat traits. *Italian journal of animal science*, 13(2), 2042–2050.

Dong-Hun, K., Soo-Hyun, C., Jin-Hyoung, K., Pil-Nam, S., Jong-Moon, L., Cheorun, J., Dong-Gyun, L. (2009). Comparison of the Quality of the Chicken Breasts from Organically and Conventionally Reared Chickens. *Korean Journal Food Science Animals Resources*, 29(4), 409–414.

Fletcher, D.L. (2002). Poultry meat quality. *World's Poultry Science Journal*, 58, 131–145.

Hall, C., Sandilands, V. (2007). Public attitudes to the welfare of broiler chickens. *Animal Welfare*, 16, 499–512.

Hamon, J.F. (2010). The alternative broiler market: history, evolution and future. *Book of abstracts XIIIth European Poultry Conference*, Tours, France, 579.

Husak, R.L., Sebranek, J.G., Bregendahl, K. (2008). A survey of commercially available broilers marketed as organic, free-range, and conventional broilers for cooked meat yields, meat composition, and relative value. *Poultry Science*, 87, 2367–2376.

Magdelaine, P., Spiess, M.P., Valceschini, E. (2008). Poultry meat consumption trends in Europe. *World's Poultry Science Journal*, 64(1), 53–64.

Popova, T., Ignatova, M., Petkov, E. (2017). Carcass quality and meat chemical composition in two lines of slow growing chickens and their crosses. *Proc. VIII International Agricultural Symposium "AGROSYM",* 2133–2139.

Preisinger, R. (2005). Development, state and perspectives of poultry production. *Zuchtungskunde*, 77, 502–507.

Radu, C. V., Popescu-Micloşanu, E. (2012). Comparative study about production and slaughtering performances in an industrial company with ROSS 308 standard chicken hybrid. *Scientific papers. Series D. Animal science*, LV, 214–219.

Stadig, L.M., Rodenburg, T.B., Reubens, B., Aerts, J., Duquenne, B., Tuyttens, F.A.M. (2016). Effects of free-range access on production parameters and meat quality, composition and taste in slow-growing broiler chickens. *Poultry Science*, 95, 2971–2978.

Tudorache, M., Van, I., Custură, I., Popescu-Micloşanu, E., Popa, A. (2012). Study on unit cost of certificate-type broilers. *Scientific papers, Series D, Animal science*, LV, 250–255.

Usturoi, M.G. (2008). *Birds growth/Creşterea păsărilor*. Iaşi, RO: Ion Ionescu de la Brad Publishing House.

Vukasovic, T. (2014). European meat market trends and consumer preference for poultry meat in buying decision making process. *Worlds Poultry Science Journal*, 70(2), 289–301.

Wang, K.H., Shi, S.R., Dou, T.C., Sun, H.J. (2009). Effect of a free-range raising system on growth performance, carcass yield, and meat quality of slow-growing chicken. *Poultry Science*, 88, 2219–2223.

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SENSORIAL EVALUATION OF NEW DEVELOPED BISCUITS ENRICHED WITH ORGANIC APPLE AND BASIL POWDERS: PRELIMINARY STUDY

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Abstract

The purpose of the study is to harness ingredients obtained from organic farming, in order to develop a new type of biscuits. Based on the original recipe, these were obtained from seeds and hemp flour and enriched with lyophilized organic apple (pieces and powder) and lyophilized organic basil powder. The organic powders were realized in the framework of the SusOrgPlus project at the Research Centre for Studies of Food Quality and Agricultural Products, and biscuits were obtained in the Bakery Pilot Station, both of University of Agronomic Sciences and Veterinary Medicine of Bucharest. The consumer acceptance testing was performed on 33 consumers and consists of the evaluation of general appearance, colour, taste, aroma, and consistency (Hedonic 5-point scale). The obtained results showed that the new biscuits enriched with lyophilized apple and basil powders were easily accepted by the consumers.

Key words*: added value, consumer acceptance, hedonic scale, new product, taste*

INTRODUCTION

Organic farming represents the most important alternative to intensive farming, because of its role to protect health and well-being of current and future generations and the environment. According to this principle of organic farming, the organic market increase year by year, all over the world, including in Romania, being characterized by diversity and constantly increasing consumers' demands (Sărăcin and Vasile, 2015). Organic farming creates more job opportunities either for members of the family or for employed personnel, in all production domains: vegetables, fruits, legumes, animals, eggs or milk, as well as for processed products for human consumption (Koufiotis et al., 2016).

Fruits and vegetables are extensively processed and the residues are often discarded. However, due to their rich composition, they could be used to minimize food waste, as value added by-products, with functional properties and in the role of antioxidants (Fereira et al., 2015; Catană et al., 2018; Rocchetti et al., 2018).

Through the development of intelligent drying processes of food, could be obtain, using raw materials from organic agriculture, high nutritional value ingredients, which can be used as natural additives (Dragomir et al., 2017). In this way, it will be extend the life of the product in the food chain and reduce the impact on the environment. In our days the food technology uses different agents of food improvement. Among them, the consumer is familiar with food additives (Zugravu et al., 2017). The collaboration between food technologist and additive producers is necessary for the optimization and development of networks and technologies, meeting the demands and high expectations of consumers (Bahaciu et al., 2019). In the following, we developed a biscuit recipe that was improved by using organic additives, obtained by controlled and gentle drying procedures, which preserve the initial characteristics of the ingredient.

In the present work, for the study, we used organic products, in the form of natural additives, respectively lyophilized apple pieces,

lyophilized apple powder and lyophilized basil powder.

The organic ingredients used were obtained at the Research Center for Studies of Food Quality and Agricultural Products from USAMV Bucharest, within the SusOrgPlus project: Intelligent food processing chains, natural additives and colourants, which aims to develop advanced processing technologies for organic products and by-products (Bujor and Bădulescu, 2019). The aim of the study is to use of these organic ingredients in a new biscuit recipe, the qualitative and sensory evaluation of the obtained products; and the analysis of the degree of consumer acceptance for the organic food products enriched with food additives and natural dyes.

Biscuits enriched with lyophilized apple powder and lyophilized basil powder, are based on fat dough, in which the classic wheat flour was partially replaced with hemp flour and hemp seeds husked (Dragomir and Nicolae, 2019; Dragomir et al., 2019). The apple powder is obtained from apples, organic Gala variety, which was dehydrated by the lyophilisation process. Apple powder it is an important source of polyphenols with high antioxidant capacity. Its presence in the recipe, balances the taste and aroma of the finished product, and the pieces of lyophilized apple give a pleasant texture and aroma to the product (Bădulescu et al., 2019).

Basil (*Ocimum basilicum* L.) belongs to aromatic plants due to their volatile compounds presented especially in leaves and flowering tops. These basil parts are used since antiquity for food preservation, flavouring, and as medicine, because of high antioxidant, antibacterial and antifungal activity of volatile oils, being good sources of natural antimicrobial and antioxidant agents, with possible application in food industry, cosmetics or medicine (Avetisyan et al., 2017).

By lyophilised basil retains the characteristics intense colour and flavour. Lyophilised basil powder is aromatic, slightly sweet, with spicy notes in taste. Because, it has a great capacity to rehydrate in the presence of water from the dough, the original character, such as the taste, colour and aroma specific to the basil, is present in the new preparation. Added the powder from the lyophilized basil aromatizes to the dough balances the taste and increases the preservation of final product. According to Złotek (2018), cakes enriched with basil (both control and elicited) may be used in human diet (also by diabetic patients) as functional foods. Husked hemp seeds and hemp flour are added for texture, taste, colour, high protein and fibber content, as well as high nutritional value (William et al., 2019; Di Cairano et al., 2018).

The use of husked hemp seeds and hemp flour, as the raw material, is considered promising. At the global level, there is a real interest in this culture, not only as a point of interest for private enterprises, but it is also the focus of large government programs (Lukin and Bitiutskikh, 2017).

MATERIALS AND METHODS

Recipes development and organic food products made

The technology of obtaining the products has been adapted according to the behaviour of the food additives and the natural organic dyes that are the object of the study.

Materials used in our study included:

- lyophilized organic apple (pieces and powder);
- lyophilized organic basil powder;
- hemp flour and husked hemp seeds.

The obtained products were tested in three repetitions until the best recipe was obtained.

All products were purchased from retail specialty stores with organic products, except for organic apple and basil powders, which were realized in the framework of the SusOrgPlus project at the Research Centre for Studies of Food Quality and Agricultural Products, from USAMV Bucharest.

The product was made in the Bakery Pilot Station of the Faculty of Animal Productions Engineering and Management, from USAMV Bucharest, within the SusOrgPlus project support.

The biscuits are obtained using a basic recipe that is used as a test control (ingredients: butter, wheat flour, unfinished brown sugar, hemp protein flour, husked hemp seeds, yolk, salt, baking powder). Biscuits obtained by adding organic lyophilized apple in the form of pieces and powder, lyophilized basil powder were used for the test sample.

Sensorial evaluation

The sensorial evaluation of biscuits was carried out in order to observe the impact of organic apple and basil powders incorporation, on its sensory characteristics.

The sensorial evaluation was conducted in three parts. A research group, consisting of five members, evaluated the biscuits to determine if the product was viable. A positive response warranted further testing with a small group of consumers to obtain quantitative data regarding acceptability and attribute analysis.

A sensory evaluation for consumer acceptance testing was performed on another two consumer groups, of un-trained panellists, using a 5-point Hedonic scale (scale: 1-dislike extremely; 2-dislike slightly; 3-neither like nor dislike; 4-like slightly; 5-like extremely) to determine the level of acceptance of biscuits enriched with organic apple and basil powders (Spence, 2016; 2018).

Consumer acceptance testing was performed in the Sensory Analysis Laboratory of the Research Center for Studies of Food Quality and Agricultural Products, USAMV Bucharest.

RESULTS AND DISCUSSIONS

Recipes development and organic food products made

For the biscuits enriched with organic apple powder and basil powders, there were used the following ingredients: butter, wheat flour, unrefined brown sugar, hemp protein flour, husked hemp seeds, yolk, salt, baking powder, 0.5% lyophilized apple pieces and 0.15% lyophilized apple powder, and 0.1% lyophilized basil powder. It was obtained, a healthy organic biscuit, with specific sensorial characteristics, and with high nutritive value.

The technology of fat-based dough includes the following stages: raw ingredients preparation for production; emulsion preparation, dough kneading, forming the pieces dough, and baking the finished product.

The production technology involved the following steps:

- The emulsion preparation: the ingredients are placed in a mixing bowl: unrefined sugar, butter, yolk, salt, and organic powders. The yolks together with lyophilized apple (pieces and powder) and lyophilized basil powder are mixed and left to rest, so that the organic powders hydrate and incorporate more easily into the dough. The ingredients are mixed, in the planetary mixer, for 15 minutes until the mixture reaches an even structure, and the mixture is creamy and fluffy.
- The dough kneading: to the mixture are added the husked hemp seeds and hemp flour, and the baking powder. The blending is done for 5 minutes. It is recommended that hemp flour be mixed with other flours in a proportion of maximum 20%, and the products will have a more pronounced taste of hemp, a brown colour.
- Forming the pieces dough: from the resulted composition is made a roll, which is wrapped in baking paper and put at cold for 10 minutes. The chilled dough is laminated and formed according to wishes.
- Baking the products: baking is oven at a temperature of 180-200°C for 10-15 minutes.

Following the evaluation of the organoleptic characteristics, the biscuits enriched with organic lyophilized apple powder and lyophilised basil powder, are very tender and extremely tasty. They have a pleasant taste of walnut and coffee and the flavour of apples is present. We find the aromatic notes specific to the introduced basil, which highlighted the product from anonymity, also observed a balanced taste of sweet-aromatic-slightly sour acid specific to dehydrated apple, introduced in two forms: small pieces and powder. Hemp flour biscuits have a less appealing appearance and a darker colour due to the use of hemp flour which has changed the overall appearance of the finished product. This colour characteristic due to their high polyphenols content, as well as their crispiness was observed also by Korus et al. (2017), Norajit et al. (2011), Šottníková et al. (2019). These aspects can be corrected by icing the biscuits, with organic dark chocolate or another ingredients.

Sensorial evaluation

The first group consisting of 33 members in panel group, of different ages, were chosen to determine the level of acceptance of biscuits enriched with organic apple and basil powders.

The panel members were requested to measure the terms identifying sensorial characteristics

and to use the score. Judgments were made through rating the products on a 5-point Hedonic scale with corresponding descriptive terms ranging from 5 ‘like extremely’ to 1 ‘dislike extremely’ (Figure 1).
The sensorial tests of the biscuits were made considering: first appearance, section appearance, colour, flavour and smell, and overall taste of the sample.

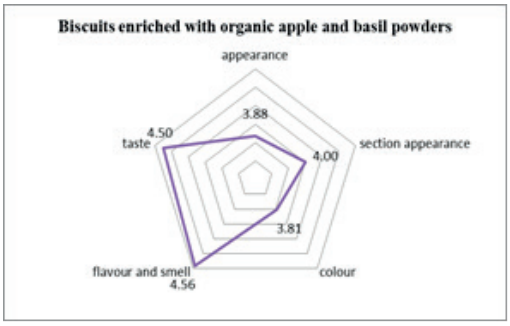

Figure 1. Consumer acceptability scores on a 5-point Hedonic scale for biscuits

The hemp flour biscuits did not receive a high score for colour (3.81) and appearance (3.88), because the use of hemp flour changed the overall appearance of the finished product. These aspects can be corrected by frosting biscuits with dark chocolate or other glaze and decoration.
In the section, the biscuits are slightly crumbly and tender, specific characteristics for this product and the apple pieces are flavoured and easy to chew. They are extremely tasty. They taste like walnut and coffee and the flavour of apples is present.
The flavour and taste received scores over 4.5. The consumers recognized the notes of lyophilized basil powder which highlighted the product, and the balance of sweet - aromatic - slightly sour taste given by the lyophilized apple introduced in two forms: pieces and powder.
We consider that the product has been accepted by the consumers very easily, and the weighted average grade of 4.12 can be modified very easily by printing a new shape of the product or by decorating with chocolate or something else.
In the second sensory evaluation session, there were analysed two biscuits samples.
One sample was prepared using the simple biscuit recipe (TC - test control) and the second one was enriched with organic apple powder and organic basil powder (TS - test sample).
The second group consisting of 10 members, was asked to evaluate the two cookie samples.
In order to reach the objective of sensory research, it was considered to organize an internal group of trained evaluators and subsequently to carry out sensory analysis tests specific to obtaining sensory profiles. For this purpose, the applications were made on food matrices using two formulas in pairs for each matrix tested: a formula made with standard ingredients (TC) and for the same matrix, another formula in which were introduced the ingredients obtained in the SusOrgPlus project (TS) (Figure 2).

Figure 2. Biscuits enriched with organic lyophilized apple powder and lyophilised basil powder: TC (right) and TS (left)

The same set of sensory descriptors, identified as representative for highlighting the differences in sensorial perception resulted on the two samples, were evaluated using the 5-point Hedonic Scale for both products in each pair of food matrices.
To achieve the partial sensory profiles, the sensory expert has completed 5 characteristic attributes for each sample. After scaling the average values of the 5 attributes and their representation on a spider diagram, there were obtained the sensory profile for each of the 2 tested products. For an easy highlighting of the profile differences within the pairs of recipes, they were represented in Figure 3.

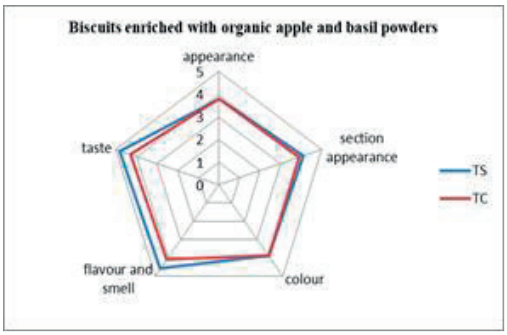

Figure 3. Spider plot of organoleptic properties of biscuits enriched with apple and basil powder made using the test control and test sample

It can be observed that out of the 5 attributes, following the sensory analysis, the TS sample received a better evaluation of taste, flavour and smell, these attributes being brought by the addition of organic ingredients tested.

Following the test, an improvement in the appearance, flavour and smell, and taste of the TC was observed compared to the TS sample (Figure 4).

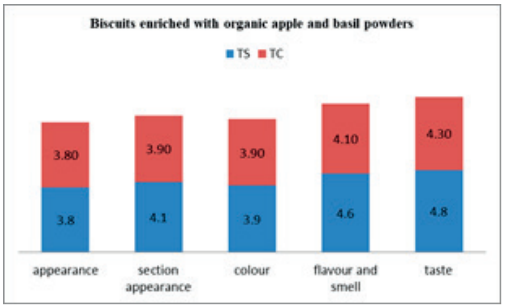

Figure 4. Results obtained after the sensory analysis of biscuit samples

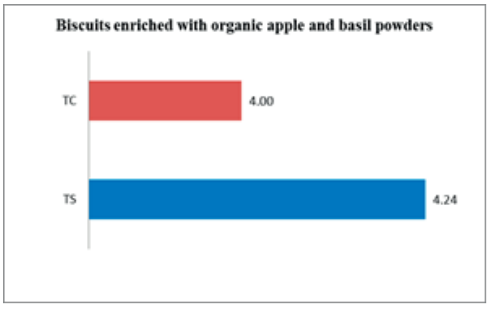

Figure 5. Sensory analysis 5-point Hedonic scale

Following the evaluation, the biscuits enriched with organic apple and basil powders (TS) achieved a score of 4.24 compared to TC which had only 4.00 (Figure 5).

Nutrient Content

Nutrient content it was calculated using a program nutritional development tool, *Softmedia programme* (http://softfedima.ro/). *Softmedia programme* makes it easy to prepare a nutrition facts panel, nutrition data sheet, ingredient statement for any food product. Formulas can be adjusted for moisture and/or fat content. Information can be printed, saved as a PDF document.

For the calculation of the nutritional value, losses in baking and cooling of 10% were taken into account. Table 1 shows the nutritional values for 100 g of biscuits enriched with organic apple and basil powders. The calculated energy value is 628.6 kcal for 100 g of product.

Table 1. Nutritional values for 100 g of Biscuits enriched with organic apple and basil powders

Average nutritional values for 100 g biscuits	
Energy value	2604.3 KJ
	628.6 kcal
Fat	52.4
Of which saturated fatty acids	32.6
Carbohydrates	31.6
Of which sugars	17.1
Fiber	3.5
Protein	5.9
Salt	0.6

They can be mentioned as products are allergenic potential due to the necessary ingredients and the product contains gluten, lactose, may contain traces of egg protein (avidin).

CONCLUSIONS

The use of organic additives in the form of powder obtained in the the SusOrgPlus project at the Research Centre for Studies of Food Quality and Agricultural Products, USAMV Bucharest, to obtaining Biscuits enriched with organic apple and basil powders, has increased sensory value and is accepted by consumers.

The evaluation of the organoleptic characteristics emphasized that biscuits enriched with organic lyophilized apple powder and lyophilised basil powder, are very tender and extremely tasty. They have a pleasant taste

of walnut and coffee and the flavour of apples is present. The basil notes are felt, and also are observed a balanced taste of sweet-aromatic-slightly sour acid specific to dehydrated apple, added in two forms: small pieces and powder.

It may be concluded from the study that the organic apple and basil powders can be successfully incorporated in biscuits up to a level of 1% to yield biscuits of enhanced nutritional quality with acceptable sensory attributes.

All the proposed activities were carried out in accordance with the achievement of the proposed objective.

The Sensory Analysis tests revealed a differentiated influence of organic ingredients SusOrgPlus in the sensory quality of food products.

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REFERENCES

Avetisyan, A., Markosian, A., Petrosyan, M., Sahakyan, N., Babayan,A., Aloyan, S., Trchounian, A. (2017) Chemical composition and some biological activities of the essential oils from basil Ocimum different cultivars. *BMC Complement Altern Med,* 17, 60 https://doi.org/10.1186/s12906-017-1587-5.

Bahaciu, G. V., Dragomir, N., Defta, N., Nicolae, C. G. (2019). Study regarding consumers behaviour towards innovative confectionery products. *Scientific Papers. Series D. Animal Science*, LXII (1), 404–409.

Bădulescu, L., Bujor-Neniţa, O. C., Dobrin, A., Stan, A., Zugravu, M., Ion, V. (2019). Bioactive compounds and quality parameters in different organic apple varieties and their natural value added powders. *Fruit Growing Research*, Vol. XXXV, http://publications.icdp.ro/index.php DOI 10.33045/fgr.v35.2019.17

Bujor-Neniţa, O., Bădulescu, L. (2019). Valorificarea produselor agroalimentare ecologice pentru obținerea de produse naturale cu valoare nutritivă ridicată. *Workshop"Ecologic pentru sănătatea noastră"*, Bio Life & Style 2019, Expoziție pentru o viață verde. Bucharest, RO: Ex Terra Aurum & INVEL-Multimedia Publishing Houses.

Catană, M., Catană, L., Iorga, E., Asănică, A.C., Belc, N. (2018). Bakery products fortified with dried fruits of *Aronia melanocarpa*. *Scientific Papers. Series B, Horticulture*, LXII, 693–702.

Di Cairano, M., Galgano, F., Tolve, R., Caruso, M.C., Condelli, N. (2018). Focus on gluten free biscuits: Ingredients and issues, *Trends in Food Science & Technology*, 81, 203–212.

Dragomir, N., Nicolae, C. G., Dragomir V. (2017). Romanian traditional products between local and global. *Analele IBNA*, 31, 103–111.

Dragomir, N., Nicolae, C. G. (2019). Produse inovative - o alternativă în valorificarea ingredientelor BIO. *Workshop"Ecologic pentru sănătatea noastră"*, Bio Life & Style 2019, Expoziție pentru o viață verde. Bucharest, RO: Ex Terra Aurum & INVEL-Multimedia Publishing Houses.

Dragomir, N., Nicolae, C. G., Bujor-Neniţa, O. C. (2019). Produse inovative obținute din ingrediente pe bază de fructe și legume ecologice. *Catalogul Conferintelor prezentate de USAMV Bucuresti la INDAGRA*. Bucharest, RO: Ex Terra Aurum & INVEL-Multimedia Publishing Houses.

Ferreira, M.S.L., Santos, M.C.P., Moro, T.M.A., Basto, G.J., Andrade, R.M.S., Gonçalves, É.C.B.A. (2015). Formulation and characterization of functional foods based on fruit and vegetable residue flour. *J. Food Sci. Technol.* 52, 822–830.

ISO 6658:2005 (2005). Sensory Analysis - Methodology - General guidance. Retrieved December 10, 2019, from https://www.iso.org/standard/36226.html

Koufiotis, I., Gyftopoulos, N., Andreou, A., Pisimisis, F., Travlos, I., Bilalis, D., Vlahos, G. (2016). Management of production and distribution of organic products. The case of organic farm managers and distributors in Athens' organic open farmers' markets. *AgroLife Scientific Journal*, 5(2), 90–94.

Korus, A., Gumul, D., Krystyjan, M., Juszczak, L., Korus, J. (2017). Evaluation of the quality, nutritional value and antioxidant activity of gluten-free biscuits made from corn-acorn flour or corn-hemp flour composites. *Eur. Food Res. Technol.,* 243, 1429–1438.

Lukin, A., Bitiutskikh, K. (2017). Investigation on the use of hemp flour in cookie production. *Bulgarian Journal of Agricultural Science*, 23(4), 664–667.

Norajit, K., Gu, B.-J., Gi-Hyung Ryu, G.-H. (2011). Effects of the addition of hemp powder on the physicochemical properties and energy bar qualities of extruded rice, *Food Chemistry*, 129(4), 1919–1925.

Rocchetti, G., Giuberti, G., Lucini, L. (2018). Gluten-free cereal-based food products: the potential of metabolomics to investigate changes in phenolics profile and their *in vitro* bioaccessibility. *Current Opinion in Food Science*, 22, 1–8.

Sărăcin V. C., Vasile A. (2015). An exploratory research regarding Romanian organic farming sector. *AgroLife Scientific Journal*, 4(2), 119–123.

Šottníková, V., Langová, R., Hřivna, L., Nedomová, Š., Jůzl , M. (2019). Quality of biscuits as affected by

addition of fibre. *Potravinarstvo Slovak Journal of Food Sciences*, 13(1), 206–211.

Spence, C. (2016). The Psychological Effects of Food Colors. In: *Handbook on Natural Pigments in Food and Beverages, I^st^ Edition, Industrial Applications for Improving Food Color*, Ed. Reinhold Carle, Ralf Schweiggert, Cap. II, 29–58. Sawston, UK: Woodhead Publishing House.

Spence, C. (2018). Background colour & its impact on food perception & behaviour. *Food Quality and Preference*, 68, 156–166.

Stone, H., Sidel, I. L. (1993). *Sensory evaluation practices, Second edition* (pp. 216–235, 240–241). San Diego, USA: Academic Press Publishing House.

William, L., P. Z., Danyang, Y., Zhongxiang, F. (2019). Hempseed in food industry: Nutritional value, health benefits, and industrial applications, Comprehensive Reviews in *Food Science and Food Safety*, 19, 282–308.

Złotek, U. (2018). Antioxidative, potentially anti-inflammatory, and antidiabetic properties, as well as oxidative stability and acceptability, of cakes supplemented with elicited basil. *Food Chemistry*, 243, 168–174.

Zugravu, C. A., Pogurschi, E. N., Patrascu, D., Iacob, P. D., Nicolae, C. G. (2017). Attitudes towards food additives: A pilot study. *Annals of the University Dunarea de Jos of Galati*, *Fascicle VI - Food Technology*, 41(1), 50–61.

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TOXIC HEAVY METALS CONTENT IN WILD BOAR AND VENISON MEAT: A BRIEF REVIEW

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Abstract

Heavy metals are prevalent in the environment and often are encountered in the food supply chain. Their presence in large game meat is commonly caused by environmental pollution and hunting techniques (ammunition for hunting), and the intake of such contaminated sub-products has demonstrated an adverse effect on consumers 'health. Regularly, the literature makes risk assessments of specific consumption scenarios regarding possible health risks to extreme game meat consumers (i.e. hunters and their family members). Therefore, tracking this metals concentration in game meat and the updated situation (especially Cd, Pb) is necessary to ensure compliance with food safety regulations and consequent consumer and environmental protection, reason for which this review addresses various gaps in current awareness (knowledge and research) on the accumulation of metal pollutants in commercially processed and consumed big game meat worldwide.

Key words: *heavy metals, game meat, contamination.*

INTRODUCTION

Heavy metals are ubiquitous in the ecosystem and can enter the food chain. Them are predominantly transferred as molecules or particulate matter via the atmosphere, mostly over distances. The amount of anthropogenically generated heavy metals has slowly increased since the beginning of the industrial revolution, but in recent decades public understanding and awareness associated with their environmental and health risks has risen sharply (Pilarczyk et al., 2020).

Heavy metals become harmful because they tend to bioaccumulate (i.e. the concentration of metal in a biological organism will increase relative to its environmental concentration over time) because the compounds accumulate in living organisms at any time they are consumed and stored faster than they are metabolized or excreted (Pascoe et al., 1994). Consequently, eating contaminated products will adversely affect consumer health. Due mainly to their persistence and biomagnification across the food chain, exposure to these chemical contaminants is of particular concern.

Specific environmental and biological parameters affect the transfer of this pollutants from the atmosphere to biota (Baker et al., 2003). The impact of gender on bioavailability, transmission and effects of contaminant has been shown (Baker et al., 2003; Gonzalez et al., 2008; Fritsch et al., 2010; Tchounwou et al., 2012). For monitoring purposes, the literature covers specific food chain levels, such as primary (herbivorous) and secondary (carnivorous) consumers (Sánchez-Chardi et al., 2009). The study of wildlife species in anthropogenic habitats that may be changed or damaged provides important information about the viability and equilibrium of the ecosystems. Moreover, the use of natural populations as environmental pollution sentinels helps to increase our awareness and strengthen the response to environmental and human health issues (Alleva et al., 2006).

This paper will address toxicological reference values for selected heavy metals (Cd and Pb), documented to occur in game meat (wild boar and venison) and relevant to human health.

Toxicological reference value and maximum level for Cd and Pb

Legislation controls the highest permissible levels of certain contaminants, including certain food elements. In the E.U., the maximum levels for certain contaminants in foodstuffs are determined by Commission Regulation (EC) (2008).

Cadmium

There is no maximum EU-regulated level of Cd in game meat. For Cd food intake, the EFSA CONTAM Panel published a tolerable weekly intake (TWI) of 2.5 μg/kg body weight (bw), although the ICRA classified it as human cancer based on occupational studies (EFSA, 2009); also, for livestock meat products (excluding offal of bovine, sheep, pig and poultry), the maximum level for cadmium is 0.05 mg/kg (EC, 2006).

Cereals and cereal sub products, vegetables, nuts and pulses, starchy roots and meat or meat products are the main contributors to dietary cadmium intake. Mean dietary intake in Europe was estimated to be 2.3 μg/kg bw/week (from 1.9 to 3.0 μg/kg bw/week), a range with upper limits slightly higher than TWI of 2.5 μg/kg bw. Population subgroups, such as vegetarians, children, smokers and people living in highly contaminated areas may exceed the TWI (EFSA, 2009).

Lead

There is no maximum EU-regulated level of Pb in game meat. In 2010, the European Food Safety Authority (EFSA, 2010) evaluated the health effects of lead in food (developmental neurotoxicity for children, respectively cardiovascular effects and nephrotoxicity for adults). Since no safe Pb intake levels could be determined, EFSA revoked the toxicological limit of 25 μg lead per kg bw/week in food and stated that any lead intake should be as minimal as reasonably achievable for humans in accordance with the ALARA principle. EFSA established lead toxicological reference values using the Benchmark Dose (BMD) framework and calculated the resulting dietary lead accumulation for: developmental neurotoxicity (0.50 μg/kg body weight per day), systolic blood pressure (1.5 μg/kg bw per day) or for prevalence of CKD (0.63 μg/kg bw per day).

In general, the exposure of the consumer to lead is primarily due to the intake of food with a relatively low lead content but with high consumption rates (i.e. fruit, vegetables and tap water). Game meat is a food item that is rarely consumed by the majority of the general population but is consumed by specific subgroups (i.e. hunter family members).

Cadmium and lead occurence in selected game meat

Across Europe, cadmium and lead have been included in monitoring programs because they are toxic and not at all essential to animals or human health. In the same time, game species are used as strong bioindicators for toxic metal emissions in biomonitoring studies (Santiago et al., 1998; Millan et al., 2008; Pérez-López et al., 2016). Mammals such as wild boar (*Sus scrofa*), roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*) and red deer (*Cervus elaphus*) are excellent bioindicators of the degree of heavy metal pollution in the environment (Amici et al., 2012; Bakowska et al., 2016; Srebočan et al., 2011; Mitrănescu et al., 2011; Lazarus et al., 2014). As the obtained values are specific to game species, the wildlife conditions, the method of harvesting, the study hypothesis, Tables 1 and 2 summarize the levels of the most important investigations concerning contamination of wild boar and venison meat. The transfer of heavy metals to animal tissues occurs primarily through the digestive tract due to ingestion of feed containing either heavy metals or soil polluted. Their concentration in free-living animals depends on a number of factors related to the area of residence, the properties of the soil, the characteristics of the species, the physiological status of the plants, the lifestyle and diet of the animals and their location relative to industrial plants.

Of all the game species mentioned above, wild boars are considered most appropriate as bioindicators due to their abundance in almost all regions of Europe, both in agricultural and forest areas. In addition, wild boars are omnivorous animals. Although 80-90% of their diet includes food retrieved from the soil (acorns, beech, nuts, herbs, grass, roots, rhizomes, or earth-worms), they also eat insects, frogs, eggs, chicks, rodents, and carrion (2-11%) (Schley and Roper, 2003; Baubet et al., 2004).

Table 1. Concentration of some heavy metals [Cd, Pb (μg g^{-1} ww)] in venison (mean value)

CC	Cd	Pb	References
Muscle tissue	1.0	0.8	Świergosz et al., 1993
	1.0	1.5	Falandysz and Gadja, 1988
	1.7	1.7	Falandysz, 1994
	3.4	2.3	Rimkus and Wolf, 1987
	0.46	0.652	Pilarczyk et al., 2020
	2.2	1.8	Jarzynska and Falandysz, 2011
	0.254^{LA}	2.079^{LA}	Mitranescu et al., 2011
	0.119	1.25	Taggart et al, 2011
	0.06	0.79	Gizejewska et al., 2017
	0.04/0.01/0.07/0.03	0.059/0.057/0.561/0.062	Bilandzic et al., 2009*
	0.43/0.05/0.07	0.57/0.26/0.12	Durkalec et al., 2015*
		$0.42^{M}/0.55^{F}$	Lehel et al., 2016
Animals offal	2.1		Falandysz, 1994
		0.21/0.71/0.11/0.55	Pokorny, 2000*
	0.19/0.15/0.11/0.20	0.11/0.08/0.77/0.07	Bilandzic et al., 2009*
		0.17	Jarzynska and Falandysz, 2011

CC = contamination category; **x** = processed data; **RV** = recommended value, **IV**= identified value; Gender **M** = male, **F** = female; **LA** = allowed limits according to Regulation (EC) no. 1881/2006; **I, II, III** = different age categories; ***** = different grounds of research.

Table 2. Concentration of some heavy metals [Cd, Pb (μg g^{-1} b.w.)] in wild boars (mean value)

CC	Cd	Pb	References
Muscle tissue	0.45	0.73	Hecht, 1986
	2.4	1.2	Rimkus and Wolf, 1987
	1.3	1.6	Falandysz and Gadja, 1988
	0.30	3.5	Venalainen, 2007
	0.11	0.316	Taggart et al, 2011
	0.79	0.126	Amici et al., 2012
	0.78^{x}	1.24	Danieli et al., 2012
	0.15	3.273	ANSES, 2018
	0.53	0.66	Pilarczyk and colab., 2020
	1.12^{RV} / 1^{IV}	$4.91^{RV}/4.8^{IV}$	Falandysz, 1994
	$0.3^{M}/0.2^{F}$	$0.13^{M}/0.10^{F}$	Roslewska et al., 2016
	$0.440^{LA}/0.310^{LA}$	$0.821^{LA}/0.504^{LA}$	Mitranescu et al., 2011
	$0.08^{I}/0.14^{II}/0.17^{III}$	$0.42^{I}/0.53^{II}/0.82^{III}$	Rudy, 2010
	0.23/0.01/0.05	1.95/1.06/0.83	Bilandzic et al., 2009*
	0.107/0.209/0.205	1.46/1.799/1.425	Florijancic et al., 2015*
	0.40/0.04/0.04	0.46/0.19/0.27	Durkalec et al., 2015*
	0.6/19.8	0.8/0.9	Świergosz et al., 1993*
Animals offal	0.49/0.32/0.30	1.2/0.62/2.02	Bilandzic et al., 2009*
	2	4	Rudy, 2010
	0.84^{x}	3.29	Danieli et al., 2012
	0.85	3.18	Amici et al., 2012
	0.326	3.83	Neila et al., 2017

CC = contamination category; **x** = processed data; **RV** = recommended value, **IV**= identified value; Gender **M** = male,
F = female; **LA** = allowed limits according to Regulation (EC) no. 1881/2006; **I, II, III** = different age categories;
***** = different grounds of research.

Heavy metals are partially taken up by wild boars due to the ingestion of earthworms that produce substantial quantities of lead and other heavy metals in their tissues (Latif et al., 2013). Also, drawing the soil clods while grazing (also known as rooting) may also play a decisive role in this process (Bakowska et al., 2016).

Unlike other food products, game meat has an additional entry for heavy metals due to hunting ammunition (Dobrowolska and Melosik, 2008; Müller-Graf et al., 2017; Taggart et al., 2011; Tsuji et al., 2009), sporadically, game meat showing higher levels of lead among analysed items (EFSA, 2010).

Literature data reveals that game meat of animals shot with lead-based ammunition contains more lead than game meat obtained with nonlead ammunition because lead bullets split into small lead particles on impact and produce fine lead splinters (Hecht, 2000; Müller-Graf et al., 2017). Various researches approach the impact of age and gender on game meat lead content. Thus, Srebočan et al. (2012) states that the highest concentration of lead observed in the muscle tissues of young animals (roe deer and wild boar) is attributed to their potential need for minerals, since calcium kinetics are strongly linked to lead kinetics.

The lead distribution within the game meat is also examined, the highest lead concentrations being found closest to the wound in game meat of roe deer, red deer and wild boar with a maximum of 4.7 mg/kg, 3.4 mg/kg, 1.6 mg/kg, respectively, when lead containing ammunition was used (BfR, 2014).

Other experiments also reported a decrease in the lead concentrations with increasing distance from the wound channel in game meat of red deer, wild boar and white-tailed deer (Dobrowolska and Melosik, 2008; Grund et al., 2010). They analysed muscle and soft tissue of red deer and wild boar for lead content immediately after being killed at different distances (5, 15, 25 and 30 cm) from the wound channel. Lead particles at a distance of 30 cm from the entry wound were detected in each sample. The highest lead content was 1095.9 mg/kg near the wound channel in a sample of wild boar meat and 3.3 mg/kg, 30 cm away from the entry wound. All animals were killed with lead-based ammunition (Dobrowolska and Melosik, 2008). In the second case, lead contamination levels in muscle tissue samples from white-tailed deer at different distances from the wound channel (5, 25 and 45 cm) were analysed. Detectable lead concentrations were found at all distances, and the highest concentrations were found closest to the wound channel (Grund et al., 2010).

Lead levels of red deer meat, in concordance with origin country are summarized in Table 3 (Froslie et al., 1984; Michalska et al., 1992; Wolkers et al., 1994; Falandysz, 1994; Drozd et al., 1997; Kottferová et al., 1998; Santiago et al., 1998; Szkoda et al., 2001; Szymczyk, 2001; Falandysz et al., 2005; Lazarus et al., 2005; Kramárová et al., 2005; Vikoren et al., 2005).

Table 3. Lead levels (mg/kg b.w.) in red deer meat from various countries (Venäläinen, 2007)

	Muscle	Liver	Kidney
NL		0.193-0.122	0.449-0.386
PL	0.09 – 0.39	0.11-0.70	0.14-0.38
ES		0.57	0.33
HR			0.58
SK	0.35	0.32-1.90	0.48-0.56

NL = Netherlands, **PL** = Poland, **ES** = Spain, **HR** = Croatia, **SK** = Slovakia.

In the case of wild boars harvested from Croatia hunting grounds, a number of research studies have obtained almost similar values for Cd content, as follow: 0.01-0.23 mg kg^{-1} (Bilandžić et al., 2009), 0.005-0.062 mg kg^{-1} (Bilandžić et al., 2010), results close to the values (0.02 mg kg^{-1}) previously reported by Michalska and Żmudzki (1992), specific to free-living wild boars from the Wielkopolska region of Poland. Moreover, the highest concentrations of Cd (0.56 mg kg^{-1}) were found to accumulate in kidneys (Roślewska et al., 2016), because this organ is essential for the excretion of toxins from the body (Drozd et al., 2001). This was a confirmation of previous Cd accumulation surveys described earlier in wild boars from Slovakia by Gasparik et al. (2012) for the kidney, liver and muscles.

Heavy metals, especially Cd and Pb, accumulate in animal organs. Analyses of different organs showed that the highest amounts of Pb (0.39 mg kg^{-1}) were accumulated in kidneys (Piskorová et al., 2003). In wild boar muscles and liver increases with age, with only the category of youngest and oldest animals considered statistically

significant differences, but with values without reaching the maximum legal level (i.e. for lead (0.045 for the youngest specimens and 0.087 mg kg^{-1} for the oldest animals) or cadmium (0.011 for the youngest and 0.018 mg kg^{-1} for the oldest animals) content (Rudy, 2010).

Different outcomes have been obtained by Szkoda and Żmudzki (2001), showing that the average lead content of wild boar meat ranged from 0.121-0.437 mg kg^{-1}, exceeding the maximum permissible level in over 20% of the samples, possible to the lead intoxication from gunshot wounds (Dobrowolska and Melosik 2008). For such a justification, muscle tissue should not be considered a valid predictor for the degree of ecosystem contamination by this element.

A recent study on the problem of heavy metals in western Ukraine shows that despite continuous emission reductions, the environment still contains high concentrations of toxic elements (Pilarczyk et al., 2020).

CONCLUSIONS

Contamination of game meat with selected heavy metals (Cd, Pb) poses a risk to human health and undermines food safety worldwide; the most common methods used in literature are risk assessments of specific consumption scenarios to provide effective mitigation methods for reducing concentrations of contaminants, game meat being a useful tool.

REFERENCES

Alleva, E., Francia, N., Pandolfi, M., De Marinis, A. M., Chiarotti, F., Santucci, D. (2006). Organochlorine and heavy-metal contaminants in wild mammals and birds of Urbino-Pesaro Province, Italy: an analytic overview for potential bioindicators. *Arch. Environ. Contam. Toxicol*., 51(1), 123-134.

Amici, A., Danieli, P. P., Russo, C., Primi, R., Ronchi, B. (2012). Concentrations of some toxic and trace elements in wild boar (*Sus scrofa*) organs and tissues in different areas of the Province of Viterbo, Central Italy. *Italian Journal of Animal Science*, 11(65), 354-362.

Baker, S., Herrchen, M., Hund-Rinke, K., Klein, W., Kordel, W., Peijnenburg, W., Rensing, C. (2003). Underlying issues including approaches and information needs in risk assessment. *Ecotoxicol. Environ. Saf.*, 56(1), 6-19.

Bakowska, M., Pilarczyk, B., Tomza-Marciniak, A., Udala, J., Pilarczyk, R. (2016). The bioaccumulation of lead in the organs of roe deer (*Capreolus capreolus* L.), red deer (*Cervus elaphus* L.), and wild boar (*Sus scrofa* L.) from Poland. *Environ. Sci. Pollut. Res. Int*., 23(14), 14373-14382.

Baubet, E., Bonenfant, C., Brandt, S. (2004). Diet of the wild boar in the French Alps. *Galemys*, 16(1), 101-113.

BfR (2014). Research project "Safety of game meat obtained through hunting" (LEMISI).

Bilandzic, N., Sedak, M., Vrataric, D., Peric, T., Simic, B. (2009). Lead and cadmiun in red deer and wild boar from different hunting grounds in Croatia. *Science of the Total Environment*, 407, 4243-4247.

Danieli, P. P., Serrani, F., Primi, R., Ponzetta, M. P., Ronchi, B., Amici, A. (2012). Cadmium, Lead and Chromium in Large Game: a local-scale exposure assessment for Hunters Consuming Meat and Liver of Wild Boar. *Archives of Environmental Contamination and Toxicology*, 244 (9791), 1-16.

Dobrowolska, A., Melosik, M. (2008). Bullet-derived lead in tissues of the wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*). *Eur. J. Wildl. Res*., 54(2), 231-235.

Drozd, L., Karpinski, M. (1997). Heavy metal content in farming red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) tissues. *Annales UMCS sec. EE Zootechnica*, 15, 309-314.

Drozd, L., Piwniuk, J., Karpiński, M. (2001). Heavy metals levels in the tissues of roe deer (*Capreolus capreolus*) from the Lublin region. *Pol. J. Food Nutr. Sci.*, 10/51(3), 59-62.

Durkalec, M., Szkoda, J., Kolacz, R., Opalinski, S., Nawrocka, A., Zmudzki, J. (2015). Bioaccumulation of lead, cadmium and mercury in roe deer and wild boars from areas with different levels of toxic metal pollution. *International Journal Environmental Resources*, 1(9), 205-212.

Falandysz, J., Gajda, B. (1988). Mercury content in muscle, liver and kidneys of slaughtered and game animals from the northern part of Poland, 1985-1986. Polish. *Roczn. Panstw. Zakl. Hig*., 39, 113-117.

Falandysz, J. (1994). Some toxic and trace metals in big game hunted in the northern part of Poland in 1987-1991. *The Sciene of Total Environment*, 141, 59-73.

Falandysz, J., Szymczyk-Kobrzynska, A., Brzostowski, A., Zalewski, K., Zasadowski, A. (2005). Concentrations of heavy metals in the tissues of red deer (*Cervus elaphus*) from the region Warmia and Mazury, Poland. *Food Additives and Contaminants*, 22(2), 141-149.

Florijancic, T., Ozimec, S., Jelkic, D., Vuksic, N., Bilandzic, N., Boskovic, G., Boskovic, I. (2015). Assessment of heavy metal content in wild boar (*Sus scrofa*) hunted in eastern Croatia. *Journal of Environmental Protection and Ecology*, 2(16), 630-636.

Fritsch, C., Cosson, R. P., Coeurdassier, M., Raoul, F., Giraudoux, P., Crini, N., de Vaufleury, A., Scheifler, R. (2010). Responses of wild small mammals to a pollution gradient: host factors influence metal and metallothionein levels. *Environ. Pollut*., 158(3), 827-840.

Froslie, A., Norheim, G., Rambaek, J. P., Steinnes, E. (1984). Levels of trace elements in liver from

Norwegian moose, reindeer and red deer in relation to atmospheric deposition. *Acta Veterinaria Scandinavica*, 25, 333-345.

Gasparik, J., Dobias, M., Capcarova, M., Smehyl, P., Slamecka, J., Bujko, J., Gasparik, J. Jr. (2012). Concentration of cadmium, mercury, zinc, copper and cobalt in the tissues of wild boar (*Sus scrofa*) hunted in the western Slovakia. *J. Environ. Sci. Health Tox. Hazard Subst. Environ. Eng*., 47(9), 1212-1216.

Gizejewska, A., Szkoda, J., Nawrocka, A., Zmudzki, J., Gizejewski, Z. (2017). Can red deer antlers be used as an indicator of environmental and edible tissues trace element contamination? *Environmental Science Pollut Resources*, 24(11), 630-638.

Gonzalez, X. I., Aboal, J. R., Fernandez, J. A., Carballeira, A. (2008). Heavy metal transfers between trophic compartments in different ecosystems in Galicia (Northwest Spain): essential elements. *Arch. Environ. Contam. Toxicol*., 55(4), 691-700.

Grund, M. D., Cornicelli, L., Carlson, L. T., Butler, E. A. (2010). Bullet fragmentation and lead deposition in whitetailed deer and domestic sheep. *Hum. Wildl. Interact.*, 4, 257-265.

Hecht, H. (1986). Unterschiede im Schwermetallgehalt in Muskelen und Organen freilebender und im Gatter gebaltener Tiere. *Fleischwirtschaft*, 66, 282-287.

Hecht, H. (2000). Auswirkungen der Geschoswahl auf die Bleibelastung des Wildbrets. Paper presented at the 'Tagung fur die Jagerschaft', 15. und 16. Februar 2000, Bundesanstalt fur alpenlandische Landwirtschaft Gumpenstein, Austria.

Jarzynska, G., Falandysz, J. (2011). Selenium and 17 other largely essential and toxic metals in muscle and organ meats of red reer (*Cervus elaphus*) - Consequences to human health. *Environment International*, 37, 882-888.

Kramárová, M., Massányi, P., Slamecka, J., Tataruch, F., Jancová, A., Gasparik, J., Fabis, M., Kovaci, J., Toman, R., Galová, J., Jurcik, R. (2005). Distribution of Cadmium and Lead in Liver and Kidney of Some Wild Animals in Slovakia. *Journal of Environmental Science and Health*, 40, 593-600.

Kottferová, J., Koréneková, B. (1998). Distribution of Cd and Pb in the Tissues and Organs of Free-Living Animals in the Territory of Slovakia. *Bulletin of Environmental Contamination and Toxicology*, 60, 730-737.

Latif, R., Malek, M., Mirmonsef, H. (2013). Cadmium and lead accumulation in three endogeic earthworm species. *Bull. Environ. Contam. Toxicol*., 90(4), 456-459.

Lazarus, M., Vickoviĉ, I., Šoštaric, B., Blanuša, M. (2005). Heavy metals in tissues of red deer (*Cervus elaphus*) from eastern Croatia. *Arhiv za Higijenu Rada I Toksikologiju*, 56, 233-240.

Lazarus, M., Prevendar Crnić, A., Bilandžić, N., Kusak, J., Reljić, S. (2014). Cadmium, lead, and mercury exposure assessment among Croatian consumers of free-living game. *Arh Hig Rada Toksikol*, 65, 281-292.

Lehel, J., Laczay, P., Gyurcso, A., Janoska F., Majoros S., Lanyi K., Marosan M. (2016). Toxic heavy metals in the muscle of roe deer (*Capreolus capreolus*) - food toxicological significance. *Environmental Science Pollut Resources*, 23(5), 4465-4472.

Michalska, K., Zmudzki, J. (1992). Metal concentrations in wild pig, roe and deer tissues of Wielkopolska region. *Medycyna Weterynaryjna*, 48, 160-162.

Millan, J., Mateo, R., Taggart, M. A., Lopez-Bao, J. V., Viota, M., Monsalve, L., Camarero, P. R., Blazquez, E., Jimenez, B. (2008). Levels of heavy metals and metalloids in critically endangered Iberian lynx and other wild carnivores from Southern Spain. *Sci. Total Environ.*, 399 (1-3), 193-201.

Mitrănescu, E., Butaru, A., Tudor, L., Latareţu, A., Furnaris, F., Ghimpeteanu, O.M., Matei, S. (2011). Researches concerning the occurrence of cadmium and lead residues in game meat. *Bulletin UASVM, Veterinary Medicine*, 62(2), 221-224.

Müller-Graf, C., Gerofke, A., Martin, A., Bandick, N., Lahrssen-Wiederholt, M., Schafft, H. A., Selhorst, T., Ulbig, E., Hensel, A. (2017). Reduction of lead contents in game meat: results of the 'Food safety of game meat obtained through hunting' research project: food safety and security. In: Paulsen, P., Smulders, F.J.M. and Bauer, A. (eds.) Game meat hygiene – food safety and security. Wageningen, the Netherlands: Wageningen Academic Publishing House, 201-212.

Neila, C., Hernandez-Moreno, D., Fidalgo, L.E., Lopez-Beceiro, A., Soler, F., Perez-Lopez, M. (2017). Does gender influence the levels of heavy metals in liver of wild boar? *Ecotoxicology and Environmental Safety*, 140, 24-29.

Pascoe G. A., Blanchet R. J., Linder G. (1994). Bioavailability of metals and arsenic to small mammals at mining waste-contaminated wetland. Archives of Environment Contamination and Toxicology, 27, 44-50.

Pérez-López, M., Rodríguez, F., Hernández-Moreno, D., Rigueira, L., Fidalgo, L. E., López-Beceiro, A. (2016). Bioaccumulation of cadmium, lead and zinc in liver and kidney of red fox (*Vulpes vulpes*) from NW Spain: influence of gender and age. *Toxicol. Environ. Chem*., 98(1), 109-117.

Pilarczyk, B., Tomza-Marciniak, A., Pilarczyk, R., Udala, J., Kruzhel, B., Ligocki, M. (2020). Content of essential and non-essential elements in wild animals from western Ukraine and the health risks associated with meat and liver consumption. *Chemosphere*, 244, 1-10.

Piskorová, L., Vasilková, Z., Krupicer, I. (2003). Heavy metal residues in tissues of wild boar (*Sus scrofa*) and red fox (*Vulpes vulpes*) in the Central Zemplin region of the Slovak Republic. *Czech J. Anim. Sci*., 48, 134-138.

Pokorny, B. (2000). Roe deer *Capreolus capreolus* as an accumulative bioindicator of heavy metals in Slovenia. *Web Ecology*, 1, 54-62.

Rimkus, G., Wolf, H. (1987). Schadstoffbelastung von Wild aus Schleswig-Holstein, 4. Cadmiun und Quecksilbergehalte. *Fleisch von Haar und Federwild. Fleischwirtschaft*, 67, 1150-1154.

Roslewska, A., Stanek, M., Janicki, B., Cygan-Szczegielniak, D., Stasiak, K., Buzala, M. (2016). Effect of sex on the content of elements in meat from wild boars (*Sus scrofa* L.) originating from the Province of Podkarpacie (South-Eastern Poland). *Journal of Elementology*, 21(3), 823-832.

Rudy, M. (2010). Chemical composition of wild boar meat and relationship between age and bioaccumulation of heavy metals in muscle and liver tissue. *Food Additives and Contaminants*, 4(27), 464-472.

Santiago, D., Motas-Guzman, M., Reja, A., Maria-Mojica, P., Rodero, B., Garcia-Fernandez, A. J. (1998). Lead and cadmium in red deer and wild boar from Sierra Morena Mountains (Andalusia, Spain). *Bull. Environ. Contam. Toxicol*., 61(6), 730-737.

Sánchez-Chardi, A., Peñarroja-Matutano, C., Borrás, M., Nadal, J. (2009). Bioaccumulation of metals and effects of a landfill in small mammals Part III: structural alterations. *Environ. Res*., 109(8), 960-967.

Schley, L., Roper, T. J. (2003). Diet of wild boar *Sus scrofa* in Western Europe, with particular reference to consumption of agricultural crops. *Mammal. Rev*., 33(1), 43-56.

Srebočan, E., Prevendar, A., Ekert-Kabalin, A., Lazarus, M., Jurasović, J., Tomljanović, K., Andreić, D., Strunjak-Perović, I., Čož-Rakovac, R. (2011). Cadmium, lead, and mercury concentrations in tissues of roe deer (*Capreolus capreolus* L.) and wild boar (*Sus scrofa* L.) from Iowland Croatia. *Czech J. Food Sci*., 29, 624-633.

Srebočan, E., Janicki, Z., Crnic, A. P., Tomljanovic, K., Sebecic, M., Konjevic, D. (2012). Cadmium, lead and mercury concentrations in selected red deer (*Cervus elaphus* L.) tissues from North-Eastern Croatia. *J. Environ. Sci. Health*, 47(13), 2101-2108.

Świergosz, R., Perzanowski, K., Makosz, U., Bilek, I. (1993). The incidence of heavy metals and other toxic elements in big game tissues. *The Science of the Total Environment Supplement*, 1, 225-231.

Szkoda, J., Żmudzki, J. (2001). Toxic elements in tissues of game animals. *Med. Wet*., 57, 883-886.

Szymczyk, K., Zalewski, K. (2001). Content of copper, zinc, lead and cadmium in some tissues of red deer (*Cervus elaphus*) in Warmia and Mazury in 1999. *Acta Toxicologica Poloniae*, 9, 105-114.

Taggart, A. M., Reglero, M. M., Camarero, P. R., Mateo, R. (2011). Should legislation regarding maximum Pb and Cd levels in human food also cover large game meat? *Environment International*, 37, 18-25.

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., Sutton, D. J. (2012). Heavy metals toxicity and the environment. *EXS*, 101, 133-164.

Tsuji, L. J., Wainman, B. C., Jayasinghe, R. K., VanSpronsen, E. P., Liberda, E. N. (2009). Determining tissue-lead levels in large game mammals harvested with lead bullets: human health concerns. *Bull. Environ. Contam. Toxicol*., 82(4), 435-439.Venäläinen, E. R. (2007). The levels of heavy metals in moose, reindeer and hares in Finland - Results of twenty years' monitoring. Faculty of Natural and Environmental Sciences - University of Kuopio, Snellmania, Auditorium. Helsinki, FI - Helsinki University Printing House.

Vikoren, T., Bernhoft, A., Waaler, T., Handeland, K. (2005). Liver concentrations of copper, cobalt, and selenium in wild Norwegian red deer (*Cervus elaphus*). *Journal of Wildlife Disease*, 41(3), 569-579.

Wolkers, H., Wensing, T., Groot Bruinderink, G. W. (1994). Heavy metal contamination in organs of red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*) and the effect on some trace elements. *The Science of the Total Environment*., 144, 191-199.

Agence Nationale de Securite Sanitaire (ANSES) (2018). Avis de l'Agence nationale de securite sanitaire de l'alimentation, de l'environnement et du travail relatif au "risque sanitaire lie a la consommation de gibier au regard des contaminants chimiques environnementaux". Saisine, SA-0109. (https://www.anses.fr/fr/system/files/ERCA2015SA0109.pdf)

European Commission (EC), 2006. Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. O.J.EU L364, 5.

Commission Regulation (EC) No 629/2008 of 2 July 2008, 2008. Amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs. Off. J. Eur. Union L 173/6, 3e7.

European Food Safety Authority (EFSA), 2009. Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on cadmium in food. EFSA J. 980, 1-139.

European Food Safety Authority (EFSA), 2010. Scientific Opinion on Lead in Food. EFSA Panel on Contaminants in the Food Chain (CONTAM). EFSA J. 8, 1570.

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STUDY REGARDING THE HONEY CONTAMINATION DEGREE ASSESSED IN A SPECIALIZED PRODUCTION UNIT

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Abstract

Honey represents a pleasant, nourishing food, with great biological and calorical value (315 kcal/100 g), easily digestible. It possesses real bactericidal properties, due to inhibin content. Examination of honey is necessary in order to assess its quality and purity, as well as to identify forbidden substances. The study was conducted in a processing unit which markets honey in the European Community. This study aimed to evaluate the contamination degree of the commercialised honey. In the summer of 2019, 3 batches of honey were analyzed (2 acacia honey batches containing 15 samples each and 1 batch containing 15 samples of polyfloral honey). Laboratory assessments were focused on determination of nitroimidazoles residues (metronidazole, dimetridazole, ronidazole), tetracyclines (oxytetracycline, tetracycline, chlortetracycline, doxycycline, demeclocycline, methacycline, minocycline), macrolides (clindamycin, erythromycin, josamycin, kitasamycin, lincomycin, oleandomycin, spiramycin, mirosamycin,tilmicosin, trimethoprim, tylosin), nitrofuran metabolites, chloramphenicol, streptomycin, dihydrostreptomycin, sulfonamides, trimethoprim, glyphosate. The methods used in the research were HPLC, LC-MS/MS, ELISA. The final results of the study that considered the a forementioned batches, sampled from various local beekeepers, proved that antimicrobial drug residues were in accordance with the national and international regulations, permitting marketing without restrictions.

Key words*: antibiotics residues, chloramphenicol, honey, streptomycin.*

INTRODUCTION

Honey represents the main product of beekeeping, being the result of nectar or manna bee processing and its storage in the honeycomb cells. Honey produced by bees exclusively from other raw materials than the one they naturally harvest, does not get into the frame of honey (Doliș, 2009). Honey is a natural food obtained in conventional or ecological systems, in units which follow the food safety principles, composed mainly of sugars and other constituents: enzymes, amino acids, organic acids, carotenoids, vitamins, minerals and aromatic substances (Petcu, 2006; Crivineanu et al., 2011; Tănăsoiu et al., 2014; Dobre, 2016; Dobre, 2017; Tăpăloagă et al., 2017; Tăpăloagă, 2018; Tudoreanu et al., 2012). It is rich in flavonoids and phenolic acids that act as natural antioxidants, being a beneficial product for consumers (Alqarni et al., 2012; Tănăsoiu et al., 2015; Șapcaliu et al., 2017; Tamas-Krumpe, 2019).

In terms of food safety, honey has to be free of chemical, toxic and carcinogenic contaminants, especially pesticides and antibiotics (Orso et al., 2015; Murariu et al., 2019; Murariu et al., 2019). The most common and important contaminations of honey are done directly (treatments applied in the hive) and indirectly (contaminants that come from the agriculture and the environment) (Mărghitaș et al., 2010). Chemical contaminants have different origin: environmental pollutants (heavy metals), chemicals used in agriculture (pesticides), toxic contamination substances and those formed during processing and storage stages (disinfectants, detergents and mycotoxin, chemicals which could migrate from packagings or packaging systems), direct treatment of bees against bacterial diseases of the bee brood, like *American foulbrood* or *European foulbrood* (Petcu, 2104a; Petcu et al., 2014b; Tofană, 2011).

Sulfonamides, tetracyclines, nitrofurans and macrolides are used by beekepers for

preventing and controlling honeybee diseases. Consequently, it is possible that antibiotics residues from honey may be the result of treatments carried out by beekeepers. The treatment of bees with antibiotics is prohibited in the European Union (EU), significant progress being made in the EU risk assessment legislation (Barganska et al., 2011).

Starting with 2000, for the EU food and products intended for marketing, it became necessary to establish a maximum residue limit (M.R.L.), paying more attention to the negative effects produced by the residues or their metabolites that are found in products intended for human consumption. For this purpose, analytical methodologies were developed for identification and quantification of these compounds (Lazăr et al., 2006). Techniques for extraction and purification of antibiotics from animal origin samples (including honey) include some forms of liquid-liquid extraction (L.L.E.) or solid phase extraction (S.P.E.). The most used technique for drug extraction is liquid-liquid chromatography. H.P.L.C. method (High Perfomance Liquid Cromatography) is the most extensive chromatographic method used for the antibiotic's analysis (Burian, 2011; Vlaic et al., 2018).

The major classes of antibiotics present in honey are: beta-lactams, amphenicols, tetracyclines, macrolides, aminoglycosides and fluoroquinolones. **Beta-lactams** are antibiotics used to treat bacterial infections, altering bacterial cell wall biosynthesis; for example: penicillin, ampicillin, cloxacillin, amoxicillin (Sapna et al., 2010). **Amphenicols** blocks the enzyme peptidyl transferase on the 50S ribosome. The most used are: thiamphenicol, florfenicol, chloramphenicol. Chloramphenicol is an antimicrobial with a carcinogenic potential and an unaccepted substance to be used on animals intended for human consumption, including beekeeping (Orso et al., 2015). **Tetracyclines** are used for the bacterial diseases' treatment of the bee brood; for example: oxytetracycline, chlortetracycline, tetracycline. The action spectrum resembles to the one of chloramphenicol. **Macrolides** include about 40 antibiotics, among which the most known are erythromycin, tylosin, oleandomycin and spiramycin. There are two groups: macrolides with 14 carbon atoms (erythromycin, oleandomycin) and macrolides with 16 carbon atoms (tylosin, spiramycin) (Mărghitaș et al., 2010). The most known **aminoglycosides** are gentamicin, lincomycin, neomycin and streptomycin. The polar nature of these macrolides makes it difficult to isolate them from the samples and determine their chromatography (Barganska et al., 2011). **Fluoroquinolones** are used as growth promoters; for example: ciprofloxacin, enrofloxacin, norfloxacin. **Organochlorine pesticides** are highly toxic chemical substances used in agriculture to destroy pests. The presence of pesticide residues in honey has needed the establishment of monitoring programs to determine the human exposure. Many studies have shown that organochlorine pesticides accumulate in plants from polluted soil and can enter the food chain not only through fat products but also through non-fat poducts, such as honey (Panseri et al., 2014).

Organophosphorus compounds have been used as pesticides for almost five decades. They continue to be used as insecticides, helminthicides, ascaricides, nematocides and to a lesser extent as fungicides and herbicides. Although they have been and continue to be extremely useful in combating agricultural pests around the world, their widespread use had led to numerous poisonings, even with human victims. The primary acute toxicity to mammals associated with exposure to organophosphorus pesticides results from the acetylcholinesterase enzyme inhibition (Sultatos, 2009).

In Europe, other more commercial products are used by beekeepers to control varroosis: **amitraz**, coumaphos, fluvalinate and thymol (Faucon et al., 1995). *Varroa destructor* is a hematophagus ectoparasite of bees and it is considered to be a major cause of bee colonies loss in Europe and North America (Surlis et al., 2018).

MATERIALS AND METHODS

In this paper the aim was to evaluate the contamination degree of honey sourced from beekeepers from the centre and Southern Romania in order to form a large and homogeneous batch). The analyzes were performed in a laboratory, external of the

processing unit. The study was conducted in the summer of 2019, on 3 batches of honey, collected by a local processor. The first batch consisted of 15 acacia honey samples from different beekeepers, analyzed before the honey homogenization process, the second one also consists of the same number and type of honey samples from other beekeepers from Romania, analyzed after the homogenization process, and the third lot consists of 15 polyfloral honey, each sample being harvasted from different beekeepers and .

The Nitroimidazole residues determination (metronidazole, dimetridazole, ronidazole) was carried out using the quantitative LC-MS/MS method (liquid chromatography-tandem mass spectrometry) (European regulation 37/2010/UE). Over the years, the LC-MS systems suffered significant changes, starting from simple analyses and reaching very accurate qualitative and quantitative analyses (Burian V., 2011). According to 470/2009/CE and 37/2010/CE regulations, the use of antibiotics in beekeeping is not allowed. The quantification limit of the method for metronidazole is 0.5 µg/kg, for dimetridazole is 2.5 µg/kg and for ronidazole is 0.5 µg/kg.

The tetracycline residue determination (oxytetracycline, tetracycline, doxycycline, chlortetracycline, demeclocycline, methacycline, minocycline) was conducted using the quantitative LC-MS/MS method. The quantification limit of the method is of 2 ppb, and according to the (EC) No 470/2009/CE and (EC) No 37/2010/UE Regulations, the use of antibiotics in beekeeping is not allowed.

The macrolide residue determination (clindamycin, erythromycin, josamycin, kitasamycin, lincomycin, oleandomycin, spiramycin, mirosamycin, tilmicosin, trimethoprim, tylosin) was carried out using the LC-MS/MS method. For these, there is no legal limit, because the use of antibiotics in beekeeping is not allowed. The quantification limit of this method is 2 ppb (Regulation (EC) No 37/2010/UE).

The Nitrofuran metabolites determination (semicarbazide from nitrofurazone, AOZ from furazolidone, AHD from nitrofurantoin) was conducted using the LC-MS/MS method. These substances are prohibited according to the Regulation (EC) No 37/2010/UE. The quantification limit of the method used is 1 µg/kg (Regulation (EC) No 2003/181/CE).

The chloramphenicol determination was conducted using the ELISA method. This is an officialy appoved method. In accordance with Regulation (EC) No 2002/657/CE, up to 5% false negative results may occur. Chloramphenicol is a prohibited substance according to Regulation (EC) No 37/2010/UE. The quantification limit of the sample is 0.1 ppb (Regulation (EC) No 2003/181/CE).

The streptomycin and dihydrostreptomycin residues detection were carried out using the LC-MS/MS method. For these, there is no legal limit, because the use of antibiotics in beekeeping is not allowed. The quantification limit of this method is 2 ppb (Regulation (EC) No 37/2010/UE).

The sulfonamides and trimethoprim detection was made using the LC-MS/MS method. There were determined: sulfadimethoxine, sulfaquinoxaline, sulfamethizole, sulfachlorpyridazine, sulfamoxole, sulfadoxine, sulfasalazine, sulfabenzamide, sulfaguanidine, sulfanilamide, sulfacetamide, sulfadiazine, sulfathiazole, sulfapyridine, sulfamerazine, sulfamethazine, sulfamethoxypyridazine, sulfamethoxazole, trimethoprim, sulfamonomethoxine, sulfaclozine, sulfisoxazole, succinylsulfathiazole, sulfaphenazole, sulfisozole, sulfisomidine. The quantification limit of the method is between 0.5-2 µg/kg. For these, there is no legal limit, because the use of antibiotics in beekeeping is not allowed (Regulation (EC) No 37/2010/UE).

The Glyphosate residues determination was conducted using LC-MS/MS method. The quantification limit of this method is 0.010 mg/kg, the maximum residue level allowed is 0.050 mg/kg (Regulation (EC) No 369/2005/UE).

RESULTS AND DISCUSSIONS

Results and discussions regarding the contamination degree of the lot 1

The analysis of the 15 acacia honey samples, which form the first batch of the present study, the following results were obtained:

Results and discussion regarding the nitroimidazole residues determination: following the method used, LC-MS/MS, for the 15 acacia honey samples, there were obtained values below the limit of quantification, respectively: metronidazole < 0.5 μg/kg, dimetridazole < 2.5 μg/kg, ronidazole < 0.5 μg/kg. Considering the limit of quantification indicated above, this result was in accordance with Regulation (EC) No 37/2010/UE.

Results and discussion regarding the tetracycline residues determinantion: regarding the results obtained from the analysis of the acacia honey samples by the LC-MS/MS method, the values obtained are below the limit of quantification, respectively < 2 μg/kg. Considering the limit, the result was in accordance with Regulation (EC) No 37/2010/UE.

Results and discussion regarding the macrolide residues determination: following the test of the 15 acacia honey samples by the LC-MS/MS method, the results are below the limit of quantification (< 2 μg/kg). Therefore, the result was in accordance with Regulation (EC) No 37/2010/UE.

Results and discussion regarding the nitrofuran metabolites determination: the results obtained by LC-MS/MS testing of the 15 acacia honey samples are below the limit of quantification, respectively (< 1 μg/kg). The result was in accordance with the Regulation (EC) No 37/2010/UE.

Results and discussion regarding the chloramphenicol determination: following the analysis of the 15 acacia honey samples by the ELISA method, the result obtained is bellow the quantification limit of the method (< 0.1 μg/kg). Considering the limit, the result was in accordance with Regulation (EC) No 37/2010/UE.

Results and discussion regarding the streptomycin and dihydrostreptomycin residues detection: the results obtained from the analysis of the acacia honey samples by the LC-MS/MS method regarding the detection of streptomycin and dihydrostreptomycin residues, are below the limit of quantification (2 ppb). Considering the limit, the result was in accordance with Regulation (EC) No 37/2010/UE (regarding the residues of pharmacologically active substances in food products of animal origin).

Results and discussion regarding the sulfonamides and trimethoprim detection: regarding the analysis of the acacia honey samples by the LC-MS/MS method, the following average results were obtained (Table 1):

Table 1. Results and discussion regarding the sulfonamides and trimethoprim detection

Analyzed parameter in μg/kg	LOQ*	Result
Sulfadimethoxine	0.5	n.n. **
Sulfaquinoxaline	0.5	n.n.
Sulfamethizole	1	n.n.
Sulfachlorpyridazine	2	n.n.
Sulfamoxole	1	n.n.
Sulfadoxine	0.5	n.n.
Sulfasalazine	2	n.n.
Sulfabenzamide	0.5	n.n.
Sulfaguanidine	2	n.n.
Sulfanilamide	2	n.n.
Sulfacetamide	2	n.n.
Sulfadiazine	1	n.n.
Sulfathiazole	0.5	n.n.
Sulfapyridine	1	n.n.
Sulfamerazine	1	n.n.
Sulfameter	1	n.n.
Sulfamethazine	1	n.n.
Sulfamethoxypyridazine	0.5	n.n.
Sulfamethoxazole	1	n.n.
Trimethoprim	0.5	n.n.
Sulfamonomethoxine	0.5	n.n.
Sulfaclozine	2	n.n.
Sulfisoxazole	1	n.n.
Succinylsulfathiazole	2	n.n.
Sulfaphenazole	2	n.n.
Sulfisozole	2	n.n.
Sulfisomidine	1	n.n.

*LOQ = limit of quantification;

**n.n. = below the limit of quantification.

Considering the limit, the result was in accordance with Regulation (EC) No 37/2010/UE.n

All the results obtained from the analysis of the batch1 (Table 2) werein accordance with Regulation (EC) No 37/2010/UE.

Table 2. The obtained results regarding the contamination degree of batch 1 of acacia honey

Performed analyses	Result	Allowed limit
β-γ-amylase activity	2.9 U/kg	Max. 5 U/kg
Nitroimidazoles	n.n.** (0.5-2.5 µg/kg)	MRL*
Tetracyclines	n.n. (2 µg/kg)	MRL
Macrolides	n.n. (2 µg/kg)	MRL
Nitrofuran metabolites	n.n. (1 µg/kg)	MRL
Chloramphenicol	n.n (0.1 µg/kg)	MRL
Streptomycin anddihydrostreptomycin	n.n. (2 µg/kg)	MRL
Sulfonamides and Trimethoprim	n.n. (0.5-2 µg/kg)	MRL

*MRL = forbidden substance (Regulation (EC) No 37/2010/UE);
**n.n. = below the limit of quantification.

Results and discussions regarding the contamination degree of the batch 2

After the analysis of the 15 acacia honey samples, which formed the batch 2, tested in the summer of 2019, the following results were obtained:

The results regarding the **glyphosate residues** determination are below the limit of quantification (0.010 mg/kg), the maximum allowed residue level being 0.050 mg/kg (Regulation (EC) No 369/2005/UE).

Regarding the results of the **nitroimidazole residues** determination, following the used method, LC-MS/MS, there were obtained values below the quantification limit in batch 2, respectively metronidazole < 0.5 µg/kg, dimetridazole < 2.5 µg/kg, ronidazole < 0.5 µg/kg. Considering the limit of quantification, the result was in accordance with Regulation (EC) No 37/2010/UE (regarding the residues of pharmacologically active substances in food products of animal origin).

The **nitrofuran metabolites** determination, bythe LC-MS/MS method showed values below the limit of quantification, respectively (< 1 µg/kg).

Following the **sulfonamides and trimethoprim residues** detection**,** by the LC-MS/MS method, there were obtained results below the quantification limit of the method, respectively below 0.5-2 µg/kg, depending on the analyzed parameter. Considering the limit of quantification, the result was in accordance with Regulation (EC) No 37/2010/UE (regarding the residues of pharmacologically active substances in food products of animal origin).

The **chloramphenicol determination** by the ELISA method, led to results below the quantification limit of the method (< 0.1 µg/kg). Taking into account the quantification limit indicated previously, the result was in accordance with Regulation (EC) No 37/2010/UE (regarding the residues of pharmacologically active substances in food products of animal origin).

The results regarding the **streptomycin and dihydrostreptomycin residues** detection**,** by the LC-MS/MS method, are below the limit of quantification (2 ppb). Taking into account the quantification limit indicated previously, this result was in accordance with Regulation (EC) No 37/2010/UE.

All the results obtained from the analysis of the batch 2 (Table 3) werein accordance with Regulation (EC) No 37/2010/UE.

Table 3. The obtained results regarding the contamination degree of batch 2 of acacia honey

Performed analyses	Result	Allowed limit
Glyphosate	n.n.** (0.010 mg/kg)	0.050 mg/kg
Nitroimidazoles	n.n. (0.5-2.5µg/kg)	MRL*
Nitrofuran metabolites	n.n. (1 µg/kg)	MRL
Chloramphenicol	n.n (0.1 µg/kg)	MRL
Streptomycin anddihydrostreptomycin	n.n. (2µg/kg)	MRL
Sulfonamides andtrimethoprim	n.n. (0.5-2 µg/kg)	MRL

*MRL= forbidden substance (Regulation (EC) No 37/2010/UE);
**n.n. = below the limit of quantification.

Results and discussions regarding the contamination degree of the batch 3

Following the analysis of the 15 polyfloral honey samples, which form the third batch of the present study, the following results were obtained:

Following the **glyphosate residues** analysis by the LC-MS/MS method, the obtained result is

below the limit of quantification (0.010 mg/kg), the maximum residue level allowed being 0.050 mg/kg (Regulation (EC) No 369/2005/UE).

The **chloramphenicol determination** by the ELISA method, led to values (maybe better in English) below the quantification limit of the method (< 0.1µg/kg). Considering the limit of quantification, the result was in accordance with Regulation (EC) No 37/2010/UE.

Following the **nitroimidazole residues** determination (metronidazole, dimetridazole, ronidazole) for the 15 polyfloral honey samples, there were obtained values below the limit of quantification, respectively: metronidazole < 0.5 µg/kg, dimetridazole < 2.5 µg/kg, ronidazole < 0.5 µg/kg. Taking into account the quantification limit indicated previously, the result was in accordance with Regulation (EC) No 37/2010/UE.

The **sulfonamides and trimethoprim residues** detection by the LC-MS/MS method showed values below the quantification limit of the method, respectively below 0.5-2 µg/kg, depending on the analyzed parameter. Considering the limit of quantification, the result was in accordance with Regulation (EC) No 37/2010/UE.

The **macrolide residues** are below the limit of quantification (< 2 µg/kg). Therefore, the result was in accordance with Regulation (EC) No 37/2010/UE.

For the **nitrofuran metabolites** determination (semicarbazide from nitrofurazone, AOZ from furazolidone, AHD from nitrofurantoin, AMOZ from furaltadon), there obtained results were below the limit of quantification, respectively (< 1 µg/kg), the result being in accordance with Regulation (EC) No 37/2010/UE.

Regarding the results of the **tetracycline residues** determinantion for the 15 polyfloral honey samples, the values obtained were below the quantification limit, respectively < 5 µg/kg. Considering the limit of quantification, the result was in accordance with Regulation (EC) No 37/2010/UE.

All the results obtained from the analysis of the batch3 were in accordance with Regulation (EC) No 37/2010/UE.

CONCLUSIONS

The results of toxic substances residues analysis (nitroimidazoles, tetracyclines, macrolides, nitrofuran metabolites, chloramphenicol, streptomycin, dihydrostreptomycin, sulfonamides and trimethoprim**)** for the acacia honey samples, which represent **Batch 1**, are below the limit of quantification.

Regarding Batch 2, consisting of 15 acacia honey samples, the value of glyphosate is < 0.050 mg/kg and the residues of: nitroimidazoles, tetracyclines, macrolides, nitrofuran metabolites, chloramphenicol, streptomycin, dihydrostreptomycin, sulfonamides and trimethoprimare below the limit of quantification.

For the polyfloral honey samples which form the Batch 3, the residues of: nitroimidazoles, tetracyclines, macrolides, nitrofuran metabolites, chloramphenicol, streptomycin, dihydrostreptomycin, sulfonamides and trimethoprim are below the quantification limit.

Following the conducted study on the 3 batches of honey, sourced from local beekeepers and analyzed in the summer of 2019, results were in accordance with the national and international legal requirements.

REFERENCES

Barganska, Z., Namiesnik, J., Slebioda, M. (2011). Determination of antibiotic residues in honey. *TrAC Trend in Analytical Chemistry*, 30, 1035-1041.

Burian (Bonta), V. (2011). Chromatographic techniques for determining contaminants in bee honey, University of Agronomic Sciences and Veterinary Medicine Cluj-Napoca, Doctoral School, Faculty of Animal Science and Biotechnology.

Alquani, A.S., Owayss, A.A., Mahmoud, A.A. (2012). Mineral content and physical properties of local and imported honeys in Saudi Arabia, *Journal of Saudi Chemical Society*, 5, 618-625.

Crivineanu, V, Goidea, A., Goran, G.V., Tudoreanu, L., Cretu, L.G. (2011). Findings on rheological and mineral parameters and heavy metal contamination of honeybees. *Lucrări Ştiinţifice Medicină Veterinară*, XLIV (1), 241-249.

Dobre, I. R., Ghiță, M., Fernoagă, C., Gajailă, G., Cotor, G. (2016). The principles and the regulation of organic beekeeping - conditions for ensuring the organic honey on the community market. *Journal of Biotechnology*, 231, S63.

Dobre, I. R., Marmandiu, A., Gajaila, G., Cotor, G., Ghiță, M., Fernoagă, C. (2017). Prevention of

diseases in the beekeeping holdings–An essential condition for obtaining organic honey. *Journal of Biotechnology*, Volume 256, Page S68, ISSN 0168-1656.

Doliș, M. (2009). *Beekeeping and sericulture / Apicultură și sericicultură*. Iași, RO: Alfa Publishing House.

Faucon, J.P., Drajnudel, P., Fleche, C. (1995). Mise en evidence d'une diminution de l'efficacité de l'Apistanutilisécontre la varroase de l'abeille (*Apis mellifera* L). *Apidologie*, 26, 291-296.

Lazăr Ștefan, Vornicu O.C., (2006). *Beekeeping/Apicultura*. Bucharest, RO: Alfa Publishing House.

Mărghitaș, L.Al. (2010). *Modern methods for determining residues and contaminants in honey and bee products/Metode moderne de determinare a reziduurilor și contaminanților din miere și produse apicole*, Bucharest, RO: Academic Pres Publishing House.

Murariu, F., Voda, A.D., Murariu, O.C. (2019), Researches on food safety assessment - supporting a healthy lifestyle for the population from NE of Romania, *Journal of Biotechnology*, 305, s68-s68.

Murariu, O.C., Irimia, L.M., Robu, M., Işan, E. (2019), Ensuring nutrition security and sustainability of food systems as basis of human healhy life. *Proceeding of the International Scientific Congress „Life sciences, a challenge for the future",* Filodiritto International Proceedings Editore, 175-180.

Orso, D., Floriano, L., Ribeiro, L., Bandeira, L., Prestes, O., Zanella, R. (2015). Simultaneous Determination of Multiclass Pesticides and Antibiotics in Honey Samples Based on Ultra-HighPerformance Liquid Chromatography-Tandem Mass Spectrometry. *Food Analytical Methods*, 9, 1638-1653.

Panseri, S., Catalano, A., Giorgia, A., Aricoli, F., Procopio, A., Chiesa, L. (2014). Occurrence of pesticide residues in Italian honey from different areas in relation to its potential contamination sources. *Food Control*, 38, 150-156.

Petcu, C.D. (2006). *HACCP-Food safety guarantor*. Bucharest, RO: Idea Design Publishing House.

Petcu, C.D. (2014a). *Packaging used in the food industry/Ambalaje utilizate în industria alimentară*. Bucharest, RO: Granada Publishing House.

Petcu, C.D., Șulea, C., Dumitrache, M. (2014b). Audit of Producers/Users of Compressed Air and other Industrial Gases used in the Food Industry, *Quality-Access to Success*, 15 (130).

Sapna, J., Nimisha, J. (2010). Antibiotics residues in Honey, New Delhi, *Center for Science and Environment.*

Savu, C., Petcu, C.D. (2002). *Hygiene and control of products of animal origin/Igiena și controlul produselor de origine animală*. Bucharest, RO: Semne Publishing House.

Sultatos, L.G. (2009). Mammalian toxicology of organophosphorus pesticides. *Journal of Toxicology and Environmental Health*, 43, 271-289

Surlis, C., James, C.C., Coffey, M., Kavanagh, K. (2018). Quantitative proteomics reveals divergent responses in *Apis mellifera* worker and drone pupae to parasitization by *Varroua destructor*. *Journal of Insect Physiology,* 107, 291-301.

Șapcaliu, A., Savu, V., Radoi, I., Tăpăloagă, D., Tanase, P., Calin, V. (2017). Evaluation of results in research made in order to obtain a phytotherapeutic product for the prophylaxis and fight against nosema in bees. *The EuroBiotech Journal*, 1(1), 36-40.

Tamas-Krumpe, O., Bobiș, O., Mărgăoan, R., Chirilă, F., Laţiu, C., Ognean, L. (2019). Correlative research regarding the total polyphenolic content, antioxidant and antibacterial activity of three types of romanian honey. *Scientific Papers. Series D. Animal Science*, LXII (2), 282-288.

Tofană, M. (2011). *Food contaminants/Contaminanți alimentari*, Cluj-Napoca, RO: Mega Publishing House.

Tănăsoiu, I.C., Drăgotoiu, D., Drăgotoiu, T., Marin, M. (2014). Researches concerning the effects of supplementary feeding of bees families during autumn, winter, spring. *Scientific Papers. Series D. Animal Science*, LVII, 112-114.

Tănăsoiu, I.C., Drăgotoiu, D., Marin, M., Drăgotoiu, T., Diniță, G. (2015). Research on the influence of eco certificated energo-protein use over the performance of bee families. *Agriculture and Agricultural Science Procedia*, 6, 265-271.

Tăpăloagă, D., Tăpăloagă, P.R. (2017). Study regarding animal organic farming in Romania - current status and trends, *Scientific Papers. Series D. Animal Science*, LX.

Tăpăloagă, D., Tăpăloagă, P.R. (2018), From conventional to organic agriculture - romanian past and future perspectives. *Scientific Papers. Series D. Animal Science*, 61(1), 239-244.

Tudoreanu, L., Codreanu, M.D., Crivineanu, V., Goran, G.V. (2012). The quality of Romanian honey varieties - mineral content and textural properties. *Bulletin USAMV*, 69(1-2), 452-58.

Vlaic, R.A., Muresan, A.E., Mureșan C.C., Petrut, G.S., Muresan, V., Muste, S. (2018). Quantitative analysis by HPLC and FT-MIR prediction of individual sugars from the plum fruit harvested during growth and fruit development. *Agronomy*, 8, 306, 1-16.

Zacharis, K., Rotsis, I., Zachariadis, P.G., Zotos, A. (2012). Dispersive liquid-liquid microextraction for the determination of organochlorine pesticides residues in honey by gas chromatography-electron capture and ion trap mass spectrometric detection. *Food Chemistr*y, 134, 1665-1672.

Zacharis, K., Rotsis, I., Zachariadis, P.G., Zotos, A. (2012). Dispersive liquid–liquid microextraction for the determination of organochlorine pesticides residues in honey by gas chromatography-electron capture and ion trap mass spectrometric detection, *Food Chemistry,* 134, 1665-1672

Commission Regulation (EC) 2002/657 laying down detailed rules for the application of Council Directive 96/23/EC on the operation of methods of analysis and interpretation of results.

Commission Regulation (EC) 2003/181 laying down minimum performance limits (MRLs) for certain residues in food of animal origin.

Regulation (EC) No 369/2005 of the European Parliament and of the Council on the maximum levels for pesticide residues in or on food and feed of plant and animal origin.

Regulation (EC) No 470/2009 of the European Parliament and of the Council laying down Community procedures for the determination of residue limits of pharmacologically active substances in foodstuffs of animal origin.

Commission Regulation (EU) 37/2010 on pharmacologically active substances and their classification according to maximum residue limits in foodstuffs of animal origin.

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MONITORING THE LEAD CONTAMINATION OF FOOD PRODUCTS OF NON-ANIMAL ORIGIN IN DIFFERENT REGIONS FROM ROMANIA IN 2019

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Abstract

The monitoring of lead contamination of food products of non-animal origin started in Romania in 2006, at the same time with entry into force of The European Commission Regulation (EC) no. 1881/2006 which regulates the maximum allowed values for this contaminant. In this study, samples that were collected and analyzed to determine lead contamination in 2019 have been analyzed by graphite furnace atomic absorption spectrometry (GFAAS)*. The samples came from Bucharest and from 12 other counties of the country. The cereals, wine, fruit, vegetables and mushrooms samples present a weaker lead contamination, compared to the fruit juice samples. Considering the results, all of the analyzed samples framed within recommended values regarding the lead contamination, and the average values falls within those of the European Union.*

Key words*: lead, food safety, contamination.*

INTRODUCTION

Lead is a contaminant that commonly appears in the environment as a result of different types of industrial activity (Tiwari and Tripathi, 2012; Tiwari et al., 2013).

The accumulation of lead in water and soil depends on many factors such as pH, mineral composition and the type of organic material in which these contaminants are found (Khan and Ghouri, 2011).The lead found in soil, based on natural cycle, may be transfered in different types of food (vegetables, fruit, cereals, etc.)

Since 2006, The International Agency for Research on Cancer (IARC) has put lead in the 2A group, meaning the group of substances which probably have a carcinogenic effect on humans.

People's exposure to lead is made through food, cigarettes, water, soil, garbage and air (WHO, 1996; Russell, 1989; Philip and Gerson, 1994).

Lead is found under both forms: organic and anorganic lead. In the environment it is found especially under the form of anorganic lead. The fact that organic forms are much more toxic than the anorganic ones is well-known (EFSA, 2012). According to IARC, the organic lead compounds are converted partially in anorganic lead compounds. (EFSA, 2012).

Lead accumulates especially in the bone tissue, being gradually released from there into the bloodstream during physiological or pathological periods of demineralisation such as gestation, lactation and osteoporosis (EFSA 2013). Lead can be transferred from mother to fetus/baby during both pregnancy and lactation. Lead affects the functioning of all systems in the body, from the circulatory system to the immune and reproductive systems (Araki et al., 1986; Papanikolaou et al., 2005).

Food is a source of lead exposure, and the main technique of determination is represented by the atomic absorption with flame or graphite furnace.

The absorption of lead in the digestive tract depends in particular on the physiological characteristics of the host as well as the characteristics of the material that can be ingested or inhaled. The absorption of lead by inhalation is higher. The absorbed lead is transported through the bloodstream and initially reaches the soft tissues such as the liver, kidneys and later the bone tissue where it accumulates with age. The half-life of lead in the blood is about 30 days and in the bone tissue it can reach up to 10 years (Landrigan et al., 1990; De Haro et al., 2001; EFSA, 2013; CodexSTAN, 1995). The lead excretion is mainly through urine and feces.

During the last years, a decrease in lead food contamination has been observed, most likely due to the increasing use of unleaded fuels as well as due to various environmental protection actions (EFSA, 2012). Regarding food, the highest concentration of lead is found in organs, then in beverages, bread and vegetables.

It is already known that the packaging or the packaging system, in gas mixtures used for packaging in controlled atmosphere are used, can influence the degree of contamination of food products (Petcu et al., 2014; Vișoescu et al., 2015). Studies are available on monitoring food contamination with mercury, iron, antibiotic residues, mycotoxins, deoxynivalenol, pesticides, genetically modified organisms) (Marin et al., 2013; Goncearov et al., 2015; Petcu et al., 2019; Pogurschi et al., 2015). The monitoring of lead contamination in our country is performed according to the Surveillance and Control Program in the field of Food Safety approved by the Order of the President of ANSVSA No. 35/2016 with subsequent amendments and completions. The monitorig of food safety in needed in order to be able tu guarantee the food safety (Savu et al., 2002; Petcu, 2006).

This study aims to monitor the lead contamination of various foods of non-animal origin in 2019. The samples were collected from different counties of the country and Bucharest, being analyzed in an accredited laboratory in Romania.

MATERIALS AND METHODS

In 2019, a number of 288 samples represented by non-animal food products (cereals, fruit, vegetables, mushrooms, fruit juices and wine) were analyzed by GFAAS in an accredited laboratory in Romania (Figure 1).

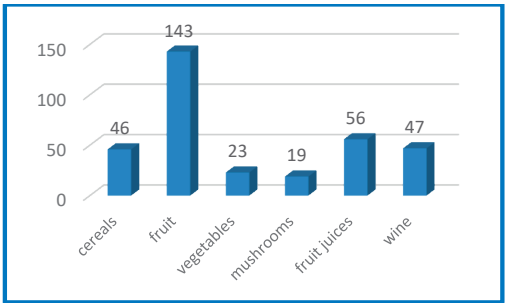

Figure 1. Types of analyzed samples in order to determinate lead residues - 2019

The samples came from Bucharest and from 8 other counties of the country.

Sampling and methods of analysis play a decisive role in determining lead contamination of foodstuff. The sampling and analysis criteria were first established in the European Union (EU) by EC Directive no. 2001/22/EC which was subsequently replaced by EC Regulation no. 333/2007 on sampling and analysis methods for the official control of lead, cadmium, mercury, 3MCPD (3-monochloropropane-diol) and bezopiren in foodstuff.

In order to protect public health, at the level of the European Union maximum limits have been established for lead in various foods, both of animal and non-animal origin by European Commission Regulation no. 1881/2006, with subsequent amendments and completions.

The maximum permitted limits have been set by reference to the edible part of a product, while the product undergoes or transforms by processing, dilution, etc., this aspect can be taken into account while reporting and interpreting the results.

All maximum permissible limits for lead are expressed in milligrams/kilogram (mg/kg), and the performance criteria and analysis

methodology are in EC Regulation no. 333/2007, can be modified and completed afterwards.
The analytical technique used was atomic absorption with graphite furnace (GFAAS), and the performance criteria corresponded to those of EC Regulation no. 333/2007 with subsequent amendments and completions, respectively: detection limit (LOD), quantification limit (LOQ), recovery, reproducibility, uncertainty.
The following definitions are used under this Regulation:
The limit of detection is the lowest measured content, from which the presence of the analyte can be deduced with reasonable statistical certainty. The limit of detection is numerically equal to three times the standard deviation of the mean of the control determinations (n>20).
The limit of quantification is the lowest analyte content that can be analyzed with reasonable statistical certainty. If both the precision and the accuracy are constant for a range of concentrations around the limit of detection, then the limit of quantification is numerically equal to six to ten times the standard deviation of the mean of the control determinations (n>20).
The repeatability is the value below which the absolute difference between the results obtained in the individual tests under repeatability conditions (for example, the same sample, the same operator, the same apparatus, the same laboratory and in a short period of time) is expected to be within a certain probability interval (usually 95%) and therefore $r = 2.8 \times sr$.
"Sr" - the standard deviation calculated from the results generated under repeatability conditions.
"RSDr" - the relative standard deviation calculated from the results generated under repeatability conditions.
"sR" - the standard deviation calculated from the results generated under reproducibility conditions.
"RSDR" - the relative standard deviation calculated from the results generated under reproducibility conditions.
The reproducibility is the value below which the absolute difference between the results obtained in individual tests under reproducibility conditions (for example, on identical material obtained by operators in different laboratories using the standardized test method) is expected to be within a certain range of probability (usually 95%); $R = 2.8 \times sR$.
The uncertainty is the extended measurement uncertainty, using a coverage factor 2 gives a confidence level of approximately 95% ($U = 2u$).
The reagents used (hydrogen peroxide, nitric acid, matrix modifier) are represented by ultrapure reagents in which the concentration of heavy metals is very low so as not to influence the results, in the sense of the appearance of false positive results.
Some studies have highlighted the existence of lead contaminants in fish samples (Hristov and Kirin, 2014).

RESULTS AND DISCUSSIONS

Results and discussions on lead contamination of grain samples
The monitored cereals regarding lead contamination are those provided in the Surveillance and Control Program in the field of food safety approved by the Order of the President of ANSVSA no. 35/2016 and for which maximum limits are set according to EC Regulation no. 1881/2006 with subsequent amendments and completions.
In 2019, a number of 46 wheat samples were analyzed.
For wheat, the maximum limit allowed according to EC regulation no. 1881/2006 is 0.2 mg/kg.
Out of a total of 46 samples, a number of 18 samples were reported with undetectable results, a number of nine samples resulted in 0.01 mg/kg, for three wheat samples. The results are: 0.02 mg/kg, two samples had the value of 0.03 mg/kg, one sample had the value of 0.04 mg/kg, and 13 samples had values lower than the quantification's limit of the method, respectively 0.007 mg/kg.
The results obtained from the analysis of lead residues coming from the wheat samples are presented in Figure 2.

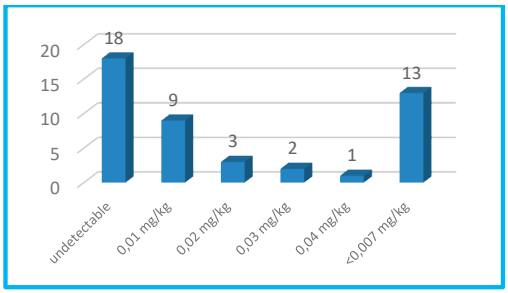

Figure 2. Results of lead contamination of cereals samples

Results and discussions on lead contamination of fruit samples

In 2019, 143 apple samples were analyzed in the study. A number of 76 samples had the result expressed as undetectable, while a number of 32 samples had the value of 0.01 mg/kg. For 18 samples the value of 0.02 mg/kg was registered, for nine samples the value of 0.03 mg/kg was registered, for two samples the value of 0.04 mg/kg was registered, and for six samples the value was < 0.007 mg/kg.

According to Reg. Ec. 1881/2006, the maximum allowed limit for lead residues in fruits is 0.1 mg/kg.

In the Figure 3 are presented the results obtained in the present study, for the determination of lead residues in apple samples.

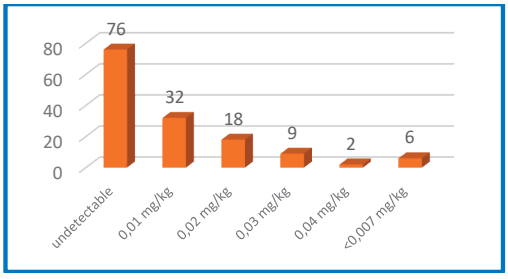

Figure 3. Results of lead contamination of apple samples

Results and discussions on lead contamination of vegetables samples

A number of 23 samples of fresh vegetables were analyzed, represented by carrots, eggplants, potatoes, cucumbers, cabbage and celery. For a number of eight samples, the results were reported with undetectable values, in ten samples the value of 0.01 mg/kg was recorded, in one sample the value of 0.02 mg/kg was recorded, in one sample the value of 0.03 mg/kg was recorded, and in two samples the value of 0.05 mg/kg was registered. A value of <0.007 mg/kg, the limit of quantification of the method, was recorded in one sample.

The maximum permissible limit for lead, according to Reg. Ec. 1881/2006 for vegetables, except *Brassica*, fresh leafy vegetables and herbs is 0.1 mg/kg, and for *Brassica* and leafy vegetables is 0.3 mg/kg. For potatoes, the maximum level applies to peeled potatoes.

Figure 4 shows the graphical distribution of the results of lead contamination of the vegetable samples analyzed during the study.

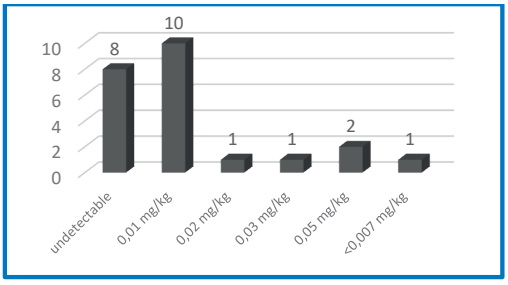

Figure 4. Results of lead contamination of vegetables samples

Results and discussions on lead contamination of mushroom samples

In the EC Regulation no. 1881/2006, subsequently amended and supplemented, the maximum permitted limits for cultivated mushrooms are set at a maximum level of 0.3 mg/kg wet weight.

In 2019, a number of 19 mushroom samples were analyzed, represented in about 90% of mushroom cases. For a number of nine samples, undetectable values of lead residues were obtained, and for the rest of the samples different values were reported according to Figure 5.

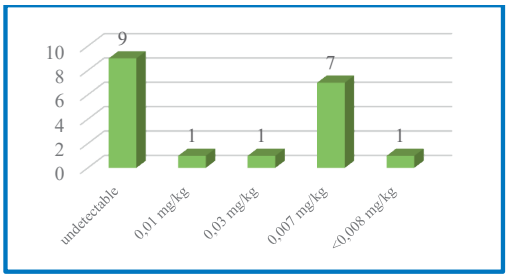

Figure 5. Results of lead contamination of mushrooms samples

Results and discussions on lead contamination of fruit juice samples

The maximum permitted limit provided in EC Regulation no. 1881/2006, with subsequent amendments and completions, is different depending on the types of fruit used. Thereby, for fruit juices, reconstituted concentrated fruit juices and fruit nectars, the accepted value is a maximum of 0.05 mg/kg.

Out of the total 56 samples of fruit juices analyzed in 2019, a number of 15 samples were reported with undetectable values, and the rest of the samples registered different values according to Table 1 and Figure 6.

Table 1. Results of lead residues from fruit juice samples

Year	Samples with undetecta-ble results	Samples with 0.01 mg/ kg	Samples with 0.02 mg/ kg	Samples with 0.05 mg/ kg	Samples with 0.007 mg/ kg
2019	15	20	13	1	7

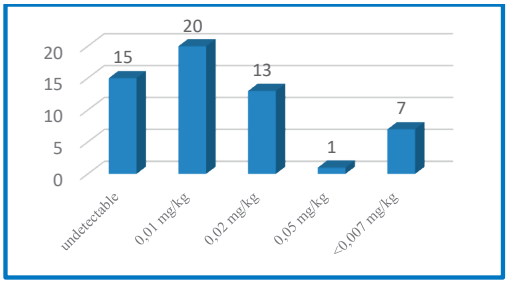

Figure 6. Results of lead contamination of fruit juice samples

Results and discussions on lead contamination of wine samples

Out of the total 47 wine samples analyzed during the study, for 29 of them the result was undetectable. The highest value was recorded in three samples for which the value of the results obtained was 0.05 mg/kg, within the limits of legal acceptability.

In Table 2 and Figure 7 present distribution of the results concerning lead contamination of the analyzed grape wine samples.

Table 2.Results of lead residues from the wine samples

Year	Samples with undetec-table results	Samples with 0.01 mg/kg	Samples with 0.02 mg/kg	Samples with 0.03 mg/kg	Samples with 0.05 mg/kg	Samples with 0.007 mg/kg
2019	29	6	2	2	3	5

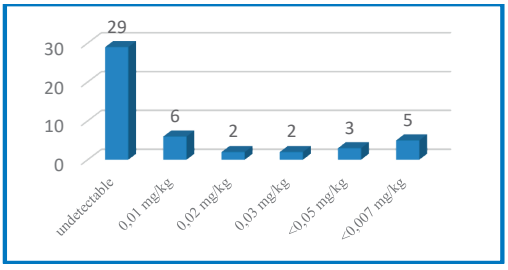

Figure 7. Results of lead contamination of wine samples

The maximum allowed limit provided in the EC Regulation no. 1881/2006, with subsequent amendments and completions, is 0.2 mg/kg for wines obtained from the fruit harvested during 2001 and 2015 and 0.15 mg/kg for wines obtained from the harvest starting with 2016. In the European regulation are defined 2 wine categories for which maximum permitted limits are set.

CONCLUSIONS

From the total samples of cereals analyzed, 39.13% had results expressed as undetectable, and the rest of the samples identified values that are much lower than the maximum allowed value.

Undetectable values were obtained at the analysis of 53.15% of apple samples, and at a percentage of 1.39% samples had two times lower results compared to the maximum allowed value, and 5.59% samples recorded values 10 times lower than the maximum allowed limit.

In the analysis of vegetables, 34.78% of the samples did not contain lead residues at a detectable level by the analysis method perfor-med, and 43.48% of the samples recorded 10

times lower values compared to the maximum allowed limit.

Almost half of the analyzed mushroom samples (47.37%) recorded an undetectable level.

Regarding the fruit juice, the lowest number of samples with undetectable level was recorded, in 26.78% of samples.

61.70% of the wine samples had undetectable content, and for some samples, even 10 times lower values than the maximum accepted average was identified.

The samples of cereals, wine, fruits, vegetables and mushrooms showed lower lead contamination compared to the samples of fruit juice.

All of the analyzed samples corresponded in terms of lead contamination.

Finally, we could say that the level of lead contamination of non-animal food products in Romania falls within the provisions of current legislation is linked to the increasingly present policy at national and international level to reduce the degree of lead contamination by effectively applying various social and economical measures. One of the most well-known measures is the gradual giving up on the usage of fuels based on lead. The fact that lead contamination has as an important source a series of industries (battery production, extraction of various ores, burning of fuels containing lead) causes pollution of water, atmosphere and different crops of cereals, vegetables and fruits which are the main sources of food.

REFERENCES

Araki, S., Aono, H., Yokoyama, K., et al. (1986). Filterable plasma concentration, glomerular filtration, tubular reabsorption and renal clearance of heavy metals and organic substances in metal workers. *Arch Environ Health*, 41, 216-221.

De Haro, L., Prost, N., Gambini, D., Bourdon, J. P., Hayek-Lanthois, M., Valli, M., Jouglard, J. (2001). Lead poisoning in adults. Experience of Poisoning Control Center of Marseille from 1993-2003. *Presse Med*., 30(37), 1817-20.

Gonciarov, M., Neagu, I., Ghimpeteanu, O. M., Petcu, C. D. (2015). General principles and regulations on obtaining products from genetically modified organisms, *Journal of Biotechnology*, 208, S72-S72.

Hristov, S., Kirin, D. (2014). Accumulation of lead (Pb) in Scardinius erythrophthalmus and Ceratophyllum demersum from freshwater ecosystem biosphere reserve Srebarna, Bulgaria, *Scientific Papers. Series D. Animal Science*, LVII, 290-297.

Khan, A. M., Ghouri, A. M. (2011). Environmental Pollution: Its Effects on Life and Remedies. *Journal of Arts, Science &Commerce*, II (2), 276-285.

Landrigan, P. J., Silbergeld, E. K., Froines, J. R., Pfeffer, R.M. (1990). Lead in the modern workplace [Editorial]. *Am J Public Health*, 80, 907-918.

Marin, M. P., Nicolae, C. G., Tăpăloagă, D., Petcu C., Tăpăloagă, P. R., Dinita, G. (2013). The metabolic utilization of iron and copper in the piglet organism, *Current Opinion in Biotechnology*, 24 (1), S51-S51.

Papanikolaou, N., Hatzidaki, E., Belivanis, S., Tzanakakis, G., Tsatsakis, A. (2005). Lead toxicity update. A brief review, *Med Sci Monit*, 11(10), RA329-336.

Petcu, C. D. (2006). *HACCP-Food safety guarantor*. Bucharest, RO: Idea Design Publishing House.

Petcu, C. D., Sulea, C., Dumitrache, M. (2014). Audit of producers/users of Compresses Air and Other Industrial Gases in the Food Industry, *Quality-Access to Success*, 15(138), 89-92.

Petcu, C. D., Georgescu, I. M., Zvorișteanu, O. V., Negreanu, C. N. (2019), Study referring to the appearance of contamination with deoxynivalenol in grains, grain flour and bakery products on the romanian market, *Scientific Papers. Series D. Animal Science*, 62(2), 214-245.

Pogurschi, E., Ciric, A., Zugravu, C., Patrascu, D. (2015) Indentification of antibiotic residues in raw milk samples coming from metropolitan area of Bucharest. *Agriculture and Agricultural Science Procedia*, 6, 242-245.

Philip, A. T., Gerson, B. (1994). Lead poisoning - Part I. *Clin Lab Med*, 14, 423-44.

Russell, J. (1989). The continuing hazard of lead in drinking water. *Lancet*, 2, 669-673.

Savu, C., Petcu, C. (2002). *Hygiene and control of animal products*. Bucharest, RO: Semne Publishing House.

Tiwari, S., Tripathi, I. P. (2012). Lead Pollution - An Overview, *Int. Res. J. Environment Sci*., 1(5), 84-86.

Tiwari, S., Tripathi, I. P., Tiwari, H. L. (2013). Effects of Lead on Environment, *International Journal of Emerging Research in Management &Technology*, 2(6).

Vișoescu, D. I., Petcu, C. D., Tăpăloagă, D. (2015). Researches regarding the influence of packaging on the quality of some dairy products, *Journal of Biotechnology*, 208, S19-S19.

***CodexSTAN 193 (1995). General Codex Standard for Contaminants and Toxins in Food and Feed, as subsequently amended and supplemented.

***EFSA Journal, (2013). EFSA Panel on Contaminants in the Food Chain (CONTAM), European Food Safety Authority (EFSA), Scientific Opinion on Lead in Food, 22 March 2013, replaces the earlier version published on 20 April 2010, Parma, Italy, 8(4), 1570.

***EFSA, (2012). Scientific Report of EFSA Lead dietary exposure in the European population-European Food Safety Authority European Food Safety Authority Parma, Italy, EFSA Journal, 7, 2831

***Order of the President of the National Sanitary Veterinary and Food Safety Authority (ANSVSA) No. 35/2016 with subsequent amendments and completions regarding the Supervision and Control Program in the field of Food Safety.
*** Regulation (EC) No Commission Regulation (EC) No 1881/2006 of 19 December 2006 laying down maximum levels for certain contaminants in foodstuffs, as subsequently amended and supplemented.
***Regulation (EC) No Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo (a) pyrene in foodstuffs as amended; subsequent additions.
***World Health Organization, (1996). Guidelines for drinking water quality, 2nd ed. Vol. 2. Health criteria and other supporting information. Geneva, 254–75 (2), Lead toxicity update. A brief Review.
***World Health Organization, 2011. Safety evaluation of certain food additives and contaminants, Geneva, Prepared by the Seventy-third meeting of the Joint FAO/ WHO Expert Committee on Food Additives (JECFA), Lead (381-497).

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EXPLORATION OF MANURE FLOUR FROM THE DEGRADATION (MFD) OF BLACK FLY LARVAE (*Hermetia illucens* L.) ON NATIVE LAYING CHICKEN CARCASSES IN SWEET LEMON (*Citrus sinensis*) MARINADE

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Abstract

The part of the piece of meat that has not been separated from the bone was known as carcass, which in the market were found in several forms such as "Dressed" (body parts without blood and feathers), "Eviscerated" (parts of the body without blood, feathers, bowels were clean) and "Ready to cook" was a carcass that has been excreted by blood, feathers, head, feet and all contents of the stomach except gizzard, liver and heart. The use of sweet orange (Citrus sinensis) as an inhibiting factor in the occurrence of meat damage so that the quality of the carcass can be maintained. This research has been carried out through an experiment using a randomized block design (RBD) (Steel and Torrie, 1994) in five replications. As the first factor (A) was the utilization of degradation manure (MFD) consisting of A1 (5% MFD), A2 (10% MFD), A3 (15% MFD) and second factor (B) was soaking time with sweet orange consists of B1 (10 minutes immersion), B2 (20 minutes immersion) and B3 (30 minutes immersion). Color, taste, aroma, texture of meat were the observation variables. The purpose of this study was to determine the effect of MFD and soaking sweet orange on the quality of non-race chicken carcasses. Another aim was to convince the public that the use of MFD flour in non-race chicken feed accompanied by soaking method with sweet oranges as a natural preservative will be able to provide a positive value and influence on the quality of the laying hen meat carcasses was another goal. The results of the analysis of variance showed that the immersion duration of sweet orange had a significantly different effect ($P<0.01$) on taste, not significantly ($P>0.05$) in texture and significantly effect ($P<0.05$) on the color of the chicken carcass. HSD test results that the soaking time of 30 minutes with a combination of the use of 15% MFD was still acceptable. In conclusion, carcass immersed for 30 minutes by giving 15% MFD by organoleptic test for taste, aroma, carcass color, can still be accepted by panellists and the public.

Key words*: aroma, color, flavor, laying chicken (non-race) carcass, MFD, sweet orange.*

INTRODUCTION

The part of meat that was cut after being separated from the head, legs and stomach contents was known as carcass (Winarno, 1993). The good carcasses range from 65-75% of its life's weight to its slender-curved chest shape like a boat, with the traits not pale, yellowish white, no sour smell, no sticky and tasted wet. Factors before slaughtering include genetic, species, breed, livestock type, sex, age and feed and factors after slaughtering including withering methods, carcass pH and storage methods greatly affect the quality of the carcass (Palupi, 1986). Whereas (Winarno, 1993) states that the quality of carcasses of chicken meat was influenced by the type of ration, age, sex, and genotype, including premortem and postmortem environmental factors. Utilization of manure from degradation (MFD) which was biodegradated by the black fly *Hermetia illucens*/Black soldier fly in 10% mixture of chicken feed can maintain the quality of chicken meat. Low cholesterol and blood triglyceride, LDL, HDL content under normal conditions were other effects of MFD utilization (Manangkot et al., 2019). MFD from *Hermetia illucens*/Black soldier fly contains protein, fat, ash (DM basis) (Barragan-Fonseca et al., 2017) suitable for poultry feed (Nyakeri et al., 2016). Furthermore, according to Kim et al., 2011, MFD *Hermetia illucens*/Black soldier fly was an alternative feed material produced from livestock waste and rotten fruit waste containing amylase, lipase and protease enzymes and then overhauled into smaller parts

such as maltose, fatty acids, glycerol, and amino acids. Sweet orange (*Citrus sinensis*) as an antioxidant which contains high vitamin C content generally only bears fruit once a year (Mirah, 1981). According to Faramade (2007), the effect of vitamin C was highly dependent on the storage temperature conditions. Meanwhile, according to Helmiyesi et al. (2008), 15 days of storage will reduce vitamin levels. Besides that, sweet orange also contains glucose, fructose, sucrose and malic acid and citric acid, which by Berlian et al (2016) and Setiawan et al. (2019) reported the ability of lemon *Citrus sinensis* to inhibit the growth of *Escherichia coli* bacteria. Rotinsulu et al. (2014) revealed that the immersion of broiler meat in lemon Cui juice (*Citrus microcarpa*) for one hour organoleptically such as color, aroma, texture, tenderness and flavor was still acceptable to consumers.

Food quality can be determined based on organoleptic assessment if done objectively using tools or subjectively based on the ability to observe the human senses as a panelist (Winarno, 1984). Organoleptic determination was done by a panel test, through the assessment of the aroma, taste, color and texture of meat (Soewarno, 1985), as well as the level of preference (Hafid and Aka, 2009). The carcasses of laying hens are strongly influenced by water content, fat, protein and carbohydrate structure. The protein coagulation, collagen gelatinization, water release and starch gelatinization were factors that affect changes in the texture of the carcass. Soaking sweet orange in laying hens carcasses will provide good organoleptic value through the panelist preference level (Hafid and Aka, 2009).

MATERIALS AND METHODS

The research material was 20 laying chicken carcasses weighing 1.5-2 kg, age 5 months, which comes from the results of maintenance by providing intensive manure feed degradation (MFD) according to treatment. Chickens were fasted before being slaughtered, dipped in hot water 60°C for 30 seconds, removed and put into cold water for 15 seconds then cleaned of feathers, cloaca, viscera of other organs such as liver, gall and heart and head and neck and legs. Then the chest was cut into 36 parts. The sweet orange juice was squeezed as much as 1.5 l divided by 3 containers of 0.5 l each. Each piece of carcass was then soaked in sweet orange juice with the following treatment A1: Soaking for 10 min, A2: Soaking for 20 min, A3: Immersion for 30 min; B1: MFD 5%, B2: MFD 10%, B3: MFD 15%. The observed variables included: taste, aroma, color and texture of the laying hens carcass. Then were organoleptically tested using 15 panelists using hedonic scale (Hafid and Aka, 2009).

RESULTS AND DISCUSSIONS

A. Effect of MFD Hermetia illucens/Black soldier fly larvae level treatment and immersion time of sweet orange (Citrus sinensis) water on organoleptic taste of chicken carcasses.

The mean of observation for the effect of MFD level and the length of soaking in sweet orange based on the level of panellist preference on the taste of carcasses of laying hen was listed in Table 1.

Table 1. Mean influence of MFD larva *Hermetia illucens*/Black soldier fly and soaking time of sweet orange water on the taste of chicken carcasses

Soaking Time (Minutes)	Level of MFD (%)			Mean
	5	10	15	
10	3.67	3.22	3.29	3.39
20	3.62	3.65	3.29	3.52
30	3.75	3.79	3.53	3.69
Mean	3.68	3.55	3.37	

Based on the level of panelists' preference for taste: 3.39-3.69 falls into the category of rather like. ANOVA showed that carcasses containing MFD flour soaked in sweet orange had a significantly different effect ($P<0.01$) on the taste of laying hens. Based on the results of

further tests using the Honestly Significant Difference (HSD) test for the effect of soaking time showed that the taste of carcasses of laying hens soaked for 30 minutes was significantly different (P<0.05) from 20 and 10 minutes for the preference level. Soak for 20 minutes was significantly higher than the 10 minutes immersion. The amount of orange juice that permeates the carcass was proportional to the length of immersion in addition to the fact that in the orange juice there were substances that can improve taste such as sugar and acid (Winarno et al., 1984). Based on the results of further HSD test, the interaction between the MFD level and the immersion time for the taste shows a picture that the taste of the laying hens carcass containing MFD at levels up to 10% for all immersion lengths (10, 20 and 30 minutes) was still acceptable panelist. The presence of protease, amylase, and lipase enzymes in MFD can suppress the growth of bacteria in the carcass so that the endurance of the carcass will be better (Manangkot, 2019) supported by previous research by Yerou et al. (2017), which use sweet orange powder (*Citrus sinensis*) as an antimicrobial and antioxidant activity in food foods. Furthermore, Berlian et al. (2016) reported that lemon juice can inhibit the growth of *E. coli* bacteria.

B. The effect of MFD Hermetia illucens/Black soldier fly level treatment and soaking time of sweet orange juice on the scent of chicken carcasses.

The influence of MFD level and soaking time in sweet orange based on panellists' preference level on the scent of broiler carcass is shown in Table 2.

Table 2. Mean effect of MFD *Hermetia illucens*/Black soldier fly flour treatment and soaking time of sweet orange juice (*Citrus sinensis*) on the scent of laying chicken carcasses

Soaking Time (Minutes)	Level of MFD (%)			mean
	5	10	15	
10	3.85	3.37	3.26	3.49
20	3.81	3.65	3.11	3.52
30	3.65	3.87	3.36	3.63
Mean	3.77	3.63	3.24	

The average effect of soaking time based on the level of panellist preference for aroma ranged from 3.49 to 3.63 (rather dislike). Furthermore, the average influence of storage duration based on the level of panellist preference for the scent of laying chicken carcasses ranged from 3.24 to 3.77 (rather dislike). ANOVA showed that the immersion time had a not significantly different effect (P>0.05) on the scent of broiler carcass, but the interaction between the immersion time and MFD level had a significantly different effect (P<0.01) on the scent of free-range chicken carcasses. Based on the results of further tests using the HSD test for interaction between the MFD level and storage time showed that there are variations in the assessment seen in the panellists, caused by differences in eating habits of the panelists. Different patterns and eating habits in human groups cause different levels of preferences (Hafid and Aka, 2009).

C. The effect of MFD Hermetia illucens/Black soldier fly level treatment and soaking time of sweet orange juice on the color and texture of chicken carcasses

The mean values of observations for the effect of MFD level and sweet orange immersion time based on panelists' level of preference on carcass color and texture are listed in Table 3.

Table 3. Mean value of influence of MFD *Hermetia illucens*/Black soldier fly and old levels soaking sweet orange juice (*Citrus sinensis*) on color and texture of laying chicken carcasses

Soaking Time (Minutes)	Level of MFD (%)			Mean
	5	10	15	
10	3.73	3.16	3.75	3.55
20	3.86	3.57	3.57	3.67
30	3.60	3.85	3.86	3.77
Mean	3.73	3.52	3.72	

The immersion time for the panelists level of preference for color and texture ranged from 3.55 to 3.77 as well as the effect of the MFD level on the preference level ranging from 3.52-3.73 or in the disliked category. Based on ANOVA results, the immersion time had a significantly different effect ($P<0.05$) on the color and texture of chicken carcasses, while the level of MFD and its interactions had a significantly different effect ($P<0.01$) on the color of chicken carcasses. Further tests using the HSD test for soaking time showed that the color of chicken carries soaked for 30 minutes was the same as 20 minutes but was higher ($P<0.05$) than the 10 minutes immersion. Presumably because the longer soaked, the absorption will be higher so that it affects the color and texture of the carcass. Dyes namely carotene and vitamin C can affect the color and texture of the carcass also allegedly because the long soaking affects the number of chemical reactions such as fat oxidation and meat dyes that change the color and texture of the carcass. In addition, the ability of preservatives to prevent chemical reactions that occur was reduced which Mirah (1981) states that poultry meat contains dyes (myoglobin) and the presence of *heme* groups with one *Fe* atom molecule that was easily degraded and hydrolyzed so that the color and texture of the carcass changes. Based on the results of further tests the level of preference by using the HSD test for interaction between the MFD level and the immersion time of sweet orange juice showed that the color and texture of the carcass soaked for 10 minutes at the MFD level was 10% lower than those immersed for 20 and 30 minutes at the MFD level 5-15% at 10, 20 and 30 minutes. While the color and texture of the carcass soaked for 10, 20 and 30 minutes at the 5% MFD level is equal to 10 and 20 minutes at the 15% MFD level even though the preference level was still lower than the 30 minutes MFD 10 and 15% immersion also 20 minutes at MFD level of 5%. Furthermore, the color and texture of the carcass soaked for 10 minutes at the MFD level of 5% was the same as the favorite level soaked for 10 and 30 minutes at the MFD level of 15% and soaked for 30 minutes at the MFD level of 10%. Rotinsulu et al. (2014) suggested that the immersion of broiler meat in lemon cui juice *Citrus microcarpa* for one hour organoleptically for color, aroma, texture, tenderness and flavor were still acceptable to consumers.

CONCLUSIONS

Laying chicken carcasses soaked in sweet orange juice for 30 minutes with an MFD level of 15% can still maintain the quality of the carcass based on organoleptic assessment of the taste, aroma, color and texture of the carcass even though based on the panelists' assessment including the dislike ones, but still acceptable.

REFERENCES

Barbagan-Fonseca, K.B., Dicke, M., van Loon, J.J.A. (2017). Nutritional value of the black soldier fly (*Hermetia illucens* L.) and suitability as animal feed- a review. *J. Insects as Food and Feed*, 3(2), 105-120.

Berlian, Z., Fatiqin, A., Agustina, E. (2016). Penggunaan perasan Jeruk nipis (*Citrus aurantifolia*) dalam Menghambat Bakteri *Escherichia coli* pada Bahan Pangan. *Jurnal Bioilmi*, 2(1), 51-54.

Faramade, O. O. (2007). Kinetics of ascorbic acid degradation in commercial orange juice produced locally in Nigeria. *African Crop Science Conference Proceedings*, 8, 1813-1816.

Harapin, H., Rahim, A. (2016). Pengaruh Pemberian Pasta Jahe (*Zingiber officinale* Rosaceae) Terhadap Kualitas Daging Ayam Kampung. *JITRO*, 3(3).

Manangkot, H.J., Rondonuwu, L.S.J., Pinontoan, O.R., et al. (2014). Black soldier fly larvae manure degradation as fish meal replacer in native chicken

ration. *Lucrări Ştiinţifice - Seria Zootehnie*, 62, 139-142.

Manangkot, H., Najoan, M., Toar, W., Rotinsulu, M., Turangan, S. (2019). The intervention of *Hermetia illucens* L. (Diptera: Stratiomyidea) in manure empowerment on blood lipid profile of native chicken. *Scientific Papers. Series D. Animal Science*, LXII (1), 256-261.

Helmiyesi, H., Hastuti, R.B., Prihastanti, E. (2008). Pengaruh Lama Penyimpanan Terhadap Kadar Gula dan Vitamin C Pada Buah Jeruk Siam(Citrus nobilis var. microcarpa). *Buletin Anatomi dan Fisiologi*, XVI (2), 33-37.

Kim, W., Bae, S., Park, K., et al. (2011). Biochemical characterization of digestive enzymes in the Black Soldier Fly, *Hermetia illucens* (Diptera: Stratiomyidae). *J Asia-Pac Entomology*, 14(1), 11-14.

Rotinsulu, M., Hadju, R., Sakul, S. (2014). Sifat Organoleptik Daging Broiler Dengan Lama Perendaman Berbeda Dalam Perasan Lemon Cui. *Jurnal Zootek*, 34(2), 139-147.

Mirah, A. D. (1981). *Pengaruh penyuntikan Papain Pada Pengempukan Daging Ayam Secara Antemortem*. Tesis. Fakultas Peternakan UNSRAT, Manado

Setiawan, M. A., Retnoningrum, M. W. (2019). Aktivitas AntiBakteri Biji Jeruk Manis (*Citrus sinensis*) Terhadap Bakteri *Eschericia coli*. DOI: https://doi.org/10.23917/bioeksperimen.v5i1.7989

Nyakeri, E.M., Ogola, H.J., Ayieko, M.A., Amimo, F.A. (2016). An open system for farming black soldier fly larva as a source of protein for small scale poultry and fish production. *J. Insects as Food and Feed*, 3(1), 51-56.

Oikeh, E., Oriakhi, K., Omoregie, E. (2013). Proximate analysis and phytochemical screening of Citrus sinensis Fruit Wastes. Bioscientist, 1(2),164-170.

Palupi, W.D.E. (1986). *Tinjauan Literatur Pengolahan Daging*. Pusat Dokumentasi Ilmiah Nasional, LIPI, Jakarta.

Sakaria, S., Wawo, B. (2004). Penyusunan Ransum Ayam Buras Secara Sederhana. Hasil Penelitian yang tidak dipublikasikan. Fakultas Peternakan Universitas Hasanudin.

Soewarno, T.S. (1985). Penilaian Organoleptik. Bhratara Aksara, Jakarta.

Steel, R.G.D, Torrie, J.H. (1994). Prinsip dan Prosedur Statistika.Diterjemahkan oleh Bambang sumantri. Edisi ke-2.PT. Gramedia Pustaka Utama, Jakarta.

Yerou, K. O., Bouhadi, K. I., Meddah, B., Touil, A. T. (2017). The use of orange (*Citrus sinensis*) peel as antimicrobial and antioxidant agent. *Journal of Fundamental and Applied Sciences*, 9(3), 1351-1357.

Winarno, F.G. (1984). Kimia Pangan dan Gizi. PT. Gramedia Utama. Jakarta.

Winarno, F.G. (1993). Pangan Gizi teknologi dan Konsumen. PT. Gramedia Pustaka Utama, Jakarta.

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INFLUENCE OF TEMPERATURE DURING SLAUGHTER ON THE CHEMICAL COMPOSITION AND GROSS ENERGY OF REFRIGERATED BROILER BREAST MEAT

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Abstract

The aim of the study was to assess whether different temperatures applied in certain points of the broilers slaughtering technological flow affects or not the chemical composition and the gross energy value of the meat. Three groups of carcasses were studied (LC, L1E and L2E), in relation with the slaughtering key points with altered temperature: scalding stage (LC = 51-53°C; L1E = 53-54°C; L2E = 55-56°C); chilling (LC = 1-4°C; L1E = 2-3°C; L2E = 1-2°C); sorting and packaging (LC < 12°C; L1E = 8-10°C; L2E = 6-8°C); final storage (LC = 0-2°C; L1E = 0-1°C; L2E = 0-1°C). Fifty carcasses of ROSS-308 chicken broilers per group, kept throughout 1 day in chill store room prior to shipping, were investigated for the proximate composition (water, dry matter, minerals, total lipids and protein content, nitrogen free extract) and gross energy value, using samples of 50 g taken from breast (Pectoralis major and Pectoralis minor muscles). Standard A.O.A.C. methods were applied for the analytical chemistry protocols in 15 replications per parameter, while the gross energy content was computed using the nutrient caloric conversion factors proposed by F.A.O. Water content reached 73.23 g/100 g in LC, 73.02 g/100 g in LE1 and 72.90 g/100 g in LE2. Dry matter content varied accordingly (26.77 g/100 g in LC to 27.10 g/100 g in LE2). Total minerals were found within the 1.08-1.10 g/100 g range. Major differences were observed between the total lipids of the control group (2.14 g/100 g) and the experimental groups, i.e. 2.65 g/100 g in LE1 group ($P<0.05$) and 2.90 g/100 g in LE2 group ($P<0.01$). Although the total proteins content decreased (LC = 23.01 g/100 g; LE1 = 22.76 g/100 g; LE2 = 22.60 g/100 g) as the experimental factor was gradually altered, there were not significant differences found. Nitrogen free extract calculations resulted in close values between groups, while the energetic value was affected due to lipid content variations ($P<0.05$ for the LC vs. LE2 comparison, i.e. 119.77 vs. 124.71 kcal/100 g). Therefore, the increase of temperature during scalding and its decrease during sorting, packaging and storage induced exudation and significant variations of lipids and energetic content.

Key words*: chicken, slaughtering, temperature, proximate composition, gross energy.*

INTRODUCTION

Poultry meat ultimate quality is affected by several pre-slaughter factors, such as fowl age (Wideman et al., 2016) farming technology and culling (Baracho et al., 2006), transportation, crating, hanging to slaughterhouse conveyor (Petracci et al., 2010) and stress leading to intense muscular efforts and spasms (Huang et al., 2018), pre-slaughter fastening (Jiang et al., 2011), type of stunning (electrical, low-pressure atmosphere, gaseous) (Kissel et al., 2015; Sirri et al., 2015; Fuseini et al., 2016; Mackie and McKeegan, 2016; Silva-Buzanello et al., 2018).

Also, the technological flow in the slaughterhouse, through its microclimate factors affects the ultimate quality of poultry meat (Wang et al., 2016).

It is known that several pathogenic bacterial species (*Salmonella* ssp., *Campylobacter* ssp.) (Giombelli and Gloria, 2014) colonise the poultry carcasses and most of them occur after slaughtering, de-feathering and evisceration (Zweifel et al., 2015). Particularly, the temperature, as one of the slaughterhouse microclimate component, is used as a decreasing factor of microbial contamination of carcasses (Lehner et al., 2014) is suspected to influence the meat quality in general (Anghinoni et al., 2019), the meat yield and its quality (Buhr et al., 2014) or the chemical composition of the poultry meat in particular, subsequently the nutritional value (Bowker et al., 2014), due to slightly induced changes in meat tissues and, therefore in their capacity to retain or exudate fluids, such as water,

cytoplasm colloids, interstitial fluids and so on (Silva-Buzanello et al., 2019).

Within this context, the original research protocol aimed to find out whether higher scalding temperatures and lower chilling and storage temperatures, used both in reducing the microbial contamination of broilers carcasses and of cut parts influence or not the chemical composition and caloricity of the meat.

MATERIALS AND METHODS

Hypothesis: increased temperature in scalding stage of broilers carcasses and decreased temperatures in chilling, sorting, storing stages does not affect the chemical composition and gross energy content in breast muscles.

Graduation of experimental factor, temperature across some key stages of the slaughter technological flow. Three groups of 50 carcasses each (LC, L1Exp and L2Exp) were randomly formed, in order to investigate the altered temperature influence on meat quality, in certain key stages of the technological flow:

- scalding: LC = 51-53°C; L1E = 53-54°C; L2E = 55-56°C;
- chilling: LC = 1-4°C; L1E = 2-3°C; L2E = 1-2°C;
- sorting and packaging: LC < 12°C; L1E = 8-10°C; L2E = 6-8°C;
- final storage: LC = 0-2°C; L1E = 0-1°C; L2E = 0-1°C.

Sampling. By the end of the flow, the carcasses were kept throughout 1 day in chill store room. Then, samples of 50 g samples of skinless breast meat (both *Pectoralis major* and *Pectoralis minor*) were harvested from each carcass in order to investigate the meat proximate composition (water, dry matter, minerals, total lipids and protein content, nitrogen free extract and to calculate the gross energy content within.

Analytical protocols. Official AOAC 983.18 method was used to prepare homogenously the meat samples before submitting them to other analytical procedures. Then, the samples were tested to assess water and dry matter content, via microwave evaporation using the AOAC official method no. 985.14. Subsequently, the dry matter compounds were assessed: crude ash (Kolar, 1992), protein (total nitrogen AOAC 928.08) and total lipids - crude fat (AOAC 991.36). Nitrogen free extract was calculated by difference, between the total dry matter and the sum of ash, fat and total nitrogen (Apata et al., 2015). Then, using the Atwater coefficients proposed by FAO, the organic content of each sample was converted into gross energy (FAO, 2003).

For every proximate composition trait and for the energy content calculation, there were run 15 analytical replications.

Statistical processing. Acquired data via analytical methods or computations were statistically analysed using the GraphPad Prism 8 Software, running ANOVA single factor analysis, followed by post-hoc Tukey computations, in accordance with the data treatment methodology proposed by Mendenhall and Sincich, 2016.

RESULTS AND DISCUSSIONS

Water content gradually decreased (by 0.3% to 0.45%) in the analysed samples as scalding temperature increased and chilling temperature was lower in experimental groups, compared to control group (Table 1). The difference between the L2Exp group and LC group was significant ($P<0.05$) suggesting a likelihood of at least 95% that water content would decrease in broilers breast meat as the temperature in scalding would increase by 3-4°C. The content of dry matter varied accordingly and reversed proportionally with the water content of samples.

Table 1. Moisture, dry matter and total mineral vs. organic compounds in the broiler breast meat, as influenced by temperature during slaughter (n = 15)

Trait	LC group (mean ± SD) (g/100 g wet weight)	L1Exp group (mean ± SD) (g/100 g wet weight)	L2Exp group (mean ± SD) (g/100 g wet weight)
Water	73.23±0.35	73.02±0.29	72.90±0.43
± % and ANOVAs	100%	-0.29% *P*=0.09 vs. LC	-0.45% *P*=0.03 vs. LC * *P*=0.09 vs. L1Exp
Dry matter	26.77±0.35	26.98±0.29	27.10±0.43
± % and ANOVAs	100%	+0.78% *P*=0.09 vs. LC	+1.23% *P*=0.03 vs. LC * *P*=0.09 vs. L1Exp
Total minerals	1.08±0.03	1.10±0.06	1.10±0.04
± % and ANOVAs	100%	+1.85% *P*=0.28 vs. LC	+1.85% *P*=0.06 vs. LC *P*=0.69 vs. L1Exp
Total organic matters	25.69±0.34	25.88±0.28	26.00±0.42
± % and ANOVAs	100%	+0.74% *P*=0.11 vs. LC	+1.20% *P*=0.04 vs. LC * *P*=0.38 vs. L1Exp

** significant differences (0.01<P<0.05)*

Total inorganic matters (minerals) content varied between 1.08 g/100 g (LC) and 1.10 g/100 g (both experimental groups), the small differences did not reach any significance threshold. In terms of total organic matters, the control group samples reached 25.69 g/100 g meat, while those in L1Exp group were 0.74% richer and those in L2Exp group contained 1.2% more organic substances (P<0.05), suggesting that the draining of water due to higher scalding temperature also induced a concentration of dry matter and, subsequently, of organic matters.

The compounds with more nutritional significance and with caloric power within (lipids, total nitrogen matters and nitrogen free extract) varied accordingly, as they are directly correlated to the total organic matters content (Table 2).

Crude fat content in L1Exp group was 23.8% higher compared to control samples (P<0.001) and 35.5% higher in L2Exp samples (P<0.001).

Table 2. Organic nutrients and the gross energy contained within, as influenced by temperature during slaughter (n = 15)

Nutrient	LC group (mean ± SD)	L1Exp group (mean ± SD)	L2Exp group (mean ± SD)
Crude fat (g/100 g wet weight)	2.14±0.18	2.65±0.15	2.90±0.17
± % and ANOVAs	100%	+23.8% $P=2 \times 10^{-9}$ vs. LC***	+35.5% $P=2 \times 10^{-12}$ vs. LC*** $P=0.0003$ vs. L1Exp***
Total nitrogen matters (g/100 g wet weight)	23.01±0.36	22.76±0.08	22.65±0.24
± % and ANOVAs	100%	-1.1% $P=0.04$ vs. LC *	-1.6% $P=0.003$ vs. LC ** $P=0.26$ vs. L1Exp
Nitrogen free extract (g/100 g wet weight)	0.55±0.09	0.47±0.24	0.45±0.40
± % and ANOVAs	100%	-17.0% $P=0.24$ vs. LC	-22.2% $P=0.39$ vs. LC $P=0.90$ vs. L1Exp
Gross energy (kcal/100 g meat)	119.77±1.92	123.05±1.10	124.71±1.45
± % and ANOVAs	100%	+2.73% $P=4 \times 10^{-6}$ vs. LC***	+4.12% $P=1 \times 10^{-8}$ vs. LC*** $P=0.002$ vs. L1Exp**

** significant differences (0.01<P<0.05)*
*** distinguished significant differences (0.001<P<0.01)*
**** very significant differences (P<0.001)*

Total nitrogen matters varied in accordance with the water content, decreasing thus with 1.1%, compared to control (P<0.05), while in L2Exp group it decreased by 1.6% vs. control group samples (P<0.001). Therefore, the hydro soluble nutrients, such as the proteins, decreased as temperature increased during scalding and was lower during chilling, sorting, packaging and storage and induced more accentuated water drips. On the contrary, the lipids trended to concentrate because they were not water soluble and did not left the tissues.

The other organic compounds soluble in water (nitrogen free extract) were lower in experimental groups compared to control one, but the differences were not found bearing statistical significance.

Gross energy content was significantly influence by the dynamics of total lipids and reached 119.77 kcal/100 g meat in LC samples, 123.05 kcal/100 g meat in L1exp samples (+2.73%; P<0.001) and 124.71 kcal/100 g meat in L2Exp group (+4.12%; P<0.001).

The proximate compounds were found within the normal variation limits for the chicken breast meat, comparable with other findings in the scientific literature, suggesting the influence of higher temperature in scalding and of lower in chilling, sorting and packaging on the decrease of water content and on the concentration of fats (Ang and Hamm, 1983; Mir et al., 2017).

However, in order to have a wider image on the temperature effect on the chemical and physical properties of the breast meat investigations should be completed with instrumental textural profile analysis, knowing the fact that higher scalding temperatures could decrease meat moisture (Bai and Wang, 2013), increase pH value and negatively affect meat tenderness (Zhuang et al., 2013).

CONCLUSIONS

Increasing the experimental factor temperature with 4-5°C in scalding stage and decreasing it by 1-4°C in chilling, packaging and storing stages led to changes in water and dry mater content of the breast meat (P<0.05).

Water loss induced concentration of lipids by 24-35% in experimental groups (P<0.001) and decrease of hydro soluble compounds, such as proteins by 1.1-1.6% (P<0.05; P<0.01) and nitrogen free extract.

The changes of proximate composition due to increased scalding temperatures and lowering the cold treatments stages values led to gross energy increase of breast meat by 2.7-4.1% in experimental groups vs. control ($P<0.001$).
Hence the variations of the experimental factor induced changes in the chemical and nutritional value of the breast meat, it would be challenging to assess, as follow-up of the research, the sensory and textural dynamics of the samples and to run correlational and regression computations, in order to determine whether such quality descriptors are influenced and in which manner, by the processing temperature during slaughtering flow.

REFERENCES

Ang, C. Y. W., Hamm, D. (1983). Comparison of commercial processing method vs hot-deboning of fresh broilers on nutrient content of breast meat. *Journal of Food Science*, 48(5), 1543-1544.

AOAC 983.18-1983. Meat and Meat Products - Preparation of Test Sample Procedure, retrieved January 2020 from http://www.aoacofficialmethod.org/index.php?main_page=product_info&products_id=558

AOAC 985.14-1991, Title: Moisture in Meat and Poultry Products – Rapid, retrieved January 2020 from http://www.aoacofficialmethod.org/index.php?main_page=product_info&cPath=1&products_id=2029

AOAC 991.36-1996, Fat(Crude) in Meat and Meat Products – Solvent, retrieved January 2020 from http://www.aoacofficialmethod.org/index.php?main_page=product_info&cPath=1&products_id=2528Apata, E. S., Koleoso, I. M., Tijani, L. A., Obi, O. O., Okere, I. A. (2015). Effect of sex on meat quality attributes of pigeon birds (*Columbia livia*) in Abeokuta metropolis. *International Journal of Agricultural Sciences and Natural Resources*, 2(2), 19-23.

Bai, J., Wang, R. (2013). Effect of Postmortem Carcass Scalding on Moisture Content of Chicken Meat. *Food Research and Development*, 7, 14.

Baracho, M. S., Camargo, G. A., Lima, A. M. C., Mentem, J. F., Moura, D. J., Moreira, J., Nääs, I. A. (2006). Variables impacting poultry meat quality from production to pre-slaughter: a review. *Brazilian Journal of Poultry Science*, 8(4), 201-212.

Bowker, B. C., Zhuang, H., Buhr, R. J. (2014). Impact of carcass scalding and chilling on muscle proteins and meat quality of broiler breast fillets. *LWT-Food Science and Technology*, 59(1), 156-162.

Buhr, R. J., Walker, J. M., Bourassa, D. V., Caudill, A. B., Kiepper, B. H., Zhuang, H. (2014). Impact of broiler processing scalding and chilling profiles on carcass and breast meat yield. *Poultry science*, 93(6), 1534-1541.

Food and Agriculture Organization of the United Nations (2003). Food energy - methods of analysis and conversion factors. Report of a Technical Workshop, Rome, 3-6 December 2002. FAO Food and Nutrition Paper, 77.

Fuseini, A., Knowles, T. G., Hadley, P. J., Wotton, S. B. (2016). Halal stunning and slaughter: Criteria for the assessment of dead animals. *Meat science*, 119, 132-137.

Giombelli, A., Gloria, M. B. A. (2014). Prevalence of *Salmonella* and *Campylobacter* on broiler chickens from farm to slaughter and efficiency of methods to remove visible fecal contamination. *Journal of food protection*, 77(11), 1851-1859.

Huang, J. C., Yang, J., Huang, M., Zhu, Z. S., Sun, X. B., Zhang, B. H., Xu, B. C. (2018). Effect of pre-slaughter shackling and wing flapping on plasma parameters, postmortem metabolism, AMPK, and meat quality of broilers. *Poultry science*, 97(5), 1841-1847.

Jiang, S., Jiang, Z., Lin, Y., Zhou, G., Chen, F., Zheng, C. (2011). Effects of different rearing and feeding methods on meat quality and antioxidative properties in Chinese Yellow male broilers. *British poultry science*, 52(3), 352-358.

Kissel, C., Soares, A. L., Oba, A., Shimokomaki, M. (2015). Electrical water bath stunning of broilers: effects on breast meat quality. *The Journal of Poultry Science*, 52(1), 74-80.

Kolar, K. (1992). Gravimetric Determination of Moisture and Ash in Meat and Meat Products: NMKL Interlaboratory Study, *Journal of AOAC INTERNATIONAL*, 75(6), 1016-1022.

Lehner, Y., Reich, F., Klein, G. (2014). Influence of process parameter on *Campylobacter* spp. counts on poultry meat in a slaughterhouse environment. *Current microbiology*, 69(3), 240-244.

Mackie, N., McKeegan, D. E. (2016). Behavioural responses of broiler chickens during low atmospheric pressure stunning. *Applied Animal Behaviour Science*, 174, 90-98.

Mendenhall, W. M., Sincich, T. L. (2016). *Statistics for Engineering and the Sciences*. CRC Press.

Mir, N. A., Rafiq, A., Kumar, F., Singh, V., Shukla, V. (2017). Determinants of broiler chicken meat quality and factors affecting them: a review. *Journal of food science and technology*, *54*(10), 2997-3009.

Petracci, M., Bianchi, M., Cavani, C. (2010). Pre-slaughter handling and slaughtering factors influencing poultry product quality. *World's Poultry Science Journal*, *66*(1), 17-26.

Silva-Buzanello, R. A. D., Schuch, A. F., Gasparin, A. W., Torquato, A. S., Scremin, F. R., Canan, C., Soares, A. L. (2019). Quality parameters of chicken breast meat affected by carcass scalding conditions. *Asian-Australasian journal of animal sciences*, *32*(8), 1186.

Silva-Buzanello, R. A. D., Schuch, A. F., Nunes Nogues, D. R., Falcão de Melo, P., Gasparin, A. W., Sanches Torquato, A., Lourenço Soares, A. (2018). Physicochemical and biochemical parameters of chicken breast meat influenced by stunning methods. *Poultry science*, *97*(11), 3786-3792.

Sirri, F., Petracci, M., Zampiga, M., Meluzzi, A. (2017). Effect of EU electrical stunning conditions on breast meat quality of broiler chickens. *Poultry science*, *96*(8), 3000-3004.

Wang, R. H., Liang, R. R., Lin, H., Zhu, L. X., Zhang, Y. M., Mao, Y. W., Luo, X. (2017). Effect of acute heat stress and slaughter processing on poultry meat quality and postmortem carbohydrate metabolism. *Poultry science*, *96*(3), 738-746.

Wideman, N., O'bryan, C. A., Crandall, P. G. (2016). Factors affecting poultry meat colour and consumer preferences-A review. *World's Poultry Science Journal*, *72*(2), 353-366.

Zhuang, H., Bowker, B. C., Buhr, R. J., Bourassa, D. V., Kiepper, B. H. (2013). Effects of broiler carcass scalding and chilling methods on quality of early-deboned breast fillets. *Poultry science*, *92*(5), 1393-1399.

Zweifel, C., Althaus, D., Stephan, R. (2015). Effects of slaughter operations on the microbiological contamination of broiler carcasses in three abattoirs. *Food Control*, *51*, 37-42.

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ZOO-SANITARY SURVEY FOR POTENTIAL MUSSEL AQUACULTURE ZONE DESIGNATION AT THE ROMANIAN COAST

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Abstract

An essential aspect for developing mussel (Mytilus galloprovincialis Lamarck, 1819) aquaculture at the Romanian Black Sea coast is the classification of culture areas from the microbiological point of view, in compliance with European Commission Regulation no. 854/2004. According to this document, the indicator species is the bacteria Escherichia coli and thresholds have been set to classify the bivalve culture areas for human consumption: for class A, no post-harvest treatment is required to reduce microbiological contamination, while for classes B and C purification, relaying or cooking by an approved method are mandatory. In this context, monthly samples were collected from three sampling points along the Romanian coast and the contamination of mussel tissue and intravalvular liquid with E. coli was analyzed. The reference method for analysis was the Most Probable Number (MPN), as mentioned by European standard EN/ISO 16649-3. The aim of this research was to assess the zoo-sanitary and food safety suitability of the most indicated areas for shellfish culture, previously selected taking into account the environmental constraints of the Romanian coast (no sheltered areas, currents, strong winter winds).

Key words*: contamination, E. coli, food safety, microbiological standards, mussels.*

INTRODUCTION

Romanian aquaculture has been historically focusing mainly on freshwater fish species, yet, recently mariculture has started to generate interest. In this context, valuable finfish species, such as turbot (*Psetta maxima*) (Niță and Nenciu, 2017), sturgeons (Russian sturgeon *Acipenser gueldenstaedti* and Siberian sturgeon *Acipenser baerii*) (Niță et al., 2018b) or golden-gray mullet (*Liza aurata*) (Niță et al., 2018a), were found to be suitable for rearing in culture conditions and provide an opportunity for market development. Moreover, in Romania, there has been a slight increase in the consumption of mussels and oysters in public nutrition (Niță et al., 2019), thus offering a great development opportunity of shellfish aquaculture.

However, significant focus should be put on zoo-sanitary conditions and public health, as shellfish can generally be considered to be a safe, healthy and nutritious food, but the consumption of bivalve mollusks harvested from contaminated waters may lead to disease due to the presence of micro-organisms (Nicolae et al., 2019). The assessment of sources and types of human and animal fecal contamination in the proximity of shellfish harvesting areas, combined with microbiological monitoring based on the use of indicator organisms (the bacteria *Escherichia coli* in the European Union) provides an assessment of the risk of contamination with bacterial and viral pathogens and are the basis for public health checks (Nenciu e al., 2020). Equally important, the increase in the demand for bivalves has encouraged the harvesting of mussels from natural populations, growing mussels on floating installations (long-line systems) and acclimatization of high-value bivalves - the Japanese oyster, for instance (Zaharia et al., 2017). The annual quantity of mussels harvested in the Romanian Black Sea coast area amounts to approximately 15 t, and the only existing mariculture farm (with interrupted activity), S.C. MARICULTURA S.R.L., can produce annually approximately 5 t of cultured mussels (Niță et al., 2019).

In the current context of Romanian legislation, the main drawback for developing shellfish aquaculture is the lack of coordination between

institutions (Sanitary Veterinary Directorate, Public Health Directorate, Romanian Waters Administration), but some steps in settling this issue have been made, as a collaboration protocol between several institutions is under elaboration. Moreover, the establishment of the Shellfish Aquaculture Demonstrative Center (S-ADC) in the frame of the National Institute for Marine Research and Development "Grigore Antipa" (NIMRD) - General Fisheries Commission for the Mediterranean (GFCM) collaboration aims at the promotion of scientific, technical and technological bases for bivalve shellfish aquaculture in Romania (Niță et al., 2018c).

Food safety monitoring of shellfish production areas in the European Community is currently regulated by the "Hygiene Package", which entered into force on 1 January 2006. This legislative package includes Regulations (EC) No. 852/2004 and No. 853/2004, which target industry professionals, Regulation No. 854/2004, aimed at competent authorities, responsible of carrying out official sanitary controls, and Regulations (EC) No. 854/2004, 2073/2005 and No. 882/2004, which refer to end-product standards required for bivalve mollusks (Nenciu et al., 2020).

The detailed implementation of classification and monitoring programs following Regulation (EC) No. 854/2004 is the responsibility of competent authorities and may vary between Member States (European Community, 2004). In Romania, the competent authority is the Sanitary-Veterinary and Food Safety National Authority, yet, up to date, no official classification has been undertaken.

In this context, the aim of this research was to assess the microbiological contamination of mussel samples collected from selected areas, in order to indicate the most suitable potential areas for mussel culture at the Romanian coast, from the zoo-sanitary and food safety perspective.

MATERIALS AND METHODS

The risk of contamination of shellfish is evaluated by reference to the sources and types of fecal contamination (human and animal) in the vicinity of the shellfish production areas (shoreline survey), on the one hand, and the results obtained based on the indicator bacteria *Escherichia coli*, from samples taken in these areas, on the other hand (Table 1). Areas are classified following a full assessment of this risk and the classification given to an area determines whether shellfish harvested in that area require post-processing treatment and, where appropriate, the level of such treatment (Anon, 2017).

Table 1. Criteria for the classification of bivalve mollusk harvesting/culture areas

Class	Criteria for the classification of bivalve mollusk harvesting areas	Post-harvest treatment required to reduce microbiological contamination
A	Samples of live bivalve mollusks from these areas must not exceed, in 80% of samples collected during the review period, **230 *E. coli* per 100 g** of flesh and intravalvular liquid. The remaining 20% of samples must not exceed 700 *E. coli* per 100 g of flesh and intravalvular liquid	None
B	Live bivalve mollusks from these areas must not exceed, in 90% of samples, **4,600 MPN *E. coli* per 100 g** of flesh and intra-valvular liquid. In the remaining 10% of samples, live bivalve mollusks must not exceed 46,000 MPN *E. coli* per 100 g of flesh and intra-valvular liquid	Purification, relaying or cooking by an approved method
C	Live bivalve mollusks from these areas must not exceed the limits of a five-tube, three dilution MPN test of **46,000 *E. coli* per 100 g** of flesh and intravalvular liquid	Relaying or cooking by an approved method

For the current research, in order to assess the risk of contamination of mussels with pathogens, mussel samples were taken from three selected sampling stations (two replicates/station - Figure 1, Table 2), on a monthly basis, during July - December 2019, covering a 6-month time frame.

The three stations (Mangalia - MG1 and MG2, Constanța - CT 1 and CT2, and Năvodari - NV1 and NV2) were selected as most suitable due to their geographical location in sheltered areas (protected by hydrotechnical constructions), which would allow for actual shellfish farms to be established. Moreover, a preliminary analysis indicated no potential land-based contamination sources.

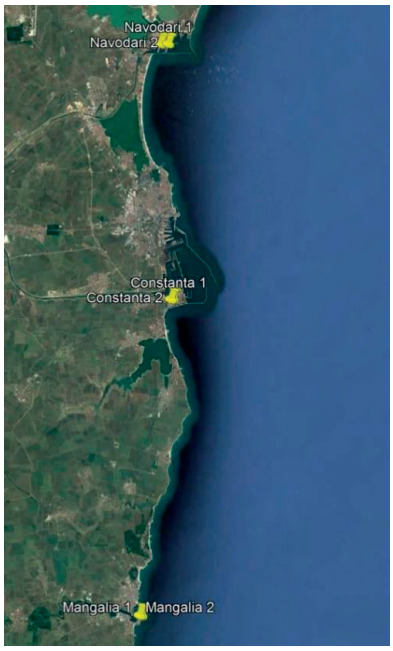

Figure 1. Map of sampling stations

The mussel samples were collected using a custom-made scraper, with stainless-steel handle, frame and blade and a collector sack made of mesh (Figure 2, left side). After scraping from the natural substrate, mussels were placed in appropriately labeled zip-lock bags and cold stored for transportation to the laboratory (Figure 2, right side). All samples were processed within 24 hours after collection, being kept in a refrigerator.

Table 2. Coordinates of sampling stations

Sampling Location						
Coord.	**MG1**	**MG2**	**CT1**	**CT2**	**NV1**	**NV2**
Lat. N	43.78951	43.78957	44.08433	44.08473	44.32421	44.32387
Long. E	28.58694	28.58424	28.64386	28.64638	28.64558	28.65562

The quality biological parameters investigated were the following: total number of germs (TNG), total coliforms (TC), fecal coliforms (FC) and *E. coli*.

The methods used for determining the microbial contamination of mussel samples were detection by culture technique on usual and selective media, except for *E. coli*, for which the European reference method was used: detection and Most Probable Number (MPN) technique specified in EN/ISO 16649-3/2015.

Figure 2. Mussel sample collection using a scraper (left side) and zip-lock bagging for cold storage and transportation to the laboratory (right side) (Original photos)

The flesh and intravalvular liquid of the mussels were excised and sampling units (10 g) were homogenized. Analysis for the detection and enumeration of *E. coli* on the homogenate took place by using a five-tube, three dilution (1 g, 0.1 g and 0.01 g) Most Probable Number (MPN) test method according to EN/ISO 16649-3. This is a two-stage, five tube by three dilution MPN method. The first stage of the method is a resuscitation requiring inoculation of minerals modified glutamate broth (MMGB) with a series of diluted bivalve mollusk homogenates (flesh and intravalvular liquid) and incubation at 37±1°C for 24±2 hours (Figure 3).

Figure 3. Preparation of samples for homogeneization of tissue and intravalvular liquid (Original photo)

E. coli was subsequently confirmed by subculturing tubes showing acid production onto tryptone bile glucuronide agar (TBGA) and detecting β-glucuronidase activity by the presence of blue or blue-green colonies.

The detection of the most probable number is made from the combination of positive and negative tubes (Figure 4).

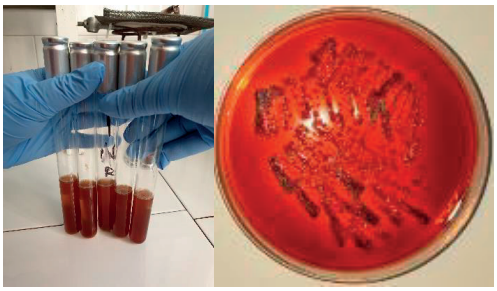

Figure 4. *E. coli* determination using the MPN technique (Original photos)

RESULTS AND DISCUSSIONS

At a preliminary examination, the organoleptic quality of all sampled mussels, related to smell and taste, was appropriate for human consumption. None of the mussels analysed had a smell or taste altered compared to the specificity of these seafood items.

The results obtained after the microbiological analysis of the sampled mussel tissue and intravalvular liquid homogenates are summarised in Tables 3 - 5 below.

In Mangalia, the southernmost station, the overall bacterial contamination (TNG) was the highest in November and December, while total coliforms (TC) recorded the peak value in August. Fecal coliforms (FC), however, reached higher values in July, explainable by the presence of tourists in the area during the summer season. Yet, regulated values for public health (<300 germs/100 g) were not exceeded. (Table 3, Figure 5).

Table 3. Values of microbial contamination of *M. galloprovincialis* samples in Mangalia: total number of germs (TNG), total coliforms (TC), fecal coliforms (FC) and *E. coli*.

Station	MG 1				MG 2			
Values (number of germs/100 g of mussel flesh and intravalvular liquid)								
2019	TNG	TC	FC	*E. coli*	TNG	TC	FC	*E. coli*
July	5000	490	230	**50**	400	5400	220	0
Aug.	1000	3500	20	0	500	230	0	0
Sept.	6000	490	170	0	6000	490	110	**50**
Oct.	2000	280	0	0	1000	79	0	0
Nov.	10000	220	20	0	10000	220	0	0
Dec.	10000	330	20	0	10000	50	0	0
*Limits**			*300*	*230*			*300*	*230*

*as per Regulation (EC) No. 854/2004

Concerning *E. coli* contamination, of all samples collected from this station, only in two the presence of this indicator bacteria was confirmed, in July and September, again most likely correlated with the presence of tourists (Table 3, Figure 5). Similarly to fecal coliforms, the recorded values did not exceed the regulated limit of 230 germs/100 g of mussel flesh and intravalvular liquid, which rates Mangalia as a Class A area for shellfish culture/harvesting.

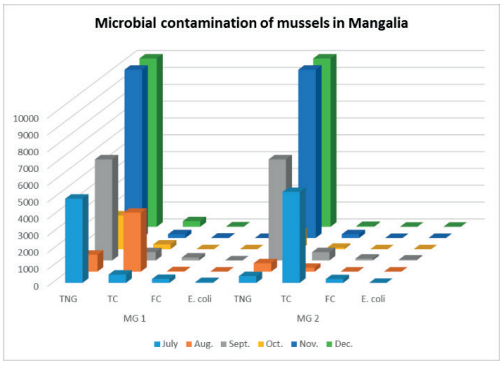

Figure 5. Microbial contamination of *M. galloprovincialis* samples in Mangalia

In the central station, Constanta, overall bacterial contamination (TNG) was higher compared to Mangalia in September, yet total coliforms (TC) and especially fecal coliforms (FC) recorded lower values (Table 4, Figure 6).

Table 4. Values of microbial contamination of *M. galloprovincialis* samples in Constanța: total number of germs (TNG), total coliforms (TC), fecal coliforms (FC) and *E. coli*

Station	CT 1				CT 2			
Values (number of germs/100 g of mussel flesh and intravalvular liquid)								
2019	TNG	TC	FC	*E. coli*	TNG	TC	FC	*E. coli*
July	13000	4300	130	**20**	10000	2300	0	0
Aug.	40000	1700	50	0	60000	1100	50	0
Sept.	100000	3300	20	0	400000	490	170	0
Oct.	400	130	50	0	1000	240	20	0
Nov.	40000	80	0	0	25000	220	0	0
Dec.	100000	230	0	0	150000	490	50	0
*Limits**			*300*	*230*			*300*	*230*

*as per Regulation (EC) No. 854/2004

With reference to *E. coli* contamination, this station recorded the lowest degree of contamination, with only one sample with 20 germs/100 g of mussel flesh and intravalvular liquid, significantly below the allowed limit for Class A (Table 4, Figure 6).

These results, corroborated with the shoreline assessment and general topography of the site, indicated that the Constanta area is the most suitable for establishing and operating a

shellfish farm, as well as for collecting wild mussels from the environment. The southern dike of the Constanta port offers an appropriate habitat for the fixation and growth of mussels, on the hand, and, on the other hand, it provides shelter against currents and storms for any potential farming installation set-up in this location.

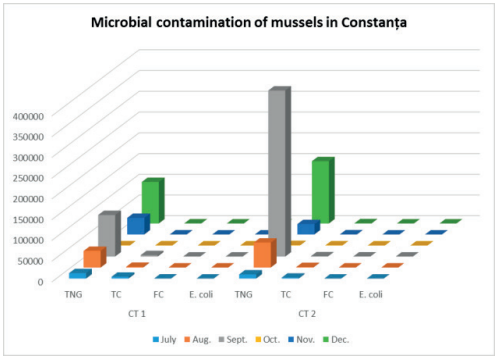

Figure 6. Microbial contamination of *M. galloprovincialis* samples in Constanța

In the Năvodari station, the highest overall microbial contamination (TNG, TC and FC) values of all stations were recorded in July, August and September, most likely due to the proximity to the Mamaia resort, a very busy holiday destination during summer (Table 5, Figure 7).

Table 5. Values of microbial contamination of *M. galloprovincialis* samples in Năvodari: total number of germs (TNG), total coliforms (TC), fecal coliforms (FC) and *E. coli*

Station	NV 1				NV 2			
Values (number of germs/100 g of mussel flesh and intravalvular liquid)								
2019	TNG	TC	FC	*E. coli*	TNG	TC	FC	*E. coli*
July	100000	400	70	0	200000	4800	220	**50**
Aug.	150000	5400	230	**20**	100000	540	17	0
Sept.	50000	330	170	0	100000	3300	210	**20**
Oct.	40000	50	0	0	50000	240	0	0
Nov.	30000	230	0	0	25000	220	0	0
Dec.	50000	230	0	0	50000	240	230	**50**
*Limits**			*300*	*230*			*300*	*230*

*as per Regulation (EC) No. 854/2004

Concerning *E. coli* contamination in Năvodari, this station recorded the highest degree of contamination, with four contaminated samples, in July, August, September and December (Table 5, Figure 7). Similarily to the other two stations, the values registered were low, below the limit of 230 germs/100 g of mussel flesh and intravalvular liquid, which rates Năvodari as well as a Class A area for shellfish culture/harvesting.

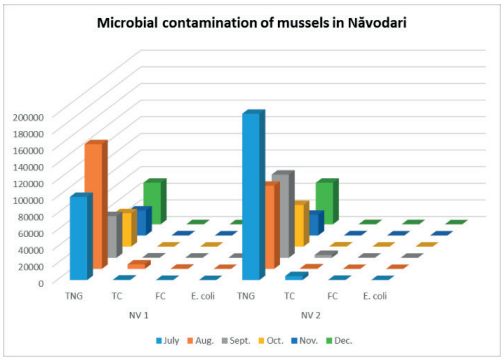

Figure 7. Microbial contamination of *M. galloprovincialis* samples in Năvodari

The results of this zoo-sanitary survey showed that all three selected sampling locations along the Romanian Black Sea coast (Mangalia, Constanța and Năvodari) are suitable for shellfish culture and/or harvesting, as the values recorded by bacterial contaminants do not pose a threat for public health and the shellfish could be safely consumed without any further purification being required.

CONCLUSIONS

The aim of this research was to assess the microbiological contamination of mussel samples collected from three selected areas, taking into account the environmental constraints of the Romanian coast (no sheltered areas, currents, strong winter winds), in order to evaluate the zoo-sanitary and food safety suitability of the most indicated areas for shellfish culture.

The overall bacterial contamination (TNG, TC and FC) in all three sampling stations was low, with values not exceeding the allowed threshold (for TC).

With reference to *E. coli* contamination, the results of this research indicate very low values in all three sampling stations. Consequently, according the criteria for the classification of bivalve mollusk harvesting/culture areas, the mussels collected from these locations can be included in Class A, thus not requiring post-harvest treatment to reduce microbiological contamination (Table 1).

Of all investigated stations during this 6 month zoo-sanitary survey, the Constanta area seems to be the most suitable for establishing and operating a shellfish farm, as well as for collecting wild mussels from the environment.
However, the trial classification itself of a certain production/culture area is not sufficient to guarantee safe human consumption. As such, the first step in an official control programme is establishing a sampling programme of bivalve mollusks in the production area (6 months). Subsequently, classified relaying and production areas must be periodically monitored to check the microbiological quality of live bivalve mollusks in relation to the production and relaying areas (weekly samples).
As far as the sampling requirements are concerned, sampling plans must be drawn-up providing for such checks to take place at regular intervals or on a case-by-case basis if harvesting periods are irregular. The geographical distribution of the representative sampling points and the sampling frequency must ensure that the results of the analysis are as representative as possible for the area considered.
The trial analyses performed on mussel samples collected from the Mangalia, Constanța and Năvodari areas were performed in NIMRD's laboratory using the reference technique, yet, in order to comply with EU regulations, the Competent Authority (namely the Romanian Sanitary-Veterinary and Food Safety National Authority) shall designate accredited laboratories for the EN/ISO 16649-3 standard that may carry out the analyses of samples taken during official controls.
It must be underlined that classification is not permanent and, once regular monitoring indicates non-compliance with the set-parameters, classification shall be suspended and the entire process must be re-run, in order to allow safe marketing on the local and European market.

ACKNOWLEDGEMENTS

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REFERENCES

Anon (2017). Microbiological monitoring of bivalve mollusc harvesting areas - Guide to good practice. Technical Application, Retrieved December 27, 2019, from www.eurlcefas.org.

European Community (2004). Regulation (EC) No. 854/2004 of the European Parliament and of the Council of 29 April 2004 Laying Down Specific Rules for the Organisation of Official Controls on Products of Animal Origin Intended for Human Consumption. *Off J Eur Union L*. 226, 83-127. Retrieved December 27, 2019, from https://eur-lex.europa.eu/.

EN ISO 16649-3 (2015). Microbiology of the Food Chain - Horizontal Method for the Enumeration of Beta-Glucuronidase-Positive *Escherichia coli* - Part 3: Detection and Most Probable Number Technique Using 5-Bromo-4-chloro-3-indolyl-ß-D-glucuronide. International Organization for Standardization, Geneva. Retrieved December 27, 2019, from https://www.iso.org/standard/56824.html.

Nenciu, M.I., Niţă, V., Ţoţoiu, A., Hamza H. (2020). Framework for setting-up a classification and monitoring program for shellfish at the Romanian Black Sea coast. *Journal of Environmental Protection and Ecology*, 21(1) (in press).

Nicolae, C.G., Popescu, A., Nenciu, M.I., Costache, M. (2018). EU regulations for organic aquaculture - a key for producing organic food. *Scientific Papers. Series D. Animal Science*, LXI (1), 333-336.

Niță, V., Nenciu, M.I. (2017). Using the recirculating technology in a pilot-system for mariculture at the Romanian Black Sea coast. *Journal of Environmental Protection and Ecology,* 18(1), 255-263.

Niță, V., Nenciu, M.I., Nicolae, C.G. (2018a). Experimental rearing of the Golden gray mullet *Liza aurata* (Risso, 1810) in a recirculating system at the Black Sea. *Agriculture for Life, Life for Agriculture Conference Proceedings*, 1(1), 149-154. DOI: 10.2478/alife-2018-0022.

Niță, V., Nenciu, M.I., Raykov, V., Nicolae, C.G. (2018b). First attempt of rearing the Siberian sturgeon (*Acipenser baerii* Brandt, 1869) in Black Sea water. *AgroLife Scientific Journal*, 7(1), 97-102.

Niță, V., Theodorou, J., Nicolaev, S., Maximov, V., Nenciu, M.I. (2018c). Capacity building and expert training in the frame of the Constanta Shellfish Aquaculture Demonstrative Center. *Cercetări Marine/Recherches Marines*, 48, 92-99.

Niță, V., Theodorou, J., Nicolaev, S., Nenciu, M.-I. (2019). Advancing shellfish aquaculture as a sustainable food procurement option in emerging Black Sea riparian countries: Romania Country Report. *Scientific Papers. Series D. Animal Science*, LXII (2), 364-370.

Zaharia, T., Niță, V., Nenciu, M.I. (2017). *Background of Romanian marine aquaculture*. Bucharest, RO: CD Press Publishing House (in Romanian).

Scientific Papers. Series D. Animal Science. Vol. LXIII, No. 1, 2020
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HELMINTH PARASITES OF TWO CYPRINID FISHES FROM TOPOLNITSA RIVER, BULGARIA

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Abstract

*During summer 2019, 15 specimens of round-scaled barbell (Barbus cyclolepis Heckel, 1837) and 12 specimens of Orpheus dace (Squalius orpheus Kottelat & Economidis, 2006) from Topolnitsa River were collected and examined with standard techniques for parasites. Helminth parasites were recorded in 9round-scaled barbell specimens (60%) and 7 Orpheus dace specimens (58.33%) from Topolnitsa Riv*er. *Only one parasite species was identified in both cyprinid fishes –the acanthocephalan species Pomphorhynchus laevis (Zoega in Müller, 1776). Bioindicator significance of established parasite species was discussed for ecological evaluation of the state of the studied freshwater ecosystem. New data for the helminths of round-scaled barbell and Orpheus dace from Topolnitsa River are presented.*

Key words*: Barbus cyclolepis, helminths, Squalius orpheus, Topolnitsa River.*

INTRODUCTION

Topolnitsa River is a river in Southern Bulgaria, left tributary of the Maritsa River. With a length of 154.8 km, Topolnitsa River is one of the longest rivers in the Maritsa catchment area. Topolnitsa River is one of the heavily polluted rivers in Bulgaria (Zhelev et al., 2013). Species composition of the ichthyofauna, heavy metal and toxic element levels in aquatic mosses, aquatic macrophytes and sediments, changes in the morphological content of the blood of amphibian species as a result of anthropogenic pollution of Topolnitsa River and its basin were studied (Yurukova and Gecheva, 2012; Gecheva et al., 2013; Kolev, 2013; Zhelev et al., 2013). Species composition of the ichthyofauna of Topolnitsa River is represented by 13 fish species belonging to 4 families (Kolev, 2013). In Topolnitsa River, two species belonging to Cyprinidae family predominate - *Barbus cyclolepis* and *Squalius orpheus* (Kolev, 2013). The area of the Topolnitsa River has been subject to constant heavy metal pollution for many years (Velcheva and Nikolov, 2009). Parasites are indicators for the ecosystem stability, biodiversity and environmental health (Marcogliese and Price, 1997; Marcogliese, 2003; 2004; 2005; Sures et al., 2017). This study aims to present the diversity of parasites of round-scaled barbell and Orpheus dace from Topolnitsa River. As a result of this survey, new data for helminths of *B. cyclolepis* and *Sq. orpheus* is presented.

MATERIALS AND METHODS

In early summer of 2019 fish and fish parasites were collected and examined from Topolnitsa River (village Yunatsite). The village Yunatsite is located on the south bank of Topolnitsa River. The River springs from the Sredna Gora Mountain in central Bulgaria. Almost the entire Topolnitsa River from the springs to above the confluence of the Mativir River is a Protected Zone (NATURA 2000 zones: Sredna Gora BG0001389).

A total of 15 specimens of the round-scaled barbell (*Barbus cyclolepis* Heckel, 1837) and 12 specimens of Orpheus dace (*Squalius orpheus* Kottelat & Economidis, 2006) from Topolnitsa River were collected and examined in 2019. Fish were caught by angling. The scientific and common names of fish hosts are used according to the FishBase database (Fröse and Pauly, 2020).

The fish samples were examined immediately after their capture for gastrointestinal parasites using standard techniques. The samples are

counted and identified using keys of Bauer et al. (1981), Bauer (1987) and Bykhovskaya-Pavlovskaya (1985). Acanthocephalans are examined as temporary slides in ethanol-glycerin and identified (Petrochenko, 1956; Ergens and Lom, 1970; Bykhovskaya-Pavlovskaya, 1985).

The ecological terms prevalence, mean intensity (MI) and mean abundance (MA) are used and calculated, based on Bush et al. (1997). The dominant structure of the component helminth communities is determined according to the criteria proposed by Kennedy (1993) based on the prevalence (P%) as: accidental (P% < 10), component (P% < 20) and core (P% >20) species.

RESULTS AND DISCUSSIONS

A total of 15 specimens of the round-scaled barbell (*Barbus cyclolepis* Heckel, 1837) and 12 specimens of Orpheus dace (*Squalius orpheus* Kottelat & Economidis, 2006) from Topolnitsa River are collected and examined in 2019. The round-scaled barbell and Orpheus dace are not included in the Red Data Book of the Republic of Bulgaria (Golemanski (Ed.), 2011). *Barbus cyclolepis* and *Squalius orpheus* are estimated as least concern species (LC=Least Concern; IUCN Red List Status). Round-scaled barbell is freshwater, benthopelagic fish species (Fröse and Pauly, 2020). This fish species inhabits upper and middle reaches of flowing rivers with sandy-gravelly bottom. *B. cyclolepis* feeds on larvae of chironomids, caddisflies, mayflies and plant detritus (Karapetkova and Zhivkov, 2006). *B. cyclolepis* is endemic fish to the Maritsa River Basin (Kolev, 2013).

Orpheus dace is freshwater, pelagic, omnivorous fish species. Young fish feeds on algae and crustaceans, and adults - insects and their larvae, fish, frogs and small rodents (Karapetkova and Zhivkov, 2006).This fish species occurs in small to larger streams and rivers with standing water to moderate current (Fröse and Pauly, 2020). *Sq. orpheus* is endemic fish to the Aegean Basin (Kolev, 2013).

A total of 15 specimens of the round-scaled barbell (*Barbus cyclolepis* Heckel, 1837) and 12 specimens of Orpheus dace (*Squalius orpheus* Kottelat & Economidis, 2006) from Topolnitsa River were collected and examined for parasites. Helminth parasites were recorded in 9 round-scaled barbell specimens (60%) and 7 Orpheus dace specimens (58.33%) from Topolnitsa River. Only one parasite species was identified in both cyprinid fishes – the acanthocephalan species *Pomphorhynchus laevis* (Zoega in Müller, 1776) (Table 1). The only established helminth species occurred as an adult. *P. laevis* is autogenic species, matured in fish. The studied two cyprinid fish hosts showed similar prevalence, mean abundance and mean intensity (Table 1).

Table 1. Ecological indices of the helminth parasites of *B. cyclolepis* and *Sq. orpheus* from Topolnitsa River (N - number of examined fish specimens, n - number of infected hosts, p - number of parasites, P% - prevalence, MA - mean abundance, MI - mean intensity)

Host / **Helminth species**	N	n	p	P%	MA±SD	MI±SD	Range
Barbus cyclolepis / *Pomphorhynchus laevis*	15	9	27	60.00	1.8±2.34	3.0±2.36	1-8
Squalius orpheus / *Pomphorhynchus laevis*	12	7	21	58.33	1.75±3.19	3.0±3.70	1-12

The established in this study *Pomphorhynchus laevis* is an intestinal parasite of many freshwater fish, most often by a family Cyprinidae and less frequently by other families (Kakacheva-Avramova, 1983). The life cycle of *P. laevis* is accomplished with the participation of the intermediate host *Gammarus pulex* (Amphipoda), and definitive host - fish (Petrochenko, 1956). *G. pulex* is a bioindicator for β-mesosaprobity (Johnson et al., 1993).

P. laevis was reported as a parasite of *B. cyclolepis* from Tundzha River (Kakacheva-Avramova, 1972). For Bulgaria, *C. brachycollis*, *C. fennica*, *Caryophyllaeides*

sp. juv., *B. rectangulum*, *Cestoidea* g. sp., *Allocreadium isoporum*, *Allocreadium isoporum macrorchis, Diplostomum spathaceum* larv., *Dactylogyrus carpathicus*, *D. dyki, D. petenyi, Gyrodactylus albaniensis*, *G. markewitschi, Paradiplozoon homoion*, *Diplozoon* sp., *Rhabdochona gnedini* (=*Rhabdochona sulaki*), *R. denudata*, *Rhabdochana hellichi, Schulmanela petruschewskii* (= *Capillaria petruschewskii*), *Nematoda* gen. sp., *Acanthocephalus anguillae*, *Neoechinorhynchus rutili* and *P. laevis* were reported as parasites of *B. cyclolepis* (Table. 2).

Table 2. Overview of helminth species of *Barbus cyclolepis* registered in Bulgaria

Authority / Helminth species	Margaritov (1965)	Kakacheva-Avramova (1965)	Kakacheva-Avramova (1972)	Kirin (2002a)	Kirin (2003)	This study
Cestoda						
Caryophyllaeus brachycollis	•	•	•	•	•	
Caryophyllaeides fennica	•	•	•		•	
Caryophyllaeides sp. juv.		•				
Bathybothrium rectangulum	•	•		•	•	
Cestoidea g.sp.	•					
Trematoda						
Allocreadium isoporum	•	•	•**			
Allocreadium isoporum macrorchis				•		
Diplostomum spathaceum larv.		•				
Dactylogyrus carpathicus			•			
Dactylogyrus dyki			•			
Dactylogyrus petenyi		•				
Gyrodactylus albaniensis		•				
Gyrodactylus markewitschi			•			
Paradiplozoon homoion			•			
Diplozoon sp.		•*				
Nematoda						
Rhabdochona gnedini	•					
Rhabdochona denudata	•	•				
Rhabdochona hellichi					•	
Schulmanela petruschewskii		•				
Schulmanela sp.			•			
Nematoda g.sp.	•					
Acanthocephala						
Acanthocephalus anguillae	•			•	•	
Neoechinorhynchus rutili		•			•	
Pomphorhynchus laevis			•			•

•* reported from Kakacheva-Avramova (1965) as *Diplozoon* sp. and *Diplozoon* sp. diporpa

•** reported from Kakacheva-Avramova (1972) as *Allocreadium isoporum isoporum*

The parasite fauna of *B. cyclolepis* from River Topolnitsa was studied from Margaritov (1965) and Kakacheva-Avramova (1965). Margaritov (1965) studied the parasite fauna of *B. cyclolepis* from the middle reaches of River Maritsa and its tributaries - Chepinska, Topolnitsa and Vacha rivers. For Topolnitsa River, the author established for round-scaled barbell four parasite species: *Caryophyllaeus brachycollis, Caryophyllaeides fennica, Rhabdochana denudata* and Nematoda g. sp. Margaritov (1965) specifically notes that acanthocephalan species has not been revealed in fish from Topolnitsa River. Kakacheva-Avramova (1965) reported *C. fennica* and *C. brachycollis* for *B. cyclolepis* from the rivers

Asenitsa, Harmanlijska, Topolnitsa, Syuyutlijka, Sushenitsa and Bedechka. The author also reported *Caryophyllaeus* sp. juv. of *B. cyclolepis*, from the Maritsa, Chepinska and Harmanlijska rivers.

Rozdina et al. (2008) studied the food spectrum and feeding of *Barbus cyclolepis* from the middle stream of the Maritsa River (Bulgaria). According to them, the food of *B. cyclolepis* is dominated by Chironomid larvae, followed by plant detritus and Gamarids.

Kakacheva-Avramova (1965) investigated the parasite fauna of *Sq. orphaeus* (reported as *Leuciscus cephalus*) from water basins in Trakia (southern Bulgaria). The author reported *C. fennica* of *L. cephalus* from the rivers Asenitsa, Harmanlijska, Topolnitsa, Syuyutlijka, Sushenitsa and Bedechka. Kakacheva-Avramova (1965) also reported *C. brachycollis* for *L. cephalus* from the rivers Asenitsa, Sjujutlijka, Chepinska, Bedechka and Topolnitsa. The author also reported *Caryophyllaeus* sp. of *L. cephalus* from the rivers Maritsa, Syuyutlijka and Harmanlijska. In most of the conducted studies of the parasite communities of the Orpheus dace from The Maritsa River Basin *Pomphorhynchus laevis* has been reported, including and in this study (Table 3). Only Kakacheva-Avramova (1965) did not report any acanthocephalan species. The number of helminth species reported for the helminth communities of *Sq. orphaeus* from The Maritsa River Basin ranged from 1 (this study) to 8 (Kirin, 2002b; Kirin et al., 2005) (Table 3).

Table 3. Overview of helminth species of *Squalius orpheus* registered in Maritsa River Basin, Bulgaria

Authority / Helminth species	Margaritov (1965)	Kakacheva-Avramova (1965)	Kirin (2000a)	Kirin (2000b)	Kirin (2001)	Kirin (2002b)	Kirin et al. (2005)	Kirin et al. (2019)	This study
Cestoda									
Caryophyllaeus brachycollis	•	•	•	•	•		•	•	
Caryophyllaeides fennica	•	•					•		
Bathybothrium rectangulum			•	•	•	•	•		
Trematoda									
Allocreadium isoporum	•	•						•	
Allocreadium isoporum macrorchis			•	•	•		•		
Clinostomum complanatum			•	•	•				
Dactylogyrus parvus		•							
Nematoda									
Nematoda sp.	•*								
Contracaecum sp.						•**			
Rhabdochona denudata	•	•				•	•	•	
Philometra abdominalis			•	•					
Acanthocephala									
Acanthocephalus anguillae	•			•	•	•	•		
Acanthocephalus tenuirostris			•	•		•	•		
Pomphorhynchuslaevis			•	•	•		•	•	•

•* reported from Margaritov (1965) as *Agamonema* sp. larva I

•** reported from Kirin (2002b) as *Contracaecum squalii* (see Moravec, 2013)

CONCLUSIONS

River Topolnitsa is a new locality of *Pomphorhynchus laevis*. The determined helminth species *P. laevis* is a core species for the helminth communities of *B. cyclolepis* and *Sq. orpheus* from the studied ecosystem.
The establishment of only one intestinal parasite in both fish hosts indicated poor species diversity within the studied freshwater habitat and negative impacts on the ecosystem.

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REFERENCES

Bauer, O. N., Musselius, V. A., Strelkov, Yu. A. (1981). *Diseases of pond fish*. Moscow, RU: LegkayaPishchevayaPromishlenost' Publishers (In Russian).

Bauer, O.N. (1987). *Key to the parasites of freshwater fishes in the fauna of the U.S.S.R*. Leningrad, RU: Academy of Sciences, USSR, Nauka.

Bush, A., Lafferty, K., Lotz, J., Shostak A. (1997). Parasitology meets ecology on its own terms. Journal of Parasitology, 83. 575-583.

Bykhovskaya-Pavlovskaya, I. (1985). *Parasites of fish.Manual on study*, Leningrad, RU: Nauka, (In Russian).

Ergens, R., Lom, J. (1970). *Causative agents of fish diseases.* Prague, CZ: Academia, 384 (In Czech).

Fröse, R., D. Pauly (Eds.) (2020). FishBase. World Wide Web electronic publication, www.fishbase.org (02 February 2020, date last accessed).

Gecheva, G., Yurukova, L., Cesa, M., Cheshmedjiev, S. (2013). Monitoring of aquatic mosses and sediments: a case study in contaminated rivers, Bulgaria. *Plant Biosystems*, 149(3). 527-536.

Golemanski, V. (Ed-in-Chief) (2011). *Red Data Book of the Republic of Bulgaria*. Sofia, BG: Joint edited of the Bulg. Acad of Sci. and Ministry of Environment and Waters, Vol. 2. - Animalia (In Bulgarian).

IUCN Red List Status, (n.d.)www.iucnredlist.org

Johnson, R. K, Wiederholm, T., Rosenberg, D. M. (1993). Freshwater biomonitoring using individual organisms, population, and species assemblages of benthic macroinvertebrates. In: Rosenberg, D.M.&Resh, V.H. (eds) (1993). *Freshwater Biomonitoring and Benthic Macroinvertebrates*. Chapman and Hall, London.

Kakacheva-Avramova, D. (1965). Helminthological studyof fishes from some water basins in Trakia. *Fauna of Trakia*, 2, 83-120 (in Bulgarian).

Kakacheva-Avramova, D. (1972). Contribution to the helminth fauna of fish from river Tundzha. Notificatios of CLF, BAS, XV, 89-105 (In Bulgarian).

Kakacheva-Avramova, D. (1983). *Helminths of freshwater fishes in Bulgaria*. Sofia, BG: Bulgarian Academy of Sciences (In Bulgarian).

Karapetkova, M., Zhivkov, M. (2006). *Fishes in Bulgaria*. Sofia, BG: GeaLibris (in Bulgarian).

Kennedy, C. (1993). The dynamics of intestinal helminth communities in eels *Anguilla anguilla* in a small stream: long-term changes in richness and structure. *Parasitology*, 107, 71-78.

Kirin, D. (2002a). Biodiversity and ecological characteristics of the helminth communities in *Barbus tauricus cyclolepis* from Luda Yana river, Bulgaria. *Comptes rendus de l' Academie bulgare des Science*, 55(5), 97-102.

Kirin, D. (2003). Biodiversity and ecologycal evaluation of the helminths communities of *Barbus cyclolepis* and *Alburnus alburnus* from Arda river, Bulgaria. *Experimental pathology and helminthology*, 6(11), 44-50.

Kirin, D. A. (2000a).Ecologofaunistical study of the helminthological communities of *Leuciscus cephalus* L. from Maritsa River. Nauchni Trudove na Sayuzana Uchenite v Bulgaria, Plovdiv, 1, 405-408 (In Bulgarian).

Kirin, D. A. (2000b). Biodiversity and ecological assessment of the status of freshwater ecosystems from the Maritsa River. Nauchni Trudove na Sayuzana Uchenite v Bulgaria, Smolyan, 1, 82–85 (In Bulgarian).

Kirin, D. A. (2001). Biodiversity and ecology of the helminths fauna in *Leuciscus cephalus* from the Maritsa River, Bulgaria. Nauchni Trudovena Plovdiv University "Paisii Hilendarski" – Animalia, 37, 79–84.

Kirin, D. A. (2002b). Ecological study of the intestinal helminth communities of *Leuciscus cephalus* (L., 1758) and appraisal of the conditions of the studied freshwater ecosystems from the Chepelarska River, Bulgaria. *Acta Zoolologica Bulgarica*, 54, 73–85.

Kirin, D. A., Koev, K., Ivanova, D., Kuzmanov, N. (2005). Biodiversity and ecological appraisal for conditions of the Stryama River, Bulgaria. *Journal of Environmental Protection and Ecology*, 6, 69–82.

Kirin, D., Chunchukova, M., Kuzmanova, D. (2019). Helminths and helminth communities of *Orpheus dace* (*Squalius orpheus* Kottelat & Economidis, 2006) from Stryama River, Bulgaria. *Scientific Papers. Series D. Animal Science*, LXII (1), 475-480.

Kolev, V. (2013). Species composition of the ichthyofauna of some tributaries of the Maritza river, *Forestry Ideas*, 19 (2), 129–139.

Marcogliese, D. J., Cone, D.K., (1997). Food webs: a plea for parasites. *Trends in Ecology & Evolution*, 12, 320–325.

Marcogliese, D. J. (2003). Food webs and biodiversity: are parasites the missing link? *Journal of Parasitology*, 89, S106–S113.

Marcogliese, D. J. (2004). Parasites: Small players with crucial roles in the ecological theatre. *Ecohealth*, 1, 151–164.

Marcogliese, D. J. (2005). Parasites of the superorganism: are they indicators of ecosystem health? *International Journal for Parasitology*, 35, 705–716.

Marcogliese, D.J., Price, J. (1997). The paradox of parasites. *Global Biodiversity*, 7, 7-15.

Margaritov, N.M. (1965). Intestinal helminths of fishes of the middle reaches of the R. Maritsa and tributaries. *Godshnik na Sofiyskia universitet Biologicheski fakultet*, 58, 129-150 (In Bulgarian)

Moravec, F. (2001).*Checklist of the metazoan parasites of fishes of the Czech Republic and the Slovak Republic (1873–2000*). Prague, CZ: Academia Publishing House.

Moravec, F. (2013). *Parasitic Nematodes of Freshwater fishes of Europe*. Praha, CZ: Academia Publishing House.

Natura 2000 (n.d.). Retrieved from http://natura2000.moew.government.bg/Home/Natura2000ProtectedSites

Petrochenko, V.I., 1956. *Acanthocephala of Domestic and Wild Animals.*Moskow, RU: NAS of SSSR (In Russian).

Rozdina, D., Raikova-Petrova, G., Marinova R. and Uzunova, E. (2008). Food spectrum andfeeding of *Barbus cyclolepis* Heckel from the middle stream of Maritza River (Bulgaria). *Bulgarian Journal of Agricultural Sciences,*14, 209-213.

Sures, B., Nachev, M.,Selbach, C., Marcogliese, D.J. (2017). Parasite responses to pollution: what we know and where we go in Environmental Parasitology. *Parasites & Vectors*, 10, 65. https://doi.org/10.1186/s13071-017-2001-3

Velcheva, I.G., Nikolov, B. N. (2009). A study on the process of distribution, accumulation and transfer of copper (Cu) in the organisms of fishes. Ecologia Balcanica, 1, 15-20. (In Bulgarian).

Yurukova, L., Gecheva, G. (2012). Aquatic macrophytes and sediment monitoring of river pollution (Topolnitsa river basin, Bulgaria). *Comptes rendus de l'Academie bulgare des Sciences*, 65(9), 1233-1238.

Zhelev, Z. M., Popgeorgiev, G.S. &Angelov, M.V. (2013). Investigating the Changes in the Morphological Content of the Blood of *Pelophylax ridibundus* (Amphibia: Ranidae) as a Result of Anthropogenic Pollution and Its Use as an Environmental Bioindicator. *Acta zoologica bulgarica*, 65(2), 187-196.

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CONTENT OF COPPER, CADMIUM AND ARSENIC IN *Chondrostoma nasus* (Linnaeus, 1758) FROM THE DANUBE RIVER

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Abstract

This study presents the result of investigations of heavy metals (Cu, Cd) and metalloids (As) in the liver, skin and muscles of Chondrostoma nasus (Linnaeus, 1758), as well as in waters and sediments of the Danube River (Kudelin village, Vidin region), Bulgaria. The highest levels of Cu (23.05 ± 16.14 mg.kg^{-1}), Cd (0.08 ± 0.04 mg.kg^{-1}) and As (9.24 ± 4.79 mg.kg^{-1}) was found in samples of liver. The content of the studied elements decreased in the following order: liver>skin>muscle. The highest values for As content (0.07 ± 0.05 mg.l^{-1}) were found in surface waters, while the highest values for Cu content (204.09 ± 121.05 mg.kg^{-1}) were found in sediments. The circulation of the studied elements in the freshwater ecosystem was investigated. The excesses in the values of the obtained results were analyzed against regulated national and international standards.

Key words*: arsenic, cadmium, Chondrostoma nasus, copper, Danube River.*

INTRODUCTION

The Danube River is 2,857 km longest river in Europe. It flows into the Black Sea (Ilie et al., 2017a). Human activity has a substantial impact on the Danube and causes pollution of its waters with various pollutants, such as heavy metals (Ilie et al., 2017b). Contamination with heavy metals such as copper, cadmium, arsenic, lead, and others is significantly close to mining areas. Subsequently, many of the pollutants in these areas may enter the rivers (Cholakova et al., 2006). Fish cannot avoid the impact of all the contaminants found in the surrounding aquatic environment (Yarsan & Yipel, 2013). Pollutants, including heavy metals, accumulate in fish (Jovanović et al., 2017). In the tissues and organs of fish, heavy metals can be found in different concentrations. Metal concentrations also depend on the fish species (Lenhardt et al., 2012). Muscles and liver of fish are the most commonly tested for heavy metal content. Muscles - because they are an important part of the human diet and the liver – because it accumulates heavy metals in very high concentrations (Jovičić et al., 2018). Few authors have conducted studies on the content of heavy metals in tissues and organs of *C. nasus* from the Danube River (Milošković et al., 2016; Subotić et al., 2019) or from rivers and dams from the Danube River Basin (e.g. from the Nitra River (Stranai & Andreji, 2007; Andreji et al., 2012), from the rivers Pek, Tisa, South Morava, West Morava, Drina (Milošković et al., 2016), from rivers in Austria (Jirsa et al., 2008), from the dam Međuvršje from the West Morava River sub-basin (Đikanović et al., 2016a; Đikanović et al., 2016b). The content of heavy metals in waters and sediments from the Bulgarian section of the Danube River have also been carried out (Kirin et al., 2013; Kirin et al., 2014; Chunchukova et al., 2016; Chunchukova & Kirin, 2017; Chunchukova & Kuzmanova, 2017; Kirin & Chunchukova, 2017; Shukerova et al., 2017), to the Serbian section (Pajević et al., 2008; Antonijević et al., 2014; Ćirić et al., 2016; Milanov et al., 2016), to the Romanian section of the river (Woitke et al., 2003; Milenkovic et al., 2005; Teodorof et al., 2007; Vosniakos et al., 2008; Urdeş et al., 2010; Vuković et al., 2011; Gati et al., 2013; Ilie et al., 2014; Vuković et al., 2014; Ionescu et al., 2015a; Ionescu et al., 2015b; Morina et al., 2015; Burada et al., 2016; Gati et al., 2016; Morina et al., 2016; Radu et al., 2016; Teodorof et al., 2016; Tudor et al., 2016; Vasile et al., 2016; Ilie et al., 2017a; Ilie et al., 2017b; Radu et al., 2017; Rusu, 2017; Begy et al., 2018; Catianis et al., 2018).

This study presents the concentrations of heavy metals (Cu, Cd) and metalloids (As) in the liver, skin, and muscles of *Chondrostoma nasus* (Linnaeus, 1758), as well as in waters and sediments of the Danube River; to trace the circulation of the studied elements in the freshwater ecosystem.

MATERIALS AND METHODS

In 2019, fish, water and sediment were collected and examined from the Bulgarian section of the Danube River (Kudelin village, Vidin region, designated as Kudelin biotope). The village of Kudelin (44 ° 11 ′ 30 ″ N, 22 ° 40 ′ 5 ″ E) is located in northwestern Bulgaria, on the border with the Republic of Serbia and the Republic of Romania, the last settlement on the territory of the Republic of Bulgaria along the river course.

Thirty samples of common nase *Chondrostoma nasus* (L., 1758), five samples of water and four samples of sediment were collected. Species belonging to the caught fish specimens were determined by Karapetkova and Jivkov (2006), Kottelat and Freyhof (2007). The scientific name of the species is found in FishBase (Froese & Pauly, 2019). The fish are caught according to the requirements of the fishing permits for scientific research issued by the Executive Agency for Fisheries and Aquaculture, the Ministry of Agriculture, Food and Forests in Bulgaria. The metric data (weight (g) in grams, maximum length (L) and maximum body width (H) in centimetres) of all examined specimens *C. nasus* were determined (Table 1).

Table 1. Metric data (L, H and g) of the examined specimens *C. nasus* from the Danube River, Kudelin biotope

C. nasus	Min. - max.	Average ± SD
L	18-35.5	31.56 ± 3.44
H	4.3-8.5	6.64 ± 0.83
g	59-435	290.03 ± 76.12

From all specimens were collected samples of liver, skin and muscle using standard methods. The samples were analyzed in an accredited laboratory for atomic absorption spectrophotometry of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia by ISP "Optima 7000" Perkin-Elmer. Based on the results obtained from chemical analyzes, the concentrations of the investigated heavy metals in the tissues and organs of *Chondrostoma nasus* ($mg.kg^{-1}$ wet weight; $mg.kg^{-1}$ dry weight) as well as in water ($mg.l^{-1}$) and sediment ($mg.kg^{-1}$ dry weight) were determined. The bioconcentration factor (BCF) and the linear correlation coefficient of Spearman (r_s) were calculated. The software was used for statistical data processing, MS Excel (Microsoft, 2010), BioDiversity Pro (McAleece, 1997) and Statistica 10 (StatSoft Inc., 2011).

RESULTS AND DISCUSSIONS

The object of study is common nase (*Chondrostoma nasus* Linnaeus, 1758) from the Danube River, Kudelin biotope, northwestern Bulgaria. *C. nasus* is a freshwater fish of the Cyprinidae family. Occurs in rivers where the stream is moderate or fast. Prefers rocky or gravelly bottom. *C. nasus* is a herbivorous species (Kottelat and Freyhof, 2007).

The results of studies on the content of copper (Cu), cadmium (Cd) and arsenic (As) in the liver, skin and muscles in mean samples of 30 specimens of *C. nasus* are presented in Table 2 and Table 3. The content of the three elements in five water samples and four sediment samples from the Danube River, Kudelin biotope, was also determined.

From the tissues and organs of *C. nasus*, the highest levels of Cu, Cd, and As were found in liver samples (for wet weight, respectively C_{Cu} = 23.05 ± 16.14 $mg.kg^{-1}$; C_{Cd} = 0.08 ± 0.04 $mg.kg^{-1}$ and C_{As} = 9.24 ± 4.79 $mg.kg^{-1}$); followed by skin (wet weight respectively C_{Cu} = 1.64 ± 0.75 $mg.kg^{-1}$; C_{Cd} = 0.07 ± 0.04 $mg.kg^{-1}$ and C_{As} = 4.59 ± 2.33 $mg.kg^{-1}$) and muscle (wet weight respectively C_{Cu} = 0.47 ± 0.11 $mg.kg^{-1}$, C_{Cd} = 0.01 ± 0.01 $mg.kg^{-1}$ and C_{As} = 1.19 ± 0.59 $mg.kg^{-1}$). Concentrations of the studied heavy metals decrease in the order: liver>skin>muscles. In the water samples was found the highest content of As (C_{As} = 0.07 ± 0.05 $mg.l^{-1}$), followed by that of Cu (C_{Cu} = 0.04 ± 0.03 $mg.l^{-1}$) and Cd (C_{Cd} = 0.001 ± 0.001 $mg.l^{-1}$). In the sediment samples (dry weight) was found the highest content of Cu (C_{Cu} =

204.09 ± 121.05 mg.kg^{-1}), followed by the content of As (C_{As} = 19.52 ± 9.76 mg.kg^{-1}) and Cd (C_{Cd} = 1.54 ± 0.35 mg.kg^{-1}) (Table 2 and Table 3).

Table 2. Content of Cu, Cd and As (mg.kg^{-1} wet weight) in tissues and organs of *C. nasus* and water (mg.l^{-1}) from the Danube River, Kudelin biotope

Chondrostoma nasus		**Cu**	**Cd**	**As**
Liver	Min. - max.	11.08-45.31	0.03-0.13	2.82-13.95
	Mean ± SD	23.05 ± 16.14	0.08 ± 0.04	9.24 ± 4.79
Skin	Min. - max.	1.02-2.73	0.03-0.11	2.20-7.75
	Mean ± SD	1.64 ± 0.75	0.07 ± 0.04	4.59 ± 2.33
Muscle	Min. - max.	0.32-0.58	0.01-0.02	0.72-1.99
	Mean ± SD	0.47 ± 0.11	0.01 ± 0.01	1.19 ± 0.59
Water	Min. - max.	0.01-0.08	0.001-0.003	0.01-0.13
	Mean ± SD	0.04 ± 0.03	0.001 ± 0.001	0.07 ± 0.05

Table 3. Content of Cu, Cd and As (mg.kg^{-1} dry weight) in tissues and organs of *C. nasus* and sediments (mg.kg^{-1} dry weight) from the Danube River, Kudelin biotope

Chondrostoma nasus		**Cu**	**Cd**	**As**
Liver	Min. - max.	28.18-111	0.09-0.34	12.61-36.81
	Mean ± SD	63.73 ± 38.01	0.23 ± 0.13	21.24 ± 11.03
Skin	Min. - max.	2.28-7.30	0.08-0.34	8.68-30.79
	Mean ± SD	4.46 ± 2.11	0.20 ± 0.12	16.64 ± 10.20
Muscle	Min. - max.	1.23 – 2.48	0.02-0.07	3.06-7.59
	Mean ± SD	1.97 ± 0.53	0.04 ± 0.02	4.75 ± 2.08
Sediments	Min. - max.	94.66 – 362.5	1.11-1.88	12.32-33.02
	Mean ± SD	204.09 ± 121.05	1.54 ± 0.35	19.52 ± 9.76

The concentration of Cu, Cd and As in the tissues and organs of *C. nasus* is compared to the norms in Ordinance No. 31 of 2004 on the maximum levels of contaminants in foodstuffs (C_{Cu} = 10 mg/kg; C_{Cd} = 0.05 mg/kg; C_{As} = 1 mg/kg) by national law, as well as to the norms indicated by WHO (C_{Cu} = 20 mg/kg) and FAO (C_{Cu} = 30 mg/kg; C_{Cd} = 0.2 mg/kg). The concentration of Cu in the liver of *C. nasus* was established to exceed the norms specified in Ordinance No. 31 and WHO by 2.30 and 1.15 times, respectively. The concentration of Cd in the liver and skin of *C. nasus* exceeds the norms specified in Ordinance No. 31 by 1.56 and 1.32 times, respectively. The concentration of As in the liver, skin and muscles of *C. nasus* exceeds the norms specified in Ordinance No. 31 by 9.24, 4.59 and 1.19 times, respectively (Figure 1).

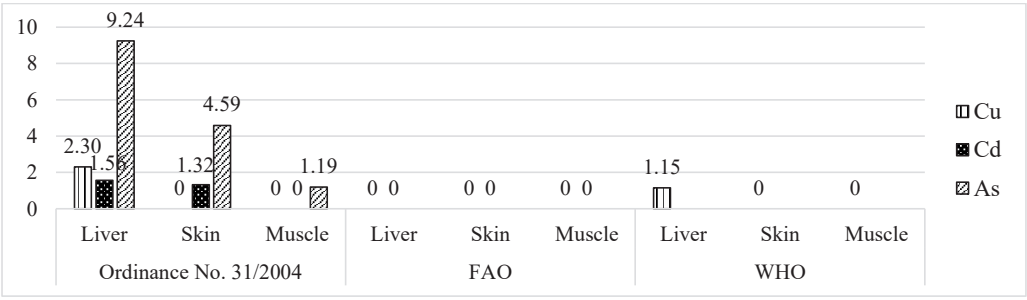

Figure 1. Exceedances of Cu, Cd and As content in liver, skin and muscle of *Chondrostoma nasus* from Danube River, Kudelin biotope according to regulatory documents

The concentration of Cu, Cd and As in the water samples is compared to the norms in Ordinance No. 18 of 2009 on the quality of water for irrigation of crops (C_{Cu} = 0.2 mg/dm^3; C_{Cd} = 0.01 mg/dm^3; C_{As} = 0.1 mg/dm^3), in Ordinance No. H-4 of 2012 on the characterization of surface water (C_{As} = 0.025 mg/l) and in the Ordinance on environmental quality standards for priority substances and certain other pollutants of 2010 (C_{Cd} = 0.0009 mg/l). The Cd concentration of the water samples was established to exceed 1.11 times the maximum permissible concentrations (MPC) specified in the Ordinance on environmental quality standards for priority substances and certain other pollutants. It was also found that the concentration of As in the water samples exceeds 2.96 times the MPC in Ordinance No. H-4 (Figure 2).

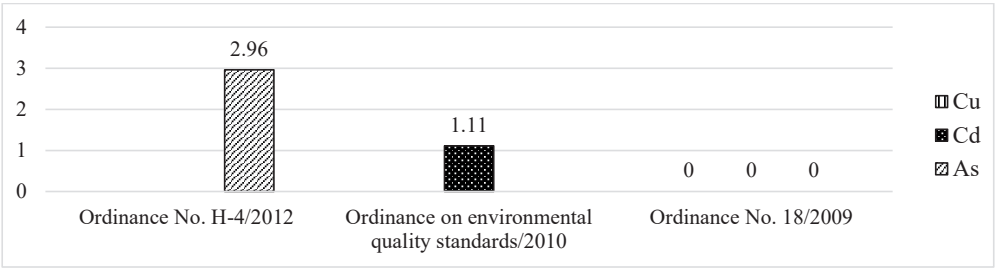

Figure 2. Exceedances of Cu, Cd and As content in surface waters of the Danube River, Kudelin biotope according to regulatory documents

The concentration of Cu, Cd and As in the sediment samples is compared to the norms in Ordinance No. 3 on the norms for permissible content of harmful substances in soils (C_{Cu} = 150 mg/kg and C_{Cd} = 2 mg/kg at pH = 7.4; C_{As} = 25 mg/kg) from national law and with the Dutch target values (C_{Cu} = 36 mg/kg; C_{Cd} = 0.8 mg/kg; C_{As} = 29 mg/kg). The concentration of Cu and Cd in the sediment samples was established to exceed 5.67 and 1.92 times the Dutch target values, respectively. Also, the concentration of Cu in sediments exceeds the MPC in Ordinance No. 3 by 1.36 times (Figure 3).

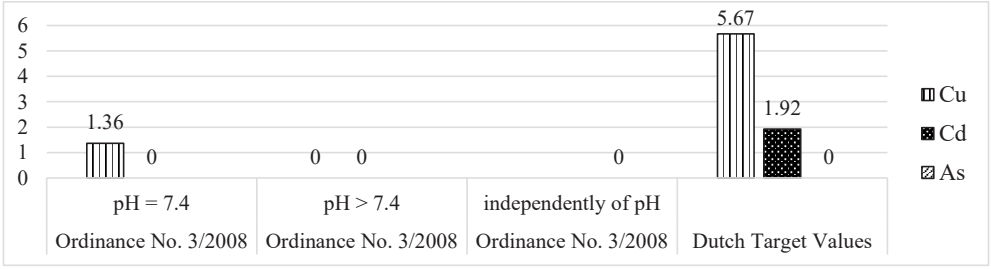

Figure 3. Exceedances of Cu, Cd and As content in sediments from the Danube River, Kudelin biotope relative to regulatory documents

The highest bioconcentration of both water (BCF = [$C_{host\ tissues}$]/[C_{water}]) and sediment (BCF = [$C_{host\ tissues}$]/[$C_{sediments}$]) was found regarding Cu, Cd and As in the samples from liver followed by those in the skin. Bioconcentration is the lowest in the muscles. The liver of the common nase bioaccumulates 4.99 times more Cu from the water compared to the accumulation of As and 7.98 times more compared to the degree of accumulation of Cd. The liver of *C. nasus* accumulates 1.60 times more As from the water, compared to that of Cd. The highest bioaccumulation of As from sediments in liver samples was established (3.48 times more than the accumulation of Cu and 7.2 times more than the accumulation of

Cd). The accumulation of Cd, both from water and from sediment in liver and skin samples, is close (statistically insignificant differences; $p>0.05$) and is 6-1 times higher than that in the muscle samples (Table 4, Table 5).

Table 4. Bioconcentration factor BCF = [$C_{host\ tissues}$]/[C_{water}]

Chondrostoma nasus/Water	BCF_{Cu}	BCF_{Cd}	BCF_{As}
C_{liver}/C_{water}	622.86	78	124.82
C_{skin}/C_{water}	44.37	66	61.97
C_{muscle}/C_{water}	12.56	13	16.12

Table 5. Bioconcentration factor BCF = [$C_{host\ tissues}$]/[$C_{sediments}$]

Chondrostoma nasus/Sediments	BCF_{Cu}	BCF_{Cd}	BCF_{As}
$C_{liver}/C_{sediments}$	0.31	0.15	1.08
$C_{skin}/C_{sediments}$	0.02	0.13	0.85
$C_{muscle}/C_{sediments}$	0.01	0.03	0.24

Positive linear correlations were found between the content of monitored elements in the liver, skin and muscles of the common nase and those in the waters and sediments of the Danube River ($r_s = 0.98 - 1.0$; $p<0.05$), proving the direct influence of the aquatic environment and the sediments on the content of the monitored elements in the tissues and organs of the common nase, except for the correlation between content of Cu in muscle and that in sediment samples ($r_s = 0.50$; $p>0.05$). A very high correlation and significance of the majority of the discussed results were found.

Studies on the content of heavy metals in tissues and organs of common nase from the Danube River are relatively small. To the present stage, such studies have not been carried out for a common nase from the Bulgarian section of the river. Jirsa et al. (2008) researched *C. nasus* from several rivers in Austria, including the Danube River, and established the highest content of cadmium and copper in liver samples. Subotic et al. (2019) studied muscle and liver of *C. nasus* from the Serbian section of the Danube River and found higher levels of Ba, Cu, Fe, and Zn in muscle samples and higher levels of Ba, Cd, Cu, and Mn in liver samples. Andreji et al. (2012) examined muscles of *C. nasus* from the Nitra River, Slovakia for heavy metal concentration and found that the metals decreased in the order: Zn>Cu>Fe>Mn>Pb>Ni>Cd. Đikanović et al. (2016a) analyzed the content of heavy metals in the liver, muscle and gills of nine fish species including *C. nasus* from the Međuvršje dam, Serbia. They established the highest concentrations of heavy metals in the liver and gills.

CONCLUSIONS

The first data for the content of Cu, Cd and As in the liver, skin and muscles of *C. nasus* is presented for the Bulgarian section of the Danube River (from the northwestern section of the river and the territory of the country). The highest content of the studied elements was found in liver samples and the lowest in the muscles. Exceedances of Cu, Cd and As content was detected in liver samples of *C. nasus*. Exceedances of Cd and As were found in skin samples, and only exceedance of As was found in muscle samples, according to national regulations. High BCF values and positive linear correlation dependencies are grounds *C. nasus* be proposed to be included as a bioindicator in biomonitoring systems regarding the content of the monitoring elements, based on their representation in liver samples. The most indicative and significant are the results for As, followed by those for Cu.

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REFERENCES

Andreji, J., Dvořák, P., Líšková, Z. D., Massányi, P., Stráňai, I., Naď, P., Skalická, M. (2012). Content of selected metals in muscle of cyprinid fish species from the Nitra River, Slovakia. *Neuroendocrinology Letters,* 33(Suppl.3), 84–89.

Antonijević, M. D., Arsović, M., Čáslavský, J., Cvetković, V., Dabić, P., Franko, M., Ilić, G., Ivanović, M., Ivanović, N., Kosovac, M., Medić, D., Najdanović, S., Nikolić, M., Novaković, J., Radovanović, T., Ranić, Đ., Šajatović, B., Špijunović, G., Stankov, I., Tošović, J., Trebše, P., Vasiljević, O., Schwarzbauer, J. (2014). Actual contamination of the Danube and Sava Rivers at Belgrade (2013). *Journal of the Serbian Chemical Society*, 79(9), 1169–1184.

Begy, R.-C., Kelemen, S., Simon, H., Tănăselia, C. (2018). The history of the sedimentation processes and heavy metal pollution in the Central Danube Delta (Romania). *Geochronometria*, 45, 97–106.

Burada, A., Teodorof, L., Ionascu, A., Topa, M. C., Georgescu, L. P., Tudor, I.-M., Ibram, O., Tudor, M. (2016). Temporal trends and evolution of heavy metals concentrations in Somova-Parches aquatic complex – last area of the Danube floodplain. *Journal of Environmental Protection and Ecology*, 17(3), 864–873.

Catianis, I., Pojar, I., Grosu, D., Scrieciu, A., Secrieru, D., Vasiliu, D., Pavel, A. B. (2018). The investigation of the water quality and bed-sediment conditions in Cutetchi Lake, Danube Delta, Romania. *Scientific Annals of the Danube Delta Institute,* Tulcea, Romania, 23, 13–22.

Cholakova, Z., Kotsev, T., Mladenova, V., Dimitrova, D., Georgieva, I. (2006). Assessment of heavy metal and arsenic concentrations in the waters of Chiprovska River catchment. Conference: Third International Conference "Global Changes and Regional Challenges", Sofia, Bulgaria, 28–29 April 2006, 180–185.

Chunchukova, M., Shukerova, S., Kirin, D. (2016). Research of the impact of the River Danube on the Srebarna biosphere reserve by the model ecosystem *Abramis brama* - macroinvertebrates - sediments. *Agricultural University of Plovdiv Agricultural Sciences/Agrarni Nauki*, VIII(19), 151–158.

Chunchukova, M., and Kirin, D. (2017). Relationships in the system *Barbus barbus* - *Pomphorhynchus tereticollis* - *Unio tumidus* in connection with the circulation of lead in the freshwater ecosystem of the Danube River, Bulgaria. *Agricultural Sciences/Agrarni Nauki*, 9(21), 71–75.

Chunchukova, M., and Kuzmanova, D. (2017). Arsenic content in parasite-host system: *Alburnus alburnus* – *Pomphorhynchus laevis* and the impact of the acanthocephalan on his host. *Agricultural Sciences/Agrarni Nauki*, 9(22), 43–47.

Ćirić, J., Janjić, J. M., Baltic, M., Milanov, R. (2016). Metal concentrations in water, sediment and three fish species from the Danube River, Serbia: a cause for environmental concern. *Environmental Science and Pollution Research*, 23(17). 17105–17112.

Đikanović, V., Skorić, S., Gačić, Z. (2016a). Concentrations of metals and trace elements in different tissues of nine fish species from the Međuvršje Reservoir (West Morava River Basin, Serbia). *Archives of Biological Sciences*, 68(4), 811–819.

Đikanović, V., Skorić, S., Jarić, I., Lenhardt, M. (2016b). Age-specific metal and accumulation patterns in different tissues of nase (*Chodrostoma nasus*) from the Medjuvršje Reservoir. *The Science of the total environment*, 566–567, 185–190.

Dutch Target and Intervention Values (the New Dutch List) (2000). Ministry of Housing, Spatial Planning and the Environment (VROM)

Froese, R., and Pauly, D. (Eds.) (2019). FishBase. World Wide Web electronic publication. Retrieved October 10, 2019, www.fishbase.org.

Gati, G., Pop, Cr., Brudașcă, F., Gurzău, A. E., Spînu, M. (2013). Assessment of the Heavy Metal Contamination in the Danube Delta from the Bioaccumulation Perspective. *Global Journal of Human Social Science, Geography, Geo-Sciences, Environmental DisasterManagement*, 13(8), 11–16.

Gati, G., Pop, C., Brudașcă, F., Gurzău, A. E., Spînu, M. (2016). The ecological risk of heavy metals in sediment from the Danube Delta. *Ecotoxicology*, 25(4), 688–696.

Ilie, M., Marinescu, F., Ghita, G., Deak, G., Tanase, G. S., Raischi, M. (2014). Assessment of heavy metal in water and sediments of the Danube River. *Journal of Environmental Protection and Ecology,* 15(3), 825–833.

Ilie, M., Marinescu, F., Ghita, G., Anghel, A.-M., Tociu, C., Popescu, I., Matei, M., Elena, H., Deák, G., Raischi, M., Cirstinoiu, C., Bogdan, U. (2017a). Spatial distribution of heavy metal contamination and ecological risk assessment in water from the Danube River. *International Journal of Environmental Science,* 2, 118–1124.

Ilie, M., Marinescu, F., Szep, R., Ghita, G., Deák, G., Anghel, A.M., Petrescu, A., Uritescu, B. (2017b). Ecological risk assessment of heavy metals in surface sediments from the Danube River. *Carpathian Journal of Earth and Environmental Sciences,* 12(2), 437–445.

Ionescu, P., Radu, V.-M., Deák, G. (2015a). Spatial and seasonal variations of heavy metals in water and sediment samples collected from the Lower Danube River. *Scientific Annals of the Danube Delta Institute,* Tulcea, Romania, 21, 43–50.

Ionescu, P., Radu, V.-M., Diacu, E. (2015b). Sediments as indicators of heavy metal contamination in the Lower Danube River. *Revista de Chimie* (Bucharest), 66(11), 1725–1727.

Jirsa, F., Leodolter-Dvorak, M., Krachler, R., Frank, C. (2008). Heavy metals in the nase, *Chondrostoma nasus* (L. 1758), and its intestinal parasite *Caryophyllaeus laticeps* (Pallas 1781) from Austrian rivers: bioindicative aspects. *Archives of Environmental Contamination and Toxicology*, 55(4), 619–626.

Jovanović, D. A., Marković, R. V., Teodorović, V. B., Šefer, D. S., Krstić, M. P., Radulović, S.B., IvanovićĆirić, J. S., Janjić J. M., Baltić M. Ž. (2017). Determination of heavy metals in muscle tissue of six fish species with different feeding habits from the Danube River, Belgrade – public health and environmental risk assessment. *Environmental Science and PollutionResearch*, 24(12), 11383–11391.

Jovičić, K., Jeftić, Ž. V., Jarić, I., Subotić, S., Hegediš, A., Lenhardt, M. (2018). Literature survey on fish tissues contamination by heavy metals and elements in the Danube River, from 1433-845 rkm. 8th International Conference "Water and Fish" - Conference proceedings, 386–393.

Karapetkova, M., and Jivkov, M. (2006). *Fish in Bulgaria*. Sofia, BG: Geya-Libris Publishing House (in Bulgarian).

Kirin, D., and Chunchukova, M. (2017). Ecological studies on the circulation of nickel in *Abramis brama* and *Pomphorhynchus tereticollis* from the freshwater ecosystem of the Danube River, Bulgaria. *Agricultural Sciences/Agrarni Nauki*, 9(21), 77–81.

Kirin, D., Hanzelova, Vl., Shukerova, S., Hristov, St., Turcekov, L., Spakulova, M. (2013). Helminth communities of fishes from the River Danube and Lake Srebarna, Bulgaria. *Scientific Papers Series D. Animal Science*, LVI, 333–340.

Kirin, D., Hanzelova, Vl., Shukerova, S., Kuzmanova, D.(2014). Biodiversity, Bioindication and Helminth communities of *Abramis brama* (Linnaeus, 1758) from the Danube River and Lake Srebarna, Bulgaria. *Turkish Journal of Agricultural and Natural Sciences*, 1, 727–733.

Kottelat, M., and Freyhof, J. (2007). *Handbook of European freshwater fishes*. Publications Kottelat, Cornol and Freyhof, Berlin. 646.

Lenhardt, M., Jaric, I., Višnjic-Jeftic, Ž., Skoric, S., Gacic, Z., Pucar, M., Hegediš, A. (2012). Concentrations of 17 elements in muscle, gills, liver and gonads of five economically important fish species from the Danube River. *Knowledge and Management of Aquatic Ecosystems*, 407(02). 1–10.

McAleece, N., Gage, J. D. G., Lambshead, P. J. D., Paterson, G. L. J. (1997). *BioDiversity Professional statistics analysis software*. Scottish Association for Marine Science and Natural History Museum London. Retrieved from http://www.sams.ac.uk.

Milanov, R., Krstić, M., Marković, R., Jovanović, D., Baltić, B., Ivanović, J., Jovetić, M., Baltić, M. (2016). Analysis of heavy metals concentration in tissues of three different fish species included in human diet from Danube River, in the Belgrade region, Serbia. *Acta Veterinaria-Beograd*, 66(1), 89–102.

Milenkovic, N., Damjanovic, M., Ristic, M. (2005). Study of heavy metal pollution in sediments from the Iron Gate (Danube River), Serbia and Montenegro. *Polish Journal of Environmental Studies*, 14(6), 781–787.

Milošković, A., Dojčinović, B., Kovačević, S., Radojković, N., Radenković, M., Milošević, D., Simić, V. (2016). Spatial monitoring of heavy metals in the inland waters of Serbia: a multispecies approach based on commercial fish. *Environmental Science and Pollution Research*, 23(10), 9918–9933.

Morina, A., Morina, F., Djikanović, V., Spasić, S., Krpo-Ćetković, J., Kostić, B., Lenhardt, M. (2015). Common barbel (*Barbus barbus*) as a bioindicator of surface river sediment pollution with Cu and Zn in three rivers of the Danube River Basin in Serbia. *Environmental Science and Pollution Research*, 23, 6723–6734.

Morina, A., Morina, F., Djikanović, V., Spasić, S., Krpo-Ćetković, J., Lenhardt, M. (2016). Seasonal variation in element concentrations in surface sediments of three rivers with different pollution input in Serbia. *Journal of Soils and Sediments*, 16(1). 255–265.

Ordinance No. 31 of 29 July 2004 on the maximum levels of contaminants in foodstuffs. Official Paper 88, 2004.

Ordinance No. 3 of 1 August 2008 on the norms for the permissible content of harmful substances in soils. Official Paper 71, 2008.

Ordinance No. 18 of 27 May 2009 on the quality of water for irrigation of crops. Official Paper 43, 2009.

Ordinance on environmental quality standards for priority substances and certain other pollutants. Official Paper 88, 2010.

Ordinance No. H-4 of 14 September 2012 on the characterization of surface water. Official Paper 22, 2013.

Pajević, S., Borišev, M., Rončević, S., Vukov, D., Igić, R. (2008). Heavy metal accumulation of Danube river aquatic plants – an indication of chemical contamination. *Central European Journal of Biology*, 3(3). 285–294.

Radu, V. M., Ivanov, A. A., Deak, G., Diacu, E. (2016). Assessment of Heavy Metals Content in Water and Sediments from the Lower Danube River Using EcoIndex. *Revista de Chimie* (Bucharest), 67(4), 649–653.

Radu, V.-M., Ivanov, A. A., Ionescu, P., Diacu, E., Popescu, I., Tociu, C. (2017). Multivariate statistical analysis of metals in Lower Danube sediments. *Bulletin of Romanian Chemical Engineering Society*, 3(1), 49–57.

Rusu, T. A. (2017). Heavy metal concentrations study in the waters of Danube Delta. 17th International Multidisciplinary Scientific GeoConference SGEM, Sofia, 17, 343–348.

Shukerova, S., Chunchukova, M., Kuzmanova, D. (2017). Cadmium content in two cyprinid fish species from the Danube River. *Agricultural Sciences / Agrarni Nauki*, 9(22), 37–42.

StatSoft Inc. (2011) (n.d.). *STATISTICA (data analysis software system), version 10.* Retrieved from www.statsoft.com.

Stranai, I, and Andreji, J. (2007). Heavy metals (Co, Ni, Cr, Pb, Cd) content in tissues of Nitra River fishes. *Slovak Journal of Animal Science*, 40(2), 97–104.

Subotić, S., Višnjić-Jeftić, Ž., Đikanović, V., Spasić, S., Krpo-Ćetković, J., Lenhardt, M. (2019). Metal accumulation in muscle and liver of the common nase (*Chondrostoma nasus*) and vimba bream (*Vimba vimba*) from the Danube River, Serbia: Bioindicative

aspects. *Bulletin of Environmental Contamination and Toxicology,* 103(2), 261–266.

Teodorof, L., Tudor, D., Nastase, C. (2007). Total mercury concentrations in surface water and sediments from Danube Delta lakes. *Scientific Annals of the Danube Delta Institute* Tulcea, Romania, 13, 155–158.

Teodorof, L., Burada, A., Despina, C., Seceleanu-Odor, D., Tudor, A. I.-M., Ibram, O., Navodaru, I., Tudor, M. (2016). Integrated indices for surface water and sediment quality, according to water framework directive. *Journal of Environmental Protection and Ecology*, 17(1), 42–52.

Tudor, I.-M., Teodorof, L., Burada, A., Tudor, M., Ibram, O., Despina, C. (2016). Long-term nutrients and heavy metals concentration dynamics in aquatic ecosystems of Danube Delta. *Scientific Annals of the Danube Delta Institute*, Tulcea, Romania, 22, 149–156.

Urdeş, L.-D., Diaconescu, C., Vlase, G., Ianiţchi, D., Diaconescu, Ş., Hangan, M. (2010). Research on interrelationship between some species of freshwater fish and helmintic larvae within aquatic ecosystems polluted with heavy metals. *Animal Science and Biotechnologies*, 43(2), 72–75.

Vasile, G., Marin, N., Petre, J., Cruceru, L. (2016). Ultrasonic nebuliser, a useful tool for improving the sensibility of trace element detection in surface water. *Journal of Environmental Protection and Ecology*, 17(1), 31–41.

Vosniakos, F., Gabriela, V., Petre, J., Cruceru, L., Nicolau, M., Mitrita, M., Iancu, V., Cruceru, I., Varga, L. A., Golumbeanu, M. (2008). Assessment of the quality of the Danube Delta aquatic ecosystem – surface water and sediment. *Journal of Environmental Protection and Ecology,* 9(2), 255–271.

Vuković, Ž., Vuković, D., Radenković, M., Stanković, S. (2011). A new approach to the analysis of the accumulation and enrichment of heavy metals in the Danube River sediment along the Iron Gate reservoir in Serbia. *Journal of the Serbian Chemical Society*, 7(3), 381–392.

Vuković, D., Vuković, Z., Stanković, S. (2014). The impact of the Danube Iron Gate dam on heavy metal storage and sediment flux within the reservoir. *CATENA*, 113.

Woitke, P., Wellmitz, J., Helm, D., Kube, P. (2003). Analysis and assessment of heavy metal pollution in suspended and sediments of the River Danube. *Chemosphere*, 51(8), 633–42.

Yarsan, E., and Yipel, M. (2013). The important terms of marine pollution Biomarkers and biomonitoring, bioaccumulation, bioconcentration, biomagnification. *Journal of Molecular Biomarkers and Diagnosis* S1: 003.

www.fao.org

www.who.int

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HELMINTHS AND HELMINTH COMMUNITIES OF THE BROWN TROUT (*Salmo trutta fario*, Linnaeus, 1758) FROM THE TAMRASHKA RIVER, BULGARIA

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Abstract

Ecoparasitological examinations of brown trout from the Tamrashka River, Aegean Water Basin, Bulgaria were carried out. Five species of helminths, one Trematoda species (Nicolla skryabini (Iwanitzky, 1928) Ślusarski, 1972) and four Nematoda species (Rhabdochona hellichi (Šramek, 1901) Chitwood, 1933; Raphidascaris acus (Bloch, 1779); Salmonema ephemeridarum (Linstow, 1872) Moravec, Santos et Brasil-Sato, 2008; Schulmanela petruschewskii (Shulman, 1948) Ivashkin, 1964) are determined. S. ephemeridarum was distinguished with the highest prevalence (50%). It is a core species for the helminth communities of the brown trout from the studied river ecosystem. *Sch. petruschewskii is a new parasite species of brown trout and S. t. fario is a new host record for Sch. petruschewskii in Bulgaria. The Tamrashka River is a new habitat for N. skryabini, Rh. hellichi, R. acus, S. ephemeridarum and Sch. petruschewskii as parasites of the brown trout in Bulgaria.*

Key words*: Aegean Water Basin, helminths, helminth communities, Salmo trutta fario.*

INTRODUCTION

The Tamrashka River is related to the Aegean Water Basin. The river and its adjacent terrestrial ecosystems (predominantly forest ecosystems) are located in one of the most beautiful sections of the Rhodopa Mountain - the Chernatitsa ridge, Bulgaria. In the past geological times, the ridge was one of the first continental droughts in the Balkans. The Tamrashka River springs from 1816 meters west of Modar Peak (Valchev, 2015). The river ecosystem refers to type R3: Mountain, Rock Type Rivers in Ecoregion 7 - Eastern Balkans (Belkinova et al., 2013). The territory is distinguished by its extremely biodiversity and the most natural complexes unaffected by human activity. At the same time, there is very little data in the scientific literature about the biodiversity, nature conservation significance and ecological status of the freshwater ecosystem of the Tamrashka River. Fish are an essential element of biodiversity in the river ecosystems. Elements of biodiversity are the parasites and the parasite communities formed in them. The most endoparasites have complex development cycles and thus reflect the state of the biodiversity and the environment. The study aims to present the results of the examinations of the gastrointestinal helminths and their helminth communities of the brown trout (*Salmo trutta fario* Linnaeus, 1758), as a typical and dominant fish species of the ichthyocenoses in the studied river ecosystem.

MATERIALS AND METHODS

During 2018, a total of 6 specimens *Salmo trutta fario* Linnaeus, 1758 are studied for gastrointestinal helminths. The fish are caught by angling under a permit issued by the Ministry of Agriculture, Food and Forestry of the Republic of Bulgaria. Ecologo-helminthological studies are performed according to Zashev & Margaritov (1966); Bauer (Ed.) (1987); Moravec (2013). All parasite specimens are fixed and stored in 70% ethyl alcohol. For species determination, the trematode specimens are included in permanent microscope slides by the methods of Georgiev et al. (1986); Scholz & Hanzelova (1998) and the specimens of nematodes - in temporary microscope slides by the method of Moravec (2013). Helminth community structure is

studied in both levels: infracommunity (total and mean number of species; total and mean number of specimens; Brillouin's diversity index; Pielou's evenness index) and component community (prevalence - P%; mean intensity - MI, for each species) (Bush et al., 1997; Magurran, 1988). According to the criteria of Kennedy (1997), the species of the component community are classified as core species (P% > 20), component (P% > 10) and accidental species (P% < 10). Calculation of the diversity measures is performed by software products Statistica 10 (StatSoft Inc., 2011) and MS Excel (Microsoft 2010).

RESULTS AND DISCUSSIONS

Fish communities

The brown trout (*Salmo trutta fario* Linnaeus, 1758; Salmonidae) inhabits clean, oxygen-rich, fast-flowing and cold waters. Brown trout reach 40 cm in length and 800 g in weight, in rare cases and more, growing relatively slowly. Young specimens feed mainly on insect larvae - daydreams, brooks, lower crustaceans, and adults - with water beetles, and small fish. The species breeds from late September to early December at 6-8^{0}C and always migrates upstream during reproduction. Sexual maturity reaches 2-4 years, and individuals of 14-21 cm length are most important for the reproduction of populations. The brown trout is a native fish species for Europe (including Bulgaria) and North Asia. The species is subject to commercial and sport fishing. *S. t. fario* is a Least concern (LC) species by the IUCN (Freyhof, 2012; Karapetkova & Zhivkov, 2006). The brown trout is a bioindicator species in freshwater ecosystems as sensitive species. *S. t. fario* is also a well-established 'model organism' for heavy metal bioaccumulation (Dvorak et al., 2020).

Helminth community structure

A total of five species of parasites belonging to two classes, four orders and four families were found in the six examined specimens of the brown trout. A total of 91 specimens of gastrointestinal helminths were studied (Table 1). The Trematoda species *Nicolla skrjabini* (Iwanitzy, 1928) Ślusarski, 1972, are distinguished by a one year developmental cycle, involving two intermediate hosts. The first intermediate hosts (for sporocysts) are the snails *Lithoglyphus naticoides* (Pfeiffer, 1828). They are localizing to the liver, glands and gills. Second intermediate hosts (for the metacercariaes) are the crustaceans *Gammarus balcanicus* Schäferna, 1923; *Obesogammarus crassus* (Sars, 1894) and *Dikerogammarus haemobaphes* (Eichwald, 1841). In them, the metacercariaes are located in the musculature of the dorsal side of the body and limbs, also in the body cavity.

Table1. Biological diversity and basic ecological characteristics of the helminth communities of *S. trutta fario*

Helminth species	N[1]	P[2]	P%[3]	MI[4]
Trematoda Class Order Fasciolida Family Opecoelidae				
Nicolla skrjabini (Iwanitzky, 1928) Ślusarski, 1972	2	7 2-5	33.34	3.5
Nematoda Class Order Spirurida Family Rhabdochonidae				
Rhabdochona hellichi (Šramek, 1901) Chitwood, 1933	2	28 6-22	33.34	14
Order Ascaridida Family Anisakidae				
Raphidascaris acus (Bloch, 1779)	1	40	16.67	40
Order Trichinellida Family Capillariidae				
Salmonema ephemeridarum (Linstow, 1872) Moravec, Santos et Brasil-Sato, 2008	3	9 1-4	50	3
Schulmanela petruschewskii (Shulman, 1948) Ivashkin, 1964	1	7	16.67	7
Total number of species	5			
Mean ± SD	1.50 ± 0.55			
Number of fish	3		3	
Number of helminth species	3		1	
Number of fish	1	1	1	3
Number of helminth specimens	11	22	40	6
Total number of specimens	91			
Mean ± SD	10.12 ± 12.80			
HB (Brillouin's diversity index)	1.26			
E (Pielou's evenness index)	0.837			

[1]N = total number of infected fish specimens.
[2]P = total number of endoparasite specimens.
[3]P% = prevalence.
[4]MI = mean intensity.

Metacercariaes are encysted, with oval or round, dark, unevenly pigmented cysts (Bauer, 1987). *G. balcanicus* is a bioindicator for χ–saprobityand refers to the relatively tolerant forms (group C) (Rusev, 1993; Peev & Gerasimov, 1999; Belkinova et al., 2013).

Definitive hosts of *N. skryabini* are different fish species of the families Cyprinidae, Percidae, Gobiidae, Siluridae, Gadidae, Esocidae, Acipenceridae, Salmonidae (Bauer, 1987). *N. skrjabini* was reported by *S. trutta fario*, including from the rivers in the Rhodopa Mountain (from Trigradska and Vacha rivers – Kakacheva-Avramova and Nedeva, 1978; from Chuprenska, Trigradska, Vacha, Shirokolashka rivers – Kakacheva-Avramova and Nedeva, 1979; from Trigradska, Vacha, Chuprenska rivers; rivers from Rhodopa Mountain in the district of the town Devin-Kakacheva-Avramova and Nedeva, 1982; Table 2). According to this study, the Tamrashka River is a new habitat of *N. skryabini* as a helminth species on the brown trout.

Rhabdochona hellichi (Šramek, 1901) Chitwood, 1933 is an intestinal parasite of many fish species of the families Cyprinidae, Salmonidae, Acipenseridae, Siluridae etc., which are the final hosts of the species (Bauer, 1987; Kakacheva-Avramova, 1983; Moravec, 2013). *Rh. hellichi* has been reported as a parasite of *S. t. fario* including from rivers of the Rhodopa Mountain (from Trigradska, Devinska, Vacha, Mugla, Shirokolashka rivers – Kakacheva-Avramova & Nedeva, 1979; Table 2). The Tamrashka River is a new habitat of *R. hellichi* as a parasite species of *S. trutta fario*.

Raphidascaris acus (Bloch, 1779) as an adult form is found in the intestine and stomach of various species of freshwater fish (*Esox lucius* Linnaeus, 1758, *Salmo trutta fario* Linnaeus, 1758, *Lota lota* (Linnaeus, 1758), *Anquilla anquilla* (Linnaeus, 1758) *Perca fluviatilis* (Linnaeus, 1758), *Sander lucioperca* (Linnaeus, 1758), *Hucho hucho* (Linnaeus, 1758), *Onchorhynchus mykiss* (Suckley, 1859), *Thymallus thymallus* (Linnaeus, 1758) etc.). Free or encapsulated larvae of *R. acus* have been established in different internal organs of freshwater fish from different families, acting as intermediate or paratenic hosts for the parasite species. The species *R. acus* has been reported as a parasite of different species of fish in Bulgaria (Bauer, 1987; Kakacheva-Avramova, 1983; Moravec, 2013). The species has been reported from *S. t. fario*, including from rivers in the Rhodopa Mountain (from the Arda River - Kirin, 2002; Table 2). The Tamrashka River is reported for the first time as a new habitat of *R. acus* as a parasite species of *S. t. fario*.

Table 2. Species of endoparasites of *S. t. fario* in Bulgaria

Species of endoparasites	Authors
Trematoda	
Bunodera lucioperca (Müller, 1776)	1, 8
Crepidistomum farionis (Müller, 1784)	10, 4, 5, 7, 8
Crepidostomum metoecus (Braun, 1900)	2, 3, 4, 6, 7, 8
Nicolla skrjabiny (Iwanitzky, 1928)	4, 5, 7
Nicolla proavita (Wišnewski, 1934) (syn. *Crowcrocaecum proavitum*)	2, 3, 4, 5, 6, 7
Nicolla wisniewski (Ślusarski, 1958)	4, 5, 6, 7, 8
Nicolla testobliqua (Wišnewski, 1933)	4, 6, 7
Cestoda	
Tetraonchus sp.	4
Acanthocephala	
Acanthocephalus anguillae (Müller, 1776)	1, 5, 7, 8
Pomphorhynchus laevis (Müller, 1776)	5
Metechinorhynchus truttae (Schrank, 1788)	4, 6, 7, 8
Nematoda	
Rhabdochona hellichi (Šramek, 1901) Chitwood, 1933	5, 8
Cucullanus truttae (Fabricius, 1794)	5, 6, 7
Raphidascaris acus (Bloch, 1779)	8
Salmonema ephemeridarum (Linstow, 1872) Moravec, Santos et Brasil-Sato, 2008 (Syn. *Ichthyobronema tennuissima*; *Cystidicoloides ephemeridarum*)	9, 10, 1, 2, 3, 4, 5, 6, 7, 8

[1]Kakacheva-Avramova, 1969.
[2]Kakacheva-Avramova, 1972.
[3]Kakacheva-Avramova, 1973.
[4]Kakacheva-Avramova & Nedeva, 1978.
[5]Kakacheva-Avramova&Nedeva, 1979.
[6]Kakacheva-Avramova & Nedeva, 1981.
[7]Kakacheva-Avramova & Nedeva, 1982.
[8]Kirin, 2002.
[9]Margaritov, 1959.
[10]Margaritov, 1964.

The life cycle of *Salmonema ephemeridarum* (Linstow, 1872) Moravec, Santos et Brasil-Sato, 2008 was carried out with the participation of the intermediate hosts *Habroflebia lauta* Eaton, 1884, *Habroleptoides modesta* (Hagen, 1864), *Ephemera danica* Müller, 1764. *H. lauta* and *E. danica* are bioindicators for 0-β-mesosaprobity of the water ecosystem. *H. modesta* is a bioindicator for 0-saprobity. Intermediate hosts refer to less sensitive forms (group B) (Rusev, 1993; Peev and Gerasimov, 1999; Belkinova et al., 2013). The adult parasite develops in definitive hosts of different species of fish from the families

Salmonidae, Thymalidae, Acipenseridae, Percidae, Esocidae, Angillidae. The species *S. ephemeridarum* was reported from *S. t. fario* in Bulgaria (Bauer, 1987; Kakacheva-Avramova, 1983; Moravec, 2013). The species was reported from the rivers in the Rhodopa Mountain (from the Vasil Kolarov Reservoir and the Yadenitsa River - Margaritov, 1959; 1964, respectively; from the Arda River - Kirin, 2002; Table 2). The helminth species was also reported as endoparasite species of brown trout from the Tundzha River and rivers of the Balkan Mountain (Kakacheva-Avramova, 1972 and Kakacheva-Avramova, 1973, respectively). The Tamrashka River is a new habitat of *S. ephemeridarum* as a parasite species of *S. t. fario*.

Schulmanela petruschewskii (Shulman, 1948) Ivashkin, 1964, accomplishes its development with the participation of intermediate hosts of oligochaetes of the species *Eisenia tetraedra* Jackson, 1931. The specific localization of the parasite is oligochaetes body cavity until it reaches an invasive stage. The final hosts for the parasite species are freshwater fish species (*Gymnocephalus cernua* (Linnaeus, 1758), *Cobitis taenia* (Linnaeus, 1758), *Lepomis gibbosus* (Linnaeus, 1758), *Sander lucioperca* (Linnaeus, 1758), *Perca fluviatilis* (Linnaeus, 1758), *Salmo trutta fario*, Linnaeus, 1758 and others). The adult form of the parasite inhabits the liver parenchyma of fish (Bauer, 1987; Kakacheva–Avramova, 1983; Moravec, 2013). The species *Sch. petruschewskii* has been reported from other freshwater fish species in Bulgaria (Kakacheva-Avramova, 1983). *S. t. fario* is a new host record for *Sch. petruschewskii* in Bulgaria. The Tamrashka River is reported for the first time as a new habitat of *Sch. petruschewskii*.

Component communities

Salmonema ephemeridarum (Linstow, 1872) Moravec, Santos et Brasil–Sato, 2008 is distinguished with the highest prevalence (P% = 50). It is the core species for the endoparasite communities of the brown trout. The species *Nicolla skryabini* (Iwanitzky, 1928) Ślusarski, 1972 and *Rhabdochona hellichi* (Šramek, 1901) Chitwood, 1933 are also core species for the helminth communities of *S. t. fario* from the Tamrashka River, but they are represented with lower prevalences (on P% = 33.34, respectively). *Raphidascaris acus* (Bloch, 1779) and *Schulmanela petruschewskii* (Shulman, 1948) Ivashkin, 1964 are component species for the endoparasite communities of the brown trout (on P% = 16.67, respectively). According to the results of the study, *Raphidascaris acus* is distinguished with the highest mean intensity (40 specimens in one specimen of fish), followed by *Rhabdochona hellichi*, MI = 14 (28 helminth specimens in two specimens of fish). The remaining species have significantly lower mean intensity (Table 1).

Infracommunities

The helminths of class Nematoda are represented by a significantly higher number of parasite species and specimens (four species with a total of 84 specimens) than those of the class Trematoda (one species with seven specimens). Three specimens of fish have two species of endoparasites, and the other three specimens of examined fish have on one endoparasite species. There are 40 specimens of *R. acus*, found in one specimen of brown trout; 22 specimens of *Rh. hellichi*, found in one specimen of fish and six specimens of some helminth species, found in another examined fish specimen. Mixed infection was detected in three specimens of fish: two specimens of *N. skrjabini* and four specimens of *S. ephemeridarum*; 5 specimens *N. skrjabini* and one specimens *S. ephemeridarum*; seven specimens *Sch. Petruschewskii* and four specimens *S. ephemeridarum*. Determined species of parasites of the brown trout (*S. trutta fario*), total number of taxa and specimens of them, peculiarities of the life cycles of the parasites, determined indices of diversity (HB = 1.26, Brillouin's diversity index) and evenness (E = 0.837, Pielou's evenness index) testify for the 0-β-mesosaprobty conditions and for the very good ecological status of the investigated freshwater ecosystem (Rusev, 1993; Peev & Gerasimov, 1999; Belkinova et al., 2013). No parasites causing dangerous diseases to fish, humans or other hosts have been identified, which cases and diseases (suspected pathogens) have been reported to other parasites by other authors (Kakacheva-Avramova and Menkova, 1979; Pekova et al.,

2017; Pekova et al., 2017a; Mitev et al., 2020). The endoparasites, identified in this study, account for 33.34% of the registered endoparasites of the brown trout in the country (Table 2). All helminth species of the brown trout identified in this study were reported by other countries and authors (Bauer, 1987; Moravec, 2013). *Salmo trutta fario* has been the subject of a number of helminthological studies in other countries. Hanzelová et al. (2001) were studied *S. t. fario* from the Morskeoko Lake in Eastern Slovakia. The authors reported for five species of helminths: *B. luciopercae*, *P. longicollis*, *N. rutili*, *P. laevis*, *C. ephemeridarum*. With highest prevalence, mean abundance and mean intensity they were fixed *N. rutili* and *P. longicollis* (P% = 86, MA = 13, MI = 15.2; P% = 53, MA = 12, MI = 22.4, respectively). Moravec (2002) studied the morphological differences between *Crepidostomum farionis* and *C. metoecus* by the methods of a scanning electron microscopy. Pellitero (1979) studied *S. t. fario* from different water ecosystems in Spain. The author established eight species of helminths belonging to classes of Nematoda and Trematoda, but he does not identify helminths belonging to classes Cestoda and Acanthocephala. The author determined the prevalences of the fixed helminth species: *Cystidicoloides tennuissima* (P% = 79.47); *Crepidostomum metoecus* (P% = 78.96); *C. farionis* (P% = 63.10); *Raphidascaris acus* (P% = 44.10); *Spinitectus gordoni* (P% = 39.47); *Capillaria coregoni* (P% = 25.96); *Nicolla* sp. (P% = 18.33); *Rhabdochona sulaki* (P% = 7.69). *C. tennuissima*, *R. acus*, *C. coregoni*, *Nicolla* sp. and *Rh sulaki* Pellitero reported as new species records for Spain and Portugal. The infection with *Rh. sulaki* was determined as accidental. The author proves the links for the following infections: *C. farionis* and *C. metoecus*; *C. farionis* and *C. tenuissima*; *C. metoecus* and *C. tenuissima*. The biocenotic relations, Pellitero explained with seasonal differences and different ecological characteristics in studied freshwater ecosystems. Moravec (2004) was studied the transmission and the seasonality of infection of the helminth species *R. acus* in *S. t. fario* from a small stream in North Bohemia, Czech Republic. The author points to *Gammarus fossarum* Koch, 1836 as an intermediate host for this parasite species and highest infection (prevalence and intensity) in August and October. Mladineo et al. (2009) were examined *S. t. fario* from the Cetina River (Croatia). The authors reported for two species of parasites: *Cyathocephalus truncatus* (Cestoda, Spathebothriidae) and *Echinorhynchus trutae* (Acanthocephala, Echinorhynchidae), parasitizing in the intestine of the examined fish. They point out that the heavy load on the body of the trout with intestinal parasites could cause their exhaustion and even death.

CONCLUSIONS

As a result of this study, *S. t. fario* is a new host record for *Sch. Petruschewskii* in Bulgaria.
The Tamrashka River is a new habitat of *N. skryabini*, *Rh. hellichi*, *R. acus*, *S. ephemeridarum*, *Sch. petruschewskii* as parasite species of *S. t.fario*. *S. ephemeridarum*, *N. Skryabini* and *Rh. hellichi* are core species in the helminth communities of brown trout.
R. acus and *Sch. petruschewskii* are component helminth species in these communities.

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REFERENCES

Bauer, O. (Ed.) (1987). *Key to the Parasites of Freshwater Fishes of the USSR*. Leningrad, RU: Nauka Publishing House (in Russian).

Belkinova, D., Gecheva, G., Cheshmedjiev, S., Dimitrova-Dyulgerova, I., Mladenov, R., Marinov, M., Teneva, I., Stoyanov, P.(2013). *Biological analysis and ecological assessment of surface water types in Bulgaria*. Plovdiv, BG: UnivPubl House "P. Hilendarskii" (in Bulgarian).

Bush, A., Lafferty, K., Lotz, J., Shostak, A. (1997). Parasitology meets ecology on its own terms. *Journal of Parasitology,* 83, 575-583.

Dvorak, P., Koushik, R., Jaroslav, A., Liskova, Z.D., Mraz, J. (2020). Vulnerability assessment of wild fish population to heavy metals in military training area: Synthesis of a framework with example from Czech Republic. *Ecological Indicators*, 110.

Freyhof, J. (2011). Salmo trutta. The IUCN Red List of Threatened Species 2011: e.T19861A9050312. Retrieved from https://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T19861A9050312.en.

Georgiev, B., Biserkov, V., Genov, T. (1986). In toto staining method for cestodes with iron acetocarmine. *Helminthologia*, 23, 279-281.

Hanzelová, V., Špakulová, M., Turčeková Ľ. (2001). Diversity of endoparasitic helminthes of fish from the lake Morskéoko, Eastern Slovakia. *Helminthologia*, 38(3), 139-143.

Kakacheva-Avramova, D. (1969). Helminths by fish from rivers of western Staraplanina mountain. II. Trematoda, Cestoda, Acanthocephala, Nematoda. *Notifications of the Centr. Helm. Lab*., XIII, 61-74 (in Bulgarian).

Kakacheva-Avramova, D. (1972). Contribution to the helminth fauna of fish from river Tundzha. *Notifications of Centr. Helm. Lab*., XV, 89-105 (in Bulgarian).

Kakacheva-Avramova, D. (1973). Helminth fauna of fish from rivers of Central and Eastern Balkan mountain. *Notifications of Helm. Lab*., XVI, 87-109 (in Bulgarian).

Kakacheva-Avramova, D. (1983). *Helminths of fresh water fishes in Bulgaria*. Sofia, BG: Bul. Acad. Sci. Publishing House(in Bulgarian).

Kakacheva-Avramova, D., Nedeva, I. (1978). Helminthological examination of fish from the Rhodope region. *Helminthologia*, 6, 44-53 (in Bulgarian).

Kakacheva-Avramova, D., Nedeva, I. (1979). Helminths of trouts. *Veterinary gathering*, 8, 15-17 (in Bulgarian).

Kakacheva-Avramova, D., Nedeva, I. (1981). Contribution to the study of fish helminths from Blagoevgrad region. *Helminthologia*, 11, 26-41 (in Bulgarian).

Kakacheva, D., Nedeva, I. (1982). Helminths of trouts (family Salmonidae) from freshwater bodies. *Veterinary-Medical Sciences*, XIX (5), 16-18 (in Bulgarian).

Karapetkova, M., Zhivkov, M. (2006). *Fishes in Bulgaria*. Sofia, BG: Gea Libris Publishing House (in Bulgarian).

Kennedy, C. (1997). Freshwater fish parasites and environmental quality, an overview and caution. *Parassitologia*, 39, 249-254.

Kirin, D. (2002). Biodiversity and ecological peculiarities of the helminth fauna in *Salmo trutta fario* from Arda River, Bulgaria. *Comptes rendus de l'Académie bulgare de Sciences*, 55(7), 83-88.

Magurran, A. (1988). *Ecological diversity and its measurement*. London, UK: Cambridge University Press Publishing House.

Margaritov, N. (1959). *Parasites of some freshwater fishes*. Varna, BG: Publishing House NIRRP (in Bulgarian).

Margaritov, N. (1964). Notes for helminth fauna of our freshwater fishes. *Bulletin of Institute of Zoology with Museum*, 15, 199-202 (in Bulgarian).

Mitev, M., Pekova, L., Valkanov, S. (2020). Dynamic follow-up and proving through computed tomography and magnetic resonance imaging of changes in secondary meningoencephalitis from suppurative left maxillary sinusitis. *Trakia J. Sci*., 2.

Mladineo, I., Zrnčić S., Oraić D. (2009). Severe helminthic infection of the wild brown trout (*Salmo trutta*) in Cetina River, Croatia; Preliminary observation. *Bull. Eur. Ass. Fish Patho*., 29(3), 86-91.

Moravec, F. (2002). External morphological differences between *Crepidostomum farionis* and *Crepidostomum metoecus* (Trematoda: Allocreadidae), parasites of salmonids, as revealed by SEM. *Folia Parasitologica*, 49, 211-217.

Maravec, F. (2004). Observations on the transmission and the seasonality of infection of the nematode *Raphidascaris acus* in *Salmo trutta fario* in a small trout stream in North Bohemia, Czech Republic. *Helminthologia*, 41(1), 91-97.

Moravec, F.(2013). *Parasitic Nematodes of Freshwater fishes of Europe*. Praha, CZ: Academia Publishing House.

Peev, D., Gerassimiv, S. (1999). *Express and long-term methods for biological monitoring*. Sofia, BG: GeaLibris Publishing House.

Pekova, L., Parousheva P., RachkovaK., Tsoneva V., Naydenov K., Mitev M., Tsachev I. (2017). Clinical Characteristic of *Listeria monocytogenes* Neuroinfections. *Nauka Infectologiya/Parasitologiya*, 1(14), 44-48 (in Bulgarian).

Pekova L., Parusheva P., Rachkova K., Naydenov K., Tsoneva V., Mitev M. (2017a). *Listeria monocytogenes* Meningoencephalitis in Immunocompetent Patient - Clinical Course and Outcome. *Ann Clin Lab Res*, 5(3), 191-193.

Rusev, V. (1993). *Principles of saprobiology*. Sofia, BG: Sofia University "St Kliment Ohridski" Publishing House.

Pelitero, A. (1979). *The helminth coenosis of the digestive tract of trout in the rivers of Leon*. Leon, ES: Institution "Fray Bernardo de Sahagun" de la Excma. Diputacion Provincial (C. S. I. C.) Publishing House.

Scholz, T., Hanzelová, V.(1998). *Tapeworms of the Genus Proteocephalus Wienland, 1858 (Cestoda: Proteocephalidae), parasites of fishes in Europe*. Praha, CZ: Academia Publishing House.

Statsoft Inc. (2011) (n.d.). STATISTICA (data analysis software system), version 10. Retrieved from www.statsoft.com.

Valchev, A. (2015). *Tamrash*. Sofia, BG: Asconi Publishing House (in Bulgarian).

Zashev, G., Margaritov, N. (1966). *Diseases of fish*. Sofia, BG: Naukai izkustvo Publishing House (in Bulgarian).

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THE USE OF CLINOPTILOLITE ZEOLITE AS A FEED ADDITIVE IN JUVENILE CARP FEED (*Cyprinus carpio*)

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Abstract

The clinoptilolite natural zeolite can be used as a dietary supplement in fish breeding to improve nutritional parameters and maintain their health. In this regard, within the Aquaculture Laboratory of the USAMV Bucharest, an experiment was carried out with juvenile carp species (Cyprinus carpio). The fish, divided into three groups, were fed with 1% and 2% zeolite feed additive, and non-additive feed, respectively, for 10 weeks. The comparative analysis of the results obtained for the morpho-productive characters (live weight, total length and maximum body height) revealed that the group fed with 2% clinoptilolite additive feed, obtained the best performances. Clinoptilolite in feed has contributed at maintaining favorable media conditions for the growth and development of fish from the controlled systems used. Although there were no significant differences in medium performances, it was found that clinoptilolite positively influenced the studied characters.

Key words*: aquaculture, feed, fish, morphological characters, zeolite.*

INTRODUCTION

Worldwide, aquaculture supplies for human consumption, over 50% of total fish production and due to the decrease of catches in fishing areas, this percentage will increase to 20% by 2032 (FAO, 2014). Global freshwater deficiency, strict regulations regarding quality of wastewater from fish farms and the limited space available are obstacles that need to be overcome for the development of aquaculture. The technologies used in aquaculture must ensure increased production and a minimal negative impact on the environment due to the toxic contaminants in the effluent waters of the recirculating systems.

Clinoptilolite, registered as a food additive - DIN53770, is declared safe for final consumers of meat, milk or eggs from animals that have zeolite in feed (EFSSA, 2007).

Due to their chemical and physical properties, zeolites and especially clinoptilolite, have a wide range of use. In recent decades, a lot of researchers have paid particular attention to the use of zeolites in biochemistry. The use of zeolite as a feed additive leads to growth rate improve and to maintenance of general state of health of animals, implicitly of the fish. In recirculating systems, fish feed, additive with zeolite, contributes to improving fish productivity, but is also a corrector of environmental conditions (Obradovic et al., 2006). Weight gain of biomass is the result of the detoxification effect of zeolite (Ortatatli and Oguz, 2001; Rizzi et al., 2003), and by slower passage of feed through the intestine, a better utilization of the nutrients is achieved (Dias et al., 1998; Eya et al., 2008). One of the important factors that influence the health of fish in recirculating aquaculture systems is the level of ammonia in the technological water (Badiola et al., 2012).

It is assumed that the rate of biomass growth is stimulated by suppressing the formation of ammonia, which is considered toxic in cells, and in the gastrointestinal tract of animals (Papaioannou et al., 2005). By using fish feed additive with zeolite, the amount of oxygen used in the oxidation of ammonia is reduced (Florian et al., 2002). The composition of the feed and the technological parameters of the

water are determining factors of the sensory quality of fish meat.
Clinoptilolite has been used as an additive in fish feed at concentrations of 1 to 10% (Edsall and Smith, 1989; Yıldırım et al., 2009; Khodanazary et al., 2013).
In Romania, research on the use of clinoptilolite in animal husbandry has been carried out only since 2000. The results of the research highlighted the favorable effects of zeolite on the feed conversion coefficient (Pogurschi et al., 2017). The quality of milk production, animal health and welfare were improved by using the Romanian volcanic tuff rich in clinoptilolite as a food additive and as a supplement in bedding. The zeolite thus used has led to the provision of optimal technological conditions for the environment (Marin et al., 2018).
Research conducted in 2017 in the Aquaculture Laboratory of USAMV Bucharest showed that the use of clinoptilolite with the granulation of 1-3 mm, in column form, ensures the filtration of water from a controlled system (Sava et al., 2017; Nicolae et al., 2017).

MATERIALS AND METHODS

The experimental research, which was carried out in the Aquaculture Laboratory of the USAMV Bucharest, followed the study of influence of the feed additive with clinoptilolite zeolite on the development of juvenile carp (*Cyprinus carpio*). The duration of the experiment was 70 days. The controlled system used consisted of three aquariums with a capacity of 220 l each, the juvenile carp being distributed in them, in lots of 36 individuals (Figure 1).
The average weight of the fish was 28.28 g/pcs in aquarium 1, 29.28 g/pcs in aquarium 2 and 26.30 g/pcs in aquarium 3. Two of the fish groups were fed with zeolite feed additive in percentages of 1% (aquarium 1) and 2% (aquarium 2), and the third lot was the control group (aquarium 3), fed with non-additive feed with zeolite. The feed dose, administered in 3 rations, was 4% of body weight in the first 42 days and 5% of body weight in the next 28 days.
Clinoptilolite zeolite used as a feed additive is a hydrated crystalline aluminosilicate, with a frame-like structure containing pores occupied by water and alkaline cations that give it a high ion exchange capacity and molecular sieve properties. Table 1 shows the chemical composition of the zeolite used.

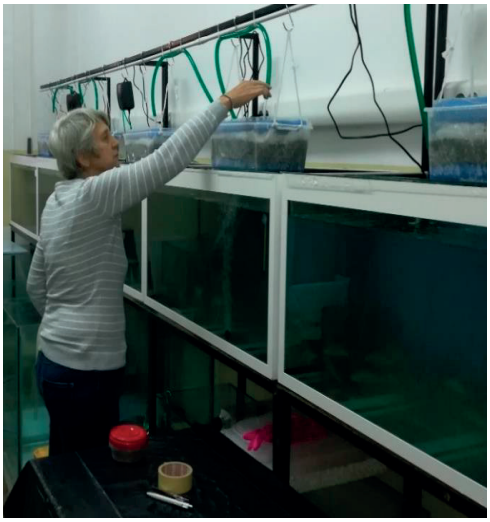

Figure 1. Controlled system for fish growth (original photo)

Table 1. The chemical composition of the clinoptilolite zeolite used

Compound	Percentage (%)
SiO_2	68.75-71.30
Al_2O_3	11.35-13.10
CaO	2.86-5.2
K_2O	3.17-3.40
Fe_2O_3	2.10-1.90
MgO	1.18-1.20
Na_2O	0.82-1.30
P.C.	9.77

When was the recipes made, the chemical composition of the ingredients and their digestibility level were taken into account, so that the level of catabolics released in the environment is as low as possible (Table 2 and Table 3).
It is known that the feeding method used and amount of daily ration are important elements, which condition the degree of acceptance and consumption of feed and efficiency of bioconversion (Misăilă, 2004).

Table 2. Raw materials in the used recipes

Raw materials	Recipe 1 - 1% zeolite	Recipe 2 - 2% zeolite	Recipe 3 - 0% zeolite
Fish meal 65%	25.00%	25.00%	25.00%
Corn	28.67%	26.63%	30.70%
Wheat	10.00%	10.00%	10.00%
Soy bean 46%	31.31%	31.68%	30.95%
Sunflower oil	2.81%	3.50%	2.13%
Zeolite	1.00%	2.00%	0.00%
Lysine HCl	0.13%	0.12%	0.14%
DL-Methionine	0.08%	0.08%	0.08%
Premix 1%	1.00%	1.00%	1.00%
Total	100.00	100.00	100.00

Table 3. The chemical composition of recipes

Nutritional value	Recipe 1 - 1% zeolite	Recipe 2 - 2% zeolite	Recipe 3 - 0% zeolite
Metabolizable energy	3000 kcal	3000 kcal	3000 kcal
Crude fat	7.02	7.64	6.41
Crude ash	6.58	6.58	6.58
Calcium	1.61	1.64	1.58
Total phosphorus	1.15	1.14	1.15
Crude cellulose	2.21	2.16	2.25
Crude protein	35.00	35.00	35.00
Total lysine	2.20	2.20	2.20
Digestible lysine	1.98	1.98	1.98
Total methionine	0.80	0.80	0.80
Digestible methionine	0.73	0.73	0.73
Arginine	2.20	2.20	2.19
Histidine	0.87	0.87	0.86
Isoleucine	1.47	1.47	1.47
Leucine	2.60	2.59	2.61
Phenylalanine	1.53	1.53	1.53
Threonine	1.36	1.36	1.36
Valine	1.68	1.68	1.68
Tryptophan	0.37	0.37	0.37

Also, through the administered food was aimed at maintaining the juvenile carp health as well as the quality of the water in the aquariums. Water filtration was performed using zeolite filters (Sava et al., 2017). Each filter used 4 kg of clinoptilolite, with a granulation of 2-3 mm. Zeolite was regenerated at 48 hours with saline solution (Nicolae et al., 2017).

Each individual was measured for three morphological characters: living body weight, maximum body height and total body length, at 2 weeks intervals. Body weight (W) was determined by weighing with a scale for small weights. Maximum body height (H) and total length (L) were measured using the graded line. Maximum body height was measured in the highest region of the body, at the level of the first radius of the dorsal fin (Nicolae et al., 2013) (Figure 2). The total length was measured on the midline of the body, from the tip of the muzzle to the midline joining the extremities of the two caudal lobes.

Figure 2. Maximum height measurement (original photo)

Statistical analysis was required to rank the categories of individuals taking into account the analysed characters: living weight, maximum body height and total length. The performance averages and their errors were established based on the following calculation relationships:

$$\overline{X} = \frac{\sum X}{n_x} \quad (1)$$

$$S_{\overline{x}} = \sqrt{\frac{S^2}{n_x}} \quad (2)$$

Where:

$\overline{X}$ = average;

ΣX = values sum;

n_x = values number/ specimen size/lot size;

$S_{\overline{x}}$ = average error;

S^2 = variant.

The Fisher test was used to determine whether or not there are significant differences between the groups of individuals made up of the amount of zeolite administered in the ration,

with respect to average body performance. Fisher value determination was done using variance analysis (ANOVA) with two sources of variation: intergroup and intragroup.
The chemical analyzes of the fish meat were carried out according to Regulation (EC) no. 152/2009 and ISO standards, by the gravimetric method for the dry substance, the Kjeldahl method, using a semi-automatic KJELTEC 2300 auto system - Tecator (Sweden), for crude protein, the organic solvent extraction method for crude fat and the gravimetric method for ash.

RESULTS AND DISCUSSIONS

At two-weeks intervals weighing was performed and the total length and maximum height of the fish body in the three aquariums were measured.
The results obtained after performing the 5 weightings are presented in Table 4.

Table 4. The average performances determined for live weight

Specification	N	Live weight (W), g				
		$\bar{X} \pm s_{\bar{X}}$				
		Interval 1 (2 weeks)	Interval 2 (2 weeks)	Interval 3 (2 weeks)	Interval 4 (2 weeks)	Interval 5 (2 weeks)
Aquarium 1	36	29.33±0.80	31.71±0.98	34.97±1.14	37.20±1.35	40.85±1.75
Aquarium 2	36	29.36±0.87	32.78±0.97	34.83±1.12	39.39±1.37	42.61±1.63
Aquarium 3	36	27.14±0.84	31.42±0.98	33.31±1.17	36.25±1.34	38.42±1.56
Total	108	28.61±0.48	31.97±0.56	34.36±0.65	37.62±0.78	40.63±6.87

Interval 1 (first two weeks of the study)
Comparing the performances recorded by individuals from each aquarium it can be observed that the average body weight of the individuals in the control aquarium deviates the most from the statistical population average, respectively by 1.47 g, this group of individuals achieving the lowest performance.
The averages of the performances achieved by individuals from the aquariums where zeolite was administered were close, the difference between the two aquariums being only 0.03 g.
Interval 2 (weeks 2-4 of the study)
Analyzing the registered performances, it can be observed that between all three aquariums there are very small differences, of maximum 1.36 g, the smallest weight being established for the control group.
Interval 3 (weeks 4-6 of the study)
The performance averages established for each group show that between groups there are very small differences, respectively between the average of the lowest performing group and the statistical population average being 1.05 g.
Interval 4 (weeks 6-8 of the study)
The best weight was determined for the aquarium 2 group, which achieved a performance with 2.19 g more than in the other aquarium where additive feed was administered with 2% clinoptilolite and 3.14 g more than the control group.
Interval 5 (weeks 8-10 of the study)
The evolution of body weight in the last study interval is similar to the situation presented for interval four. Individuals who received 2% clinoptilolite in ration differ from the control group by an additional 4.19 g. The poorest performance was determined for the control group, which achieved with 2.21 g less than the statistical population average.
Simultaneous analysis of the performances recorded in all three aquariums, throughout the experiment showed that the individuals in the aquarium where zeolite was administered 2% in ration achieve the best weights in 4 of the 5 intervals, and the lowest performances were established for the individuals in the control group (Figure 3).

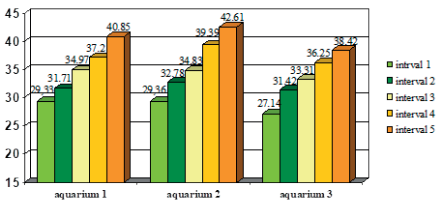

Figure 3. Diagram of live weight recorded performances

It can conclude that the administration of 2% clinoptilolite influences the character of the study, live weight. Regarding the second character that represented the object of this study, respectively the total body length, in the first interval, the best performance is described for the group of individuals in the aquarium 2 (Table 5).

Table 5. The average performances determined for total body length

Specification	N	Total body length (L), mm				
		$\bar{X} \pm s_{\bar{X}}$				
		Interval 1	Interval 2	Interval 3	Interval 4	Interval 5
Aquarium 1	36	11.85±0.12	12.13±0.34	12.76±0.13	13.15±0.15	13.49±0.17
Aquarium 2	36	12.13±0.11	12.47±0.11	12.79±0.12	13.30±0.13	13.62±0.14
Aquarium 3	36	11.81±0.13	12.09±0.13	12.45±0.15	13.01±0.16	13.10±0.18
Total	108	11.83±0.07	12.12±0.13	12.66±0.08	13.15±0.08	13.40±0.09

Individuals in aquarium 1 achieve a total length of only 0.05 mm longer than the control group, for which the lowest performance was established. The situation is similar for next four intervals.

The largest difference was observed in the second interval, respectively in Aquarium 2. The average of total body length was 0.35 mm higher compared to the average calculated for the statistical population (Figure 4).

The averages of performances recorded by all three groups of individuals for the maximum body height character are very close (Figure 5). Between the best and the poorest performance, a difference of only 0.1 mm in the first interval, 0.06 mm in the second, 0.11 mm in the third, 0.16 mm in the fourth and 0.17 mm in the last interval was achieved (Table 6).

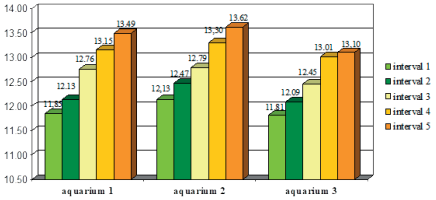

Figure 4. Diagram of total length recorded performances

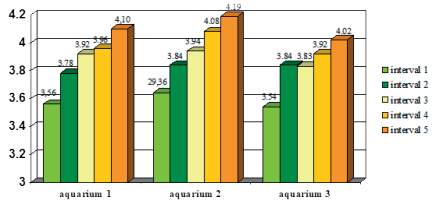

Figure 5. Diagram of maximum body height recorded performances

Table 6. The average performances determined for maximum body height

Specification	N	Maximum body height (H), mm				
		$\bar{X} \pm s_{\bar{X}}$				
		Interval 1	Interval 2	Interval 3	Interval 4	Interval 5
Aquarium 1	36	3.56±0.04	3.78±0.05	3.92±0.05	3.96±0.05	4.10±0.07
Aquarium 2	36	3.64±0.05	3.84±0.06	3.94±0.06	4.08±0.06	4.19±0.07
Aquarium 3	36	3.54±0.04	3.84±0.04	3.83±0.04	3.92±0.05	4.02±0.06
Total	108	3.58±0.03	3.82±0.03	3.90±0.03	3.10±0.03	4.10±0.04

The results of the test of the significance of the differences between the averages of the performances for weight, length and height, determined for the three groups, taking into account the evolution in each interval, are presented in Table 7.

Table 7. Fisher test for studied characters

Character	Period	The average of the squares intergroups	The average of the squares intragroups	Fisher test
Live weight	Interval 1	58.528	25.320	2.311ns
	Interval 2	18.400	34.213	0.538ns
	Interval 3	30.587	46.477	0.658ns
	Interval 4	93.192	66.143	1.409ns
	Interval 5	159.728	97.034	1.646ns
Total length	Interval 1	1.106	0.501	2.207ns
	Interval 2	1.519	0.543	2.797ns
	Interval 3	1.291	0.649	1.989ns
	Interval 4	0.795	0.790	1.006ns
	Interval 5	2.654	0.957	2.773ns
Maximum body height	Interval 1	0.095	0.078	1.218ns
	Interval 2	0.037	0.083	0.446ns
	Interval 3	0.129	0.101	1.277ns
	Interval 4	0.253	0.116	2.181ns
	Interval 5	0.276	0.167	1.652ns

The calculated Fisher values for all three characters are smaller than table values. This shows that between all three groups of individuals there are no significant differences in terms of average performances, in any of all three characters studied. The low values of morpho-productive parameters of juvenile carp from the experience are due to the relatively low water temperature (18-20ºC), the experiment being carried out between November 2019 - January 2020.

At the end of the study period, of 10 weeks, the chemical composition of fish meat was analysed, in terms of the proportion of dry matter (DM), crude protein (CP), crude fat (CF) and ash (A). The results obtained are presented in Table 8.

Table 8. Chemical analysis of fish meat

Zeolite (%)	DM 65ºC (%)	DM103ºC (%)	CP (%)	CF (%)	A (%)
0	27.78	96.26	58.67	28.99	5.78
1	27.03	96.93	60.01	28.25	5.60
2	27.80	96.85	58.52	31.50	5.30

Chemical analysis of juvenile carp meat showed that the highest percentage of crude protein was recorded by the group fed with feed additive with 1% zeolite. The group fed with feed additive with 2% zeolite had the highest percentage of crude fat.

CONCLUSIONS

In the present study the influence of the feed additive with the clinoptilolite zeolite on the development of juvenile carp (*Cyprinus carpio*) was highlighted.

Even if there were no significant differences by comparing the recorded performances, it can be concluded that the administration of clinoptilolite influences productive characters.

The best results regarding live weight, total length and maximum body height characters were achieved by the group fed with 2% zeolite recipe.

It is important to note that no deaths were recorded during the experiment.

Research has shown the benefits of using clinoptilolite as a feed additive in juvenile carp feed.

ACKNOWLEDGEMENTS

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REFERENCES

Badiola, M., Mendiola, D., Bostock, J. (2012). Recirculating aquaculture systems (RAS) analysis: main issues on management and future challenges. *Aquaculture Engineering*, 51, 26–35.

Dias, J., Huelvan, C., Dinis, M. T., Metailler, R. (1998). Influence of dietary bulk agents (silica, cellulose and a natural zeolyte) on protein digestibility, growth, feed intake and feed transit time in European seabass

(*Dicentrarchus labrax*) juveniles. *Aquatic Living Resources*, 11, 219–226.

Edsall, D. A., Smith, C. E. (1989). Effects of dietary clinoptilolite on levels of effluent ammonia from hatchery coho salmon. *The Progressive Fish-Culturist*, 51, 98–100.

EFSSA, (2007). Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed on safety of Zeolite as a feed additive for dairy cows. *The EFSSA Journal*, 523, 1–11.

Eya, J. C., Parsons, A., Haile, I., Jagidi, P. (2008). Effects of dietary zeolytes (Bentonite and Mordenite) on the performance juvenile rainbow trout *Onchorhynchus mykiss*. *Australian Journal of Basic and Applied Sciences*, 2, 961–967.

FAO, (2014). The State of World Fisheries and Aquaculture. The Food a nd Agriculture Organization of the United Nations (FAO), Rome, 223.

Florian, M., Hicks, B., Groves, D. (2002). Zeolite additive for animal feed method of using same. World Intellectual Property Organization, WO 02/41703 A2.

Khodanazary, A., Boldaji, F., Tatar, A., Dastar, B. (2013). Effects of dietary zeolyte and perlite supplementations on growth and nutrient utilization performance, and some serum variables in common carp, (*Cyprinus carpio*). *Turkish Journal of Fisheries and Aquatic Sciences*, 13, 495–501.

Marin, M., Pogurschi, E., Nicolae, C. (2018). Use of volcanic tuff in dairy farms - solution for environmental protection. *Journal of Environmental Protection and Ecology*, 19(2), 620–627.

Misăilă, C. (2004). Ecological fish feeding strategies in aquaculture. *Analele Ştiinţifice ale Universităţii „Al.I.Cuza" Iaşi, s. Biologie animală*, L, 243–256.

Nicolae, C. G., Grosu, H., Costache, M., Marin, M., Radu, D., Badilita, A., Niţă, V. (2013). Genetic evaluation in a population of Frasinet cyprinids used for selection to maximize meat production. *Scientific Papers. Series D. Animal Science*, LVI, 358–361.

Nicolae, C. G., Sava, S. C., Marin, M. P., Pogurschi, E., Sava, B. A. (2017). Innovative solutions for removing nitrogen compounds from water of recirculating aquaculture system using clinoptilolite natural zeolytes. *Current Trends in Natural Sciences*, 16(11), 105–109.

Obradovic, S., Adamovic, M., Vukasinovic, M., Jovanovic, R., Levic, J. (2006). The applications effects of natural zeolite in feed and water on production results of Oncorhynchus Mykiis (Walbaum), *Romanian Biotechnological Letters*, 11(6), 3005–3013.

Ortatatli, M., Oguz, H. (2001). Ameliorative effects of dietary clinoptilolite on pathological changes in broiler chickens during aflatoxicosis. *Research in Veterinary Science*, 71, 59–66.

Papaioannou, D., Katsoulos, P. D., Panousis, N., Karatzias, H. (2005). The role of natural and synthetic zeolytes as feed additives on the prevention and/or the treatment of certain farm animal disease: a review. *Microporous and Mesoporus Materials*, 84, 161–170.

Pogurschi, E., Marin, M., Zugravu, C., Nicolae, C. G. (2017). The potential of some Romanian zeolites to improve bioeconomy results. *Scientific Papers.Animal Science Series*, 67, 151–155.

Rizzi, L., Simioli, M., Roncada, P., Zaghini, A. (2003). Aflatoxin b1 and clinoptilolite in feed for laying hens: effects on egg quality, mycotoxin residues in livers and hepatic mixed-function oxygenase activities. *Journal of Food Protection*, 66, 860–865.

Sava, S. C., Nicolae, C. G., Marin, M., Sava, B. A. (2017). Innovative model based on clinoptilolite use in water purification in recirculating aquaculture systems (RAS). Catalog of The XV Edition of the International Exhibition of Research, Innovation and Inventions PROINVENT Cluj-Napoca, Romania, 192–193.

Yıldırım, O., Turker, A., Senel, B. (2009). Effects of natural zeolyte (clinoptilolite) levels in fish diet on water quality, growth performance and nutrient utilization of tilapia (*Tilapia zillii*) Fry. *Fresenius Environmental Bulletin*, 18, 1567–1571.

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SLAUGHTER AND MORPHOPHYSIOLOGICAL CHARACTERISTICS OF MALE STERLET (*Acipenser ruthenus* Linnaeus, 1758) OF DIFFERENT AGE, REARED IN A CAGE FARM

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Abstract

A comparative analysis of indicators related to the meat production of a male Sterlet at the age of six and seven summers raised on an industrial cage farm located in Southeastern Bulgaria was performed in the study. The slaughter yield and meat quantities, as well as indices relating to the exterior and interior of the fish, were calculated. Older fish have been found to be significantly heavier than younger ones, but age does not significantly affect the weight of individual body parts and exterior dimensions. It is a reliable source of variation only on the relative proportion of fillets without the abdomen in the cleaned carcass. The difference in favor of younger fish was 8.2% (p≤0.05). Fish of different ages do not differ significantly in the indexes related to exterior, interior and fatness. A significant difference in favor of younger fish (p≤0.05) was found only on the spleen-somatic index.

Key words*: aquaculture, exterior indices, interior indices, meat production, sturgeon.*

INTRODUCTION

The condition of wild sturgeon populations is steadily deteriorating (Bronzi and Rosenthal, 2014). The development of sturgeon breeding is of particular importance for their conservation and restoration (Vasileva, 2015).

Bronzi et al. (2019) note that more and more production (caviar and meat) comes from Sturgeon aquaculture. In Bulgaria, Sturgeon breeding is developing well (Nikolova, 2019). The country ranks 8th in caviar production and 12th in Sturgeon biomass production (Bronzi et al., 2019).

A number of sturgeon species are cultivated in Bulgarian aquaculture (MAFF, 2019), and traditionally farmed Sterlet. Sterlet is the smallest representative of the Acipenseridae family. The life span is about 20 years, reaching a maximum length of 70-90 cm and a weight of 2-4 kg (Chebanov and Galich, 2013). Sterlet is successfully cultivated in different technologies, in different regions (Volkova, 2006; Rybnikár et al., 2011; Khudyi et al., 2014). Ak et al. (2019) identify the species as suitable for multicultural farming, and Skvortsova and Pavlova (2017) identify it as the most popular target for industrial fish farming.

At the same time, according to Saraiva (2020), knowledge of this species, both in the wild and in aquaculture, is not sufficient. The author notes the need for scientific research on this species in its cultivation on aquaculture farms. They affect the productivity of fish by the rearing technologies used (Prokeš et al., 2011; Akbulut et al., 2013), which is why it is important to conduct studies in specific conditions (Nikolova, 2013). In combination with genetic factors, environmental factors determine both body growth and body proportions in fish (Kirpichnikov, 1979; Kapusta et al., 2013).

In Bulgaria, Sterlet is reared in pure condition and used to produce hybrids. In Sturgeon farms with a full reproduction cycle, after the selection of producers to complete the reproductive herds, the remaining male individuals are used for meat. In this connection, the regularities of the development of the species and the meat-forming qualities, which are determined by them at different stages of life, are of interest. We have set ourselves the goal of conducting a slaughter and morphophysiological characteristic of male Sterlets at different ages, when reared in an industrial cage farm located in Southeastern Bulgaria.

MATERIALS AND METHODS

The study was carried out with male individuals of Sterlet (*Acipenser ruthenus* Linnaeus, 1758) from a net-cage farm located in a warm water reservoir. According to its type, the reservoir refers to large and deep reservoirs. Its area is 16.07 km^2, its volume is 532.9 x 106 m^3. The reservoir is located in South-Easthern Bulgaria, at 41°37 'N latitude and 25°20' E longitude. It falls into the South Rhodopian climatic region. The average altitude is about 280 m. Fish of different age groups were reared in separate net-cages. The cages were 8 × 8 m in size, the water depth being 6 m. Each cage had a double polyamide net. The average stocking density during the vegetation period was 4.28-5.20 kg/m³. Feeding was performed with a commercial granulated sturgeon feed (Table 1).

Table 1. Composition of commercial feed

Indices	Value	Indices	Value
Protein, %	46	Vitamin A, IU.kg^{-1}	10 000
Fat, %	15	Vitamin C, mg.kg^{-1}	520
Crude fibre, %	1.4	Vitamin E, mg.kg^{-1}	200
Ash, %	6.5	Vitamin D3, IU.kg^{-1}	2 303
Total P, %	1.03	Gross energy, MJ.kg^{-1}	21.0
Ca, %	1.4	Digestible energy, MJ.kg^{-1}	19.2
Na, %	0.3%		

Five individuals were randomly selected from each age group (six - (Ar_{5+}) and seven – summer - old (Ar_{6+}) at the end of the vegetation period (in November) for the morphophysiological analysis. Classical methods for exterior measurements and slaughter analysis of fish have been applied.

Total weight, kg - TW; Total length, cm - TL; Standard length, cm - SL; Fork length, cm - FL; Maximum body height, cm - BH; Maximum body width, cm - BT; Maximum body girth, cm - aO; Eviscerated weight (TW without intestines), kg - EW; Carcass weight (TW without intestines, whole head, fins and tail), kg - CW; Fins and tail, kg - FT; Head without gills, kg - Hw; Gills, kg - G; Bone plates, kg - Bp; Swim bladder, kg - Sb; Pyloric appendage, kg - Pa; Chord, kg - Ch; Fillet with skin, kg - FS; Fillet with skin without belly flap, kg - FSwB; Total viscera/insides/, kg - TV; Gonads, kg - GO; Heart, kg - Ht; Liver, g - LW; Spleen, kg - SW were measured for each fish.

The following indices were calculated: IHB - High-backed index - (SL/BH); IBB - Broad-backed index (BT/SL) * 100; IH - Hardness index - (aO/SL) * 100, %; CFF - Fulton's coefficient - (TW/SL^3) * 100, %; CFC - Clarc's coefficient (EW/SL^3) * 100, %; IC - Condition index - (TW/(SL * BH * aO)) * 100, %; ICR - Modified Fulton's coefficient by Jones et al. (1999) (according to Richter et al., 2000) - ($TW/(SL^2BH)$) * 100, %; VSI - Viscerosomatic index - (TV/TW) * 100, %; HSI - Hepatosomatic index - (LW/TW) * 100, %; GSI - Gonadosomatic index - (GO/TW) * 100, %; SSI - Spleensomatic index - (SW/TW) * 100, %; HtSI - Heartsomatic index - (Ht/TW) * 100, %; Sv1 - Slaughter value 1 (EW/TW) * 100, %; Sv2 - Slaughter value 2 (TW without intestines and gills/TW) * 100, %; Sv3 - Slaughter value 3 (CW/TW) * 100, %; Fy1 - Relative share of the fillet with skin from the live weight - (FS/TW)*100, %; Fy2 - Relative share of the fillet with skin from the carcass weight - (FS/CW)*100, %; My1 - Relative share of the fillet with skin without belly flap from the live weight - (FSwB/TW)*100, %; My2 - Relative share of the fillet with skin without belly flap from the carcass weight - (FSwB/CW)*100, %.

IBM SPSS Statistics 21 was used for statistical data processing.

RESULTS AND DISCUSSIONS

The exterior characteristics of the analyzed fish are presented in Table 2. Although the absolute values for these indicators are higher for fish of the older age group, age is not a reliable source of variation. A similar regularity was observed by Reshetnikov and Popova (2015), who, based on Coregonidae's own studies, found that plastic signs in fish are less closely related to age than to size and growth rate.

Table 2. Exterior characteristics of fish, cm

Indices	Ar_{5+}			Ar_{6+}		
	LS	±Se	SD	LS	±Se	SD
TL	68.72	0.836	1.672	73.26	1.223	2.446
FL	61.30	0.751	1.502	65.94	1.041	2.082
SL	57.70	0.826	1.652	60.54	2.087	4.174
BH	8.950	0.334	0.669	9.460	0.266	0.532
BT	8.580	0.566	1.132	11.68	0.985	1.969
aO	26.40	1.059	2.118	30.96	0.772	1.544

Prokeš et al. (2011) indicate that the most intense growth of Sterlet is at 1-2 years of age, and the adult period begins after 4-5 years of age. In the conditions of our studied by us, the Sterlet maintains good growth rates until later in life, with seven-summer-old fish 1.5 times ($p<0.01$) heavier than six-summer-olds (Table 3). Logically, in older fish, the absolute weights of individual body parts are higher. CW was heavier by 51.1%, whole fillet (FS) by 44%, cleaned fillet (FSwB) - 34.4%, all insides (TV) - 63.6%, fins and tail (FT) - 33.9% and head without gills (Hw) - 15.8%. Only six-summer-old fish, heavier by 2.9%, have bone shields (Bp), but the difference is not significant. Age is not a reliable source of variation in the weight characteristics of individual body parts as a whole.

Table 3. Weight characteristics, g

Indices	Ar_{5+}			Ar_{6+}		
	LS	±Se	SD	LS	±Se	SD
TW	1667.6**	136.3	267.8	2456.6**	192.8	337.9
CW	1055.2	82.75	165.5	1594.1	117.8	235.6
FS	927.6	90.52	181.0	1335.7	112.0	224.0
FSwB	825.2	83.81	167.6	1108.7	82.85	165.7
TV	291.0	60.83	121.7	476.0	54.84	109.7
GO	153.1	56.96	113.9	292.5	66.97	133.9
LW	55.71	5.454	10.91	74.84	2.066	4.132
SW	2.542	0.087	0.175	3.364	0.331	0.663
Ht	1.962	0.132	0.263	2.564	0.382	0.765
FT	65.75	1.558	3.116	88.01	5.862	11.72
Hw	222.7	8.491	16.98	257.9	18.37	36.74
G	29.70	1.654	3.308	40.57	5.846	11.69
Bp	62.57	5.442	10.88	60.76	8.299	16.60
Sb	2.410	0.275	0.549	6.400	1.151	2.302
Pa	0.858	0.212	0.425	2.354	0.392	0.784
Ch	20.35	3.246	6.493	26.43	6.396	12.79

**P<0.01

In terms of the weight of the individual internal organs, there are also differences in favor of older fish, respectively, in the liver - 34.3%; spleens - 32.3%; heart - 30.7%, but they are not significant. The biggest (91%) is the difference in the weight of the gonads in favor of older fish, which is also not significant. The development of gonads in fish can vary greatly within the same age and body weight. Thus, Skvorcova and Pavlova (2017), by comparing the morphological characteristics of Sterlet with relatively equal body weight, from different aquaculture farms, found more than 5 times the difference in the development of gonads. The authors point out that, as a whole, the weight of internal organs increases in parallel with the increase in the body weight of the fish.

The development of the fish can be determined by the dynamics of the relative proportions of the individual parts of the body (Table 4). In Sterlet, the head makes up for 12.62% (seven summers) to 15.35% (six summers) of body weight. Despite the fact that the head refers to conditionally consumable products, in sturgeon it has value because it makes a certain type of canned food (Lisovskaja et al., 2009). Sturgeon fish are also often prepared whole for the purpose, especially for smaller species. In all cases, when using the head, the gills are removed, which in our case reduces its weight by 11.8-13.6%.

Table 4. Relative proportions of the individual parts of the body, % of the body weight

Indices	Ar_{5+}			Ar_{6+}		
	LS	±Se	SD	LS	±Se	SD
Hw	13.55	0.882	1.764	10.51	0.371	0.743
G	1.798	0.094	0.187	1.650	0.199	0.399
FT	4.007	0.259	0.518	3.584	0.033	0.067
Sb	0.145	0.015	0.029	0.260	0.037	0.074
Pa	0.052	0.014	0.029	0.096	0.016	0.032
Ch	1.236	0.190	0.379	1.102	0.266	0.533
Bp	3.873	0.555	1.110	2.488	0.318	0.636

When Sterlet is prepared as a whole dish, chords are removed in addition to the gills (Ratushniy and Aminov, 2017). Chord in sturgeon species can be referred to consumable products, and in Russia it is known as 'vyaziga' (Vlasova and Frensis, 2007). The relative proportion of chord in our study ranged from 1.1 to 1.2 and decreased with age.

Fish is a unique product that is subject to a full processing. Waste from fish processing, except for the production of fishmeal, can be used in various fields of production. Swimming bladders from Sturgeon fish are used to clarify beverages and for the production of fish glue, the latter being 15-20% by weight of feedstock (Koochekian et al., 2006).

The use of non-traditional raw materials is expanding in various industries. Thus, there is a growing interest in medicine for fish swimming bladders. Ivanova and Petrova (2015) noted that, in medicine, fish collagen and its hydrolysis products are widely used for the treatment of wounds, skin burns, ulcers, pulps, osteoarthritis and hypertension. The authors obtained positive results from a study

of the possibility of using the Siberian sturgeon swimming bladder as a source of collagen for the production of medical supplies (adhesive and film for superficial wounds).

In our study, at six- and seven-summer-olds, the swimming bladder accounts for 0.145 and 0.260%, respectively, of the body weight, with its relative proportion increasing almost twice with age. In older fish, the relative share of the pyloric appendage is also higher, with a difference of 1.8 times.

In general, it can be summarized that the proportion of head without gills and gills decreases with age. The same applies to other parts of the body, except the pyloric appendage and the swimming bladder, where the difference is in favor of older fish.

Slaughter indices are decisive in the evaluation of animals grown for meat. They depend on both genetic and paratype factors. Thus, Souza et al. (2015), when studying the effect of rainbow trout body weight on processing yield and chemical composition, found that fish with a lower body weight had higher yield for the whole eviscerated fish and head, but a lower yield for the viscera.

We have calculated three of the main ones recommended by Todorov and Ivancheva (1992) in fish farming, yields - slaughter (Sv1), consumable (Sv2) and for the canning industry (Sv3) (Table 5). Sv1 and Sv2 were higher in the Sterlet at an earlier age by 2.9% and 2.7%, respectively, and Sv3 were higher than those at an older age (2.4%), but the differences were not significant.

Table 5. Slaughter indicators, %

Indices	Ar_{5+}			Ar_{6+}		
	LS	±Se	SD	LS	±Se	SD
Sv1	82.9	2.29	4.58	80.6	1.83	3.65
Sv2	81.1	2.28	4.55	78.9	1.84	3.68
Sv3	63.4	1.71	3.41	64.9	1.64	3.28
Fy1	55.4	1.30	2.60	54.3	2.32	4.63
Fy2	49.2	1.34	2.68	45.2	2.16	4.36
My1	87.5	2.15	4.30	83.6	1.75	3.49
My2	77.8*	2.13	4.26	69.6*	1.86	3.73

*P<0.05

Whole Sterlet fillet, at the studied age, constitutes 54.3-55.4% of the body weight, with the difference in Fy1 favoring younger fish being 1.1%. The difference in Fy2 is greater - 4%. This shows that the proportion of valuable muscles decreases with age, but the differences found are not significant. Higher, but again not significant, in six-summer-old fish, the relative share of whole (My1) and of the filet in the cleaned carcass (My2) is also high. Age is a significant source of variation only on My2. The difference in favor of younger fish is 8.2% ($p \leq 0.05$).

The morphophysiological method for assessing regularity of growth and development is often applied to both fish and warm-blooded animals, although the principles of manifestation of morphophysiological regularity are different (Bolshakov, 2019). In fish studies, indices related to constitution, exterior, fatness, and development of internal organs are sources of information about the condition of individuals and their well-being (Smirnov et al., 1972; Dzyubuk and Klyukina, 2014 etc.).

Dekic et al. (2016) point out condition factor and organosomatic indices of fish as tools for assessing the impact of environmental factors on fish. In fish farmed on aquaculture farms, morphophysiological indicators are examined using different technologies. Thus, Molchanova and Khrustalyov (2017) found in rainbow trout reared in RAS changes on a number of plastic features. Kuritsyn et al. (2017) have applied the morphophysiological method for analyzing the physiological status, health status and general well-being of rainbow trout and muksun when grown in cages. The authors state that the absolute and relative dimensions of the internal organs of different fish species differ and depend on the conditions under which the fish are farmed. For example, the weight of the heart is related to the activity and energy balance in body; liver weight - with metabolic processes; the spleen is an important hematopoietic organ, also associated with fish metabolism; the relative share of all the viscera can be used as a biotest for physiological features related to nutrition, etc.

Lenhardt et al. (2012), when comparing the morphological characteristics of wild and cultivated aquaculture Sterlet, found significant differences on 11 traits. Farm-raised fish had a shorter pectoral fins and stockier body. Jankowska et al. (2007) also found differences in slaughter yield, proximate composition and flesh color of cultivated and wild perch.

Table 6 shows the morphometric and morphophysiological indices of six- and seven-summer-old Sterlets. The high backed index (IHB) is practically the same for fish of both age groups. For the bold backed index (IBB), the difference (4.4%) is in favor of older individuals. Fish of this age are also more compact (with a higher hardness index - IH), with a difference of six summers at 5.5%. Seven-summer-old fish are also higher in all condition-related indices (CFF; CFC; IC; ICR). The Fulton coefficient (CFF) for the two age categories ranges from 0.865 to 1.115, and in principle it can vary considerably. In accessible literature, much of the CFF information is about early age Sterlet.

Table 6. Morphometric and morphophysiological indices

Indices	Ar_{5+}			Ar_{6+}		
	LS	±Se	SD	LS	±Se	SD
IHB	6.472	0.230	0.459	6.403	0.183	0.367
IBB	14.87	0.934	1.868	19.30	1.509	3.018
IH	45.75	1.656	3.313	51.22	1.004	2.007
CFF	0.865	0.051	0.103	1.115	0.082	0.165
CFC	0.714	0.024	0.048	0.901	0.078	0.155
IC	12.19	0.323	0.646	13.83	0.440	0.880
ICR	5.565	0.170	0.341	7.099	0.370	0.741
VSI	17.06	2.288	4.576	19.37	1.825	3.650
HSI	3.350	0.268	0.536	3.092	0.230	0.459
GSI	8.746	2.592	5.184	11.75	2.520	5.041
SSI	0.156*	0.016	0.032	0.138*	0.016	0.032
HtSI	0.119	0.009	0.018	0.106	0.019	0.038

*P<0.05

Lenhardt et al. (2012) indicate average values of about 0.42 in a growing up Sterlet from natural populations, and for aquaculture farm Sterlets - 0.30. In a study by Skvorcova and Pavlova (2017) of a Sterlet with an average age of 2.5 years reared on different aquaculture farms, CFF was similar, with a high degree of variation of the indicator, ranging from 0.33 to 1.18 on one of the farms and from 0.57 to 0.65 on another. CFF remains an important index in fish research, despite its shortcomings. Kolisnyk et al. (2014) indicate that CFF shows not only the level of nutrition and quality of the natural food base, but also the ability of fish to absorb available food. CFF correlates with body composition, showing that slender fish contained less fat (Rønsholdt, 1995). CFF depends on fish age, gender, fullness of gut, amount of fat reserve and degree of muscular development etc. (Barnham and Baxter, 1998). The authors, pointing out the stage of maturation impact on CFF, indicate that gonads can take up to 15% of fish body weight. In our study, the gonadosomatic index (GSI) was 8.75-11.75%.

Regardless of the found age differences, between the six- and seven-summer-old Sterlets the differences in indexes related to the exterior and fatness are not significant. A significant difference in favor of fish at an earlier age ($p < 0.05$) was found only on the spleensomatic index (SSI). With age, the index decreases from 0.156 to 0.138. Kuritsyn et al. (2017) indicate that SSI of rainbow trout is highly labile. The authors found that with age, the heartsomatic index (HtSI) decreased and the viscerosomatic index (VSI), hepatosomatic index (HSI) and CFF increased. A number of studies show that the growth and development of fish is not directly dependent on its age. Thus, in a study of the rainbow trout, Reinitz (1983) found that age cannot be determined as a major determinant of body composition. The content of the individual components (protein, fat etc.) is determined not by the age of the fish, but by its body weight. Shearer (1994) also notes the importance of fish body weight as a factor in forming body composition.

CONCLUSIONS

Seven-summer-old fish are significantly heavier than six-summer-old fish, but age does not significantly affect the weight of individual body parts and exterior indicators.

The slaughter yield Sv1 (eviscerated weight to total weight) and consumable slaughter value (Sv2 - total weight without intestines and gills to total weight) are higher in fish at an earlier age, 82.9% vs. 80.6% and 81.1% vs. 78.9%, respectively. The slaughter value for the canning industry (Sv3 - carcass weight to total weight) at six and seven summers of age is 63.4 and 64.9%, respectively. However the differences between these indicators are not significant.

It is a significant source of variation only on the relative proportion of fillets without the abdomen in the cleaned carcass. The difference in favor of fish at an earlier age was 8.2% ($p \leq 0.05$). Fish of different ages do not differ significantly in the indexes related to exterior, interior and fatness. A significant difference

($p \leq 0.05$) in favor of younger fish was found only on the spleensomatic index.

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REFERENCES

Ak, K., Kurtoğlu, İ.Z., Serezli, R., Kayış, S., Yandi, İ. (2019). Introduce the Siberian Sturgeon (*Acipenser baerii*) to Turkish aquaculture industry: Duoculture possibility with Rainbow Trout. *Ege Journal of Fisheries and Aquatic Sciences*, 36(3), 211- 217.

Akbulut, B., Feledi, T., Lengyel, S., Ronyai, A. (2013). Effect of feeding rate on growth performance, food utilization and meat yield of Sterlet (*Acipenser ruthenus* Linne, 1758). *Journal of Fisheries Sciences.com*, 7(3), 216–224.

Barnham, C.A., Baxter, A.F. (1998). Condition Factor, K, for Salmonid Fish. Fisheries notes, 1-3. http://bamboorods.ca/Trout%20condition%20factor.pdf

Bolshakov, V.N. (2019). Schwartz 's Environmental Rule - A Look Through Decades. *Principles of Ecology*, 3, 4-11. DOI: 10.15393/j1.art.2019.9642 (Ru).

Bronzi, P., Chebanov, M., Michaels, J.T., Wei, Q., Rosenthal, H., Gessner, J. (2019). Sturgeon meat and caviar production: Global update 2017. *Journal of Applied Ichthyology*, 35(1), 257-266.

Bronzi, P., Rosenthal, H. (2014). Present and future sturgeon and caviar production and marketing: A global market overview. *Journal of Applied Ichthyology*, 30, 1536–1546.

Chebanov, M.S., Galich, E.V. (2013). *Sturgeon Hatchery Manual*, FAO, Ankara, 303 pp.

Dekić, R., Savić, N., Manojlović, M., Golub, D., Pavličević, J. (2016). Condition factor and organosomatic indices of Rainbow trout (*Onchorhynchus mykiss,* Wal.) from different brood stock. *Biotechnology in Animal Husbandry,* 32(2), 229-237.

Dzyubuk, I.M., Klyukina, E.A. (2014). Morphophysiological analysis of ruffe (*Gymnocephalus cernuus* L.) local population near the Raantasaari island in lake Ladoga by variation statistics methods. *Vestnik Nijegorodskogo Universiteta Lobachevskogo,* N.I., 4(1), 214-221 (Ru).

Ivanova, S.F., Petrova, N.N. (2015). Study of collagen from swim bladders of sturgeon (Acipenseridae) and white fish (Coregonidae). *Vestnik SVFU*, 5(49), 51-59 (Ru).

Jankowska, B., Zakęś, Z., Żmijewski, T., Szczepkowski, M., Kowalska, A. (2007). Slaughter yield, proximate composition and flesh color of cultivated and wild perch (*Perca fluviatilis* L*.). Czech Journal of Animal Science*, 52, 260–267.

Kapusta, A., Partyka, K., Szczepkowska, B., Jarmołowicz, S., Hopko, M., Piotrowska, I., Kowalska, A., Zakęś, Z. (2013). Impact of diet and culture conditions on the body shape of crucian carp (*Carassius carassius* L.). *Journal of Applied Animal Research*, 41(4), 462-469.

Khudyi, O., Kolman, R., Khuda, L., Marchenko, M., Terteryan, L. (2014). Characterization of growth and biochemical composition of Sterlet, *Acipenser ruthenu*s L., juveniles from the Dniester population reared in RAS. *Archives of Polish Fisheries*, 22(4), 249-256.

Kirpichnikov, V.S. (1979). *Genetic bases of fish selection*. Leningrad, RU: Nauka Publishing House 391.

Kolisnyk, N., Osoba, I., Hrytsyniak, I. (2014). Analysis of individual biological characteristics of amur carp reproduced using cryopreserved sperm. *Fishfarming science of Ukraine*, 4, 70-77.

Koochekian, A., Ghorban, Z., Jourdehi, A. (2006). Production of Isin glass from the swim bladder of sturgeons. *Journal of Applied Ichthyology*, 22(1), 419-421.

Kuritsyn, A.E., Efremov, S.A., Makarova, T.A. (2017). Morphophysiological features of rainbow trout (*Oncorhynchus mykiss* Walbaum) and muksun (*Coregonus muksun* Pallas) grown in a cage fish farm. *Izvestiya TSHA*, 38, 84-94 (Ru).

Lenhardt, M., Jaric, I., Cvijanovic, G., Kolarevich, J. (2012). Comparison of morphological characters between wild and cultured Sterlet (*Acipenser ruthenus* L.), *Slovenian Veterinary Research*, 49(4), 177-184.

Lisovskaja, D.P., Roschina, E.V., Galun, L.A., Kirilenko N.M. (2009). Production technologies. *Minsk: Visshaja shkola*, 400 (Ru).

MAFF (Ministry of Agriculture, Food and Forestry) (2019). *Annual Report on the State and Development of Agriculture (Agrarian Report*), Sofia, 246. (Bg).

Molchanova, K.A., E.I. Khrustalyov (2017). Determination of morphometric parameters of rainbow trout grown in different types of fish farms. *KSTU NEWS*, 44, 38-45 (Ru).

Nikolova, L.N. (2013). Impact of Some Technological Factors on The Growth of Carp Fish Cyprinidae Reared in Autochthonous Polyculture. *Bulgarian Journal of Agricultural Science*, 19(6), 1391-1395.

Nikolova, L.N. (2019). Modern aspects of sturgeon culture in republic of Bulgaria. *Conference proceedings, NACEE (Network of Aquaculture Centers in Central-Eastern Europe) International Scientific and Practical Conference*. May 22, 2019. Mukachevo, Ukraine, 43-48. http://if.org.ua/images/konf/2019myk/mykach2019.pdf

Prokeš, M., Baruš, V., Mareš, J., Peňáz, M., Baránek, V. (2011). Growth of sterlet *Acipenser ruthenus* under experimental and farm conditions of the Czech Republic, with remarks on other sturgeons. *Acta Univ. Agric. Silvic. Mendelianae Brun*., 59, 281-290. https://doi.org/10.11118/actaun201159060281.

Ratushniy, A.S., Aminov, S.S. (2017). All of the food from A to Z, *Moscou: Dashkov and Ko*, 440 (Ru).

Reinitz, G. (1983). Relative effect of age, diet, and feeding rate on the body composition of young rainbow trout (*Salmo gairdneri*), *Aquaculture*, 35, 19-27.

Reshetnikov, Yu.S., Popova, O.A. (2015). About field ichthyological methods and errors in our conclusions, *Trudi VNIRO*, 156, 114-131 (Ru).

Richter, H., Lückstädt, C., Focken, U.L., Becker, K. (2000). An improved procedure to assess fish condition on the basis of length-weight relationships. *Archive of Fishery and Marine Research*, 48(3), 226-235.

Rønsholdt, B. (1995). Effect of size/age and feed composition on body composition and phosphorus content of Rainbow trout, *Oncorhynchus mykiss*, *Water Science and Technology*, 31(10), 175-183.

Rybnikár, J., Prokeš, M., Mareš, J., Cileček, M. (2011). Early development and growth of Sterlet (*Acipenser ruthenus*) in the Czech Republic. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, LIX, 26(5), 217-225.

Saraiva, J.L. (2020). *Acipenser ruthenus* (Short Profile). In: FishEthoBase, Fish Ethology and Welfare Group. World Wide Web electronic publication. Version 1.39. www.fishethobase.net.

Shearer, K.D. (1994). Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. *Aquaculture*, 119(1), 63-88.

Skvorcova, E.G., Pavlova, Ya.V. (2017). Comparative morphological characterization of sterlet from different aquaculture populations. *Vestnik APK Verhnevoljia, Zootehniya i veterinariya*, 4(40), 18-24.

Smirnov, V.S., Bojko, A.M., Rijkov, L.P., Dobrinskaja, L.A. (1972). Application of morphophysiological indicators in the fish ecology, *Proc. Of SevNiorh, Petrozavodsk: Karelija*, 7, 190 (Ru).

Souza, M.L.R., Macedo-Viegas, E.M., Zuanon, J.A.S., Carvalho, M.R.B., Goes, E.S.R. (2015). Processing yield and chemical composition of rainbow trout (*Oncorhynchus mykiss*) with regard to body weight. *Acta Scientiarum. Animal Sciences Maringá*, 37(2), 103-108.

Todorov, M., Ivancheva, E. (1992). *Manual for seminars in fish farming*. Sofia, BG: Zemizdat Publishing House, 147 (Bg).

Vasileva, L.M. (2015). Modern problems of sturgeon in Russia and the world. *Technologies of food and processing industry of agro-industrial complex - healthy food products*, 2, 30-36 (Ru).

Vlasova, Z.I., Frensis, E.P. (2007). *Skomorohy in the monuments of writing*. Sant-Petersburg, RU: Nestor-Istorija Publishing House, 680 (Ru).

Volkova, A.Yu. (2006). Biology and ecology of sturgeon growing in net cage in the European North. *PhD Thesis*, Petrozavodsk, from http://aquacultura.org/upload/files/pdf/library/disser.pdf (Ru).

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WATER QUALITY PARAMETERS WHICH INFLUENCE RAINBOW TROUT (*Oncorhynchus mykiss*) WELFARE IN CLASSIC SYSTEMS

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Abstract

Water quality parameters determines the success or the failure of a fish culture operation, so any proper-prepared plan for aquaculture must describe the quality and quantity of water available for this purpose. The aim of this study was to evaluate the physical and chemical water parameters in Rainbow trout culture, in the summer season from three salmonid farms, due to the environmental changes that are nowadays happening and affect the fish culture operation. The water samples were collected from Bistrița-Năsăud County, from three trout farms: Strâmba, Șoimul de Jos and Fiad, in the summer of 2018, between May and August. For the determination of water parameters (dissolved oxygen, total dissolved solids, electrical conductivity and resistivity, pH, temperature, salinity) was used the Hanna HI 9828/4-01 Multi-Parameter and for the chemical parameters (total ammonia, nitrates, nitrites) samples were transferred to the USAMV laboratories. The samples collection was made in each farm from three different places: catchment, basins and evacuation water areas. The results of the study showed that the parameters observed from the three farms were different, with major differences in month of July and August. The environment of fish aquaculture is a complex system with critical parameters as are the temperature, suspended solids, dissolved oxygen, nitrite and ammonia. However, the temperature and the dissolved oxygen are the most important parameter, requiring continuous monitoring in aquaculture production systems, due to the fish aerobic metabolism which requires an elevated level of dissolved oxygen at optimum temperatures.

Key words*: pH, nitrates, welfare, water parameters*

INTRODUCTION

Water quality can be defined as a set of physical, chemical, biological and bacteriological characteristics, expressed in values, which allow a sample to be classified in a category. To determine the quality of water, out of all the physical, chemical, biological and bacteriological characteristics, a smaller number is used, using only those that are more significant (Diersing, 2009).

The welfare of salmonids depends to a large extent on the quality of the aquatic environment in which the fish live, thus needing to know the physico-chemical parameters of the water is essential (Ashley, 2007; Lawrence et al., 2014).

Any variation of these parameters above or below the biological limits of salmonids leads to a state of stress, a condition that, if extended over a longer period, leads to the installation of pathological conditions and mass mortality (Delfosse et al., 2016).

Salmonids are poikilotherm organisms, so they do not need a large amount of energy for thermal regulation of the body, compared to homeotherms (Stanković et al., 2015; Page & Burr, 2011).

The growth, development and reproduction of salmonids, implies the knowledge of the minimum and maximum limits of the values of the medial parameters, specific to the species of interest, as well as the application of an effective management regarding the fish production (Cocan et al., 2018). The fish welfare level in an aquatic environment is considered satisfactory if the values of the water quality parameters do not deviate from the optimum values necessary for the fish development. In this interval, the optimum conditions are highlighted when the intensity of the physiological processes registers the

highest level, and the physiological functions are performed in a normal way (Relic et al., 2010).

The environmental factors that have major implications in the physiological processes of salmonids are represented by: water temperature, dissolved oxygen, water pH, nitrates and nitrates, ammonia, carbon dioxide, dissolved solids, hydrogen sulphide, phosphates, etc. (Brune, 2005).

The biggest problem of welfare in salmon farming is given by the poor quality of the water or its deterioration. This deterioration of water quality can lead to both acute violations and a chronic reduction in the welfare state of salmonids (Relic et al., 2010). Rainbow trout go through several distinct stages throughout their lives (eggs, fry, young, juvenile, adult, brood fish), each with its own requirements regarding optimal environmental conditions. Degradation or absence of optimal habitat for any of the early stages will certainly affect the next stages of the fish life cycle (Hay et al., 2006).

Fish are conscious animals, which can feel pain, suffering and stress, so they require increased attention from farmers, especially in the case of salmonids, as it is known that in captivity, the life span of animals is reduced (Volpato et al., 2009; Uiuiu et al., 2019) and that environmental conditions influence their welfare.

Thus, the values for the physico-chemical factors that ensure the welfare of the Rainbow trout, must be within the following limits: temperature (T°C) 16-20°C, transparency (Tr) 1.5-1.8 m, pH 6.0-8.5 units, totally dissolved solids (TDS) max. 10 mg/l, dissolved oxygen (DO) ≥ 9.0 mg/l, carbon dioxide (CO_2) < 10 mg/l, hydrogen sulphide (H_2S) absent - mg/l, ammonia (NH_3) < 0.07 mg/l, chemical consumption of oxygen (COD) < 15.0 mg/l, biochemical oxygen consumption (BOD) < 30.0 mg/l, Nitrates (NO_2^-) < 0.05 mg/l, nitrates (NO_3^-) < 15.0 mg/l, Phosphates PO_4^{3-}) < 0.3 mg/l, Iron (Fe) < 0.5 mg/l (Hay et al., 2006).

MATERIALS AND METHODS

In order to determine the qualitative parameters (physico-chemical parameters) of the water which influence the welfare of salmonids, water samples were taken from three salmonid farms from Bistrița-Năsăud County (Fiad, Strâmba, and Șoimul de Jos trout farms), in the summer season, between May and August.

The following physico-chemical parameters were monitored: pH (pH), temperature (°C), resistivity (Ω/cm), conductivity (μS/cm), total dissolved solids (TDS), salinity, dissolved oxygen (mg/L), ammonia (NH_3), nitrites (NO_2^-) and nitrates (NO_3^-) during the summer season.

The pH, temperature, resistivity, conductivity, total dissolved solids, salinity and dissolved oxygen of the water were monitored at daybreak, at 8 A.M. from different points of the basins (catchment, centre and evacuation of water from the basins).

To determine the parameters listed above, the Hanna HI 9828/4-01 multiparameter was used, which according to the manufacturer, for the temperature parameter has a measurement range from -5.00 to 55.00°C, resolution 0.01°C and an accuracy of ± 0.15°C. For pH, the measurement range is between 0.00 and 14.00 units, ± 600.0 mV (pH) and ± 2000.0 mV.

For dissolved oxygen, the measurement range is 0.00-50.00 mg/l, for conductivity (0.000-200,000 μS/cm), for total dissolved solids (0-400000 mg/l), for resistivity (0-1.0000 MΩ/cm) and salinity (0.00-70.00 PSU).

Ammonia, nitrites and nitrates were monitored monthly, water samples being collected, transported and analysed in the Fisheries Hygiene laboratory within the USAMV Cluj-Napoca, within a maximum of four hours from their collection.

The methods used to determine the nitrogen-based elements were the reference ones corresponding to the current requirements. For the determination of ammonia (NH^3) - STAS 9800-2/71, nitrites (NO_2^-) - SR ISO 6777/1996 and nitrates (NO_3^-)-SR ISO 7890: 1-1998 was used the spectrometric method.

For the statistical analysis, the Kolmogorov-Smirnov and Shapiro-Wilk tests were used, to test the normality of the distributions.

To perform the correlation analysis, we used Pearson's coefficient to see how strong the relationship is between measured indicators, between calendar months (May, June, July, August) and between farms (Fiad, Strâmba, Șoimul de Jos). The final results were presented both in text and table form.

RESULTS AND DISCUSSIONS

In our study, we analysed the following parameters: pH, temperature, resistivity, conductivity, total dissolved solids, salinity, dissolved oxygen, nitrites, nitrates and ammonia.

In Table 1 are presented the mean values of the physico-chemical parameters of the water in the Fiad farm, in Table 2 for Șoimul de Jos farm and in Table 3 for Strâmba trout farm, during the summer season. The values recorded for all the parameters are within the limits necessary to ensure the welfare of the rainbow trout.

Analysing the values registered all together, in the three salmonid farms, in the case of the temperature parameter we observe that Fiad farm records the highest water temperatures (May - 13.47°C; June - 17.12°C; August - 16.66°C), followed by Strâmba farm (July - 15.29°C) and the lowest temperatures are observed in Șoimul de Jos farm (May - 10.47°C; June - 13.35°C; July - 13.75°C; August - 14.47°C (Tables 2 and 3). The fluctuating values of the temperature are directly influenced by the values of the atmospheric air, values that exert a direct action on the temperature of the water in the basins, so that the heating or cooling of the atmospheric air prints the same course for the water temperature because the depth of the basins is small (1-1.5 m).

For the dissolved oxygen (DO) parameter, due to the negative correlation between temperature and oxygen, the lowest levels of dissolved oxygen are recorded in Fiad farm (May - 9.50 mg/l; June - 9.06 mg/l; July - 9.36 mg/l; August - 9.1 mg/l) and the highest levels of dissolved oxygen are observed in Strâmba farm (May - 10.08 mg/l) and Șoimul de Jos farm (May - 10.79 mg/l; June - 9.29 mg/l; July - 9.95 mg/l) (Table 1). Dissolved oxygen had variations and oscillations during each month, oscillations that are directly influenced by the temperature, so it is observed that if the value of the temperature increases the dissolved oxygen level decreases and vice versa.

Table 1. Physico-chemical parameters of water recorded in Fiad trout farm

Parameters		Summer Month			
		May	June	July	August
pH	X±SD	7.84±0.15	7.71±0.43	6.65±0.22	7.07±0.35
	V%	2%	6%	3%	5%
Temperature (°C)	X±SD	13.47±0.13	17.12±0.39	14.6±0.02	16.66±0.25
	V%	1%	2%	0%	2%
Resistivity (Ω/cm)	X±SD	4746.33±303.62	5153.67±79.41	5910.33±46.2	4244.67±43.43
	V%	6%	2%	1%	1%
Conductivity (µS/cm)	X±SD	214.33±13.01	189±12.12	176.67±7.64	242.67±11.72
	V%	6%	6%	4%	5%
TSD (mg/l)	X±SD	0.12±0.02	0.11±0.01	0.09±0.01	0.12±0.001
	V%	14%	13%	6%	1%
Salinity (PSU)	X±SD	0.1±0.01	0.09±0	0.08±0.01	0.11±0
	V%	6%	0%	7%	0%
Dissolved oxygen (mg/L)	X±SD	9.5±0.1	9.06±0.1	9.36±0.09	9.1±0.22
	V%	1%	1%	1%	2%
Nitrites - NO_2 (mg/dm^3)	X±SD	0.17±0.02	0.003±0	0.02±0.01	0.06±0
	V%	10%	0%	35%	1%
Nitrates - NO_3 (mg/dm^3)	X±SD	3.29±0	4.08±0.13	3.94±0	3.02±0.01
	V%	0%	1%	0%	1%
Ammonia - NH_3 (mg/l)	X±SD	0.01±0.0003	0.002±0.0002	0.005±0.0043	0.03±0.02
	V%	6%	11%	88%	53%

* X±SD – Mean ± Standard Deviation; V% - Coefficient of variation; TSD – Total dissolved solids

Table 2. Physico-chemical parameters of water recorded in Șoimul de Jos trout farm

Parameters		Month			
		May	June	July	August
pH	X±SD	7.42±0.21	7.46±0.18	7.59±0.18	7.66±0.07
	V%	3%	2%	2%	1%
Temperature (°C)	X±SD	10.47±0.24	13.35±0.1	13.75±0.2	14.47±0.23
	V%	2%	1%	1%	2%
Resistivity (Ω/cm)	X±SD	5981±1289.12	6058±2083.67	5653±297.65	4485±262.5
	V%	22%	34%	5%	6%
Conductivity (µS/cm)	X±SD	172.67±38.21	180±81.63	187.67±6.51	207.7±11.93
	V%	22%	45%	3%	6%
TSD (mg/l)	X±SD	0.09±0.02	0.1±0.04	0.1±0.004	0.12±0.005
	V%	22%	38%	4%	4%
Salinity (PSU)	X±SD	0.08±0.02	0.08±0.01	0.08±0.01	0.09±0
	V%	18%	8%	7%	0%
Dissolved oxygen (mg/L)	X±SD	10.79±0.2	9.29±0.16	9.95±0.2	9.95±0.08
	V%	2%	2%	2%	1%
Nitrites - NO_2 (mg/dm^3)	X±SD	0.005±0.002	0.004±0.001	0.001±0.0003	0.09±0.09
	V%	35%	36%	25%	1%
Nitrates - NO_3 (mg/dm^3)	X±SD	9.29±0	11±1.42	2.2±0.87	9.82±0.003
	V%	0%	13%	40%	3%
Ammonia - NH_3 (mg/l)	X±SD	0.004±0.002	0.002±0.001	0.003±0.002	0.001±0.001
	V%	48%	50%	68%	87%

* X±SD – Mean ± Standard Deviation; V% - Coefficient of variation; TSD – Total dissolved solids

Following the pH analysis, we can see that in May, the highest pH value is found in Fiad farm and the lowest in Strâmba farm (Tables 1 and 3). In June, the Șoimul de Jos farm registers the lowest pH value and the highest value in the Fiad farm (Table 2). In July and August, the highest pH value is found in the farm Șoimul de Jos and the lowest in the farm Fiad. The monthly variation of the pH parameter is very small, which indicates that there is a very good capacity for water buffering and that the values fluctuations are within the normal limits of the rainbow trout life cycle.

We have noticed that in the case of the resistivity and the conductivity parameters, in all summer months we registered high values of the variability coefficient. As we mentioned in the Material and methods section the samples were taken from different points of the basins (entrance, centre and evacuation of water from the basins). The resistivity or the conductivity helps us to monitor the purity level of the water. Resistivity is the reciprocal of conductivity and either may be used to inexpensively monitor the ionic purity of water. Resistivity or conductivity of water is a measure of the ability of the water to resist or conduct an electric current. (Light et al., 2005). The ability of water to resist or conduct an electric current is directly related to the amount of ionic material (salts) dissolved in the water. Dissolved ionic material is commonly referred to as total dissolved solids or TDS.

Water with a relatively high TDS will have a low resistivity and a high conductivity. The opposite is true for water with low TDS. The relationship of electrical conductivity and TDS is non-linear being both ionic concentration and temperature dependent.

Therefore, in our experiment, this it is confirmed by the registered values during the summer season. The highest values were recorded at the catchment of water in the basins, followed by the centre and the evacuation of basins.

As for the nitrogen cycle in water ecosystems, we registered that there is no exceedance of the maximum allowed values in the case of any recorded parameter.

Nitrite is an important pollutant in aquatic systems (Nicolae et al., 2017). Major sources of NO_2^- contamination are microbial processes, nitrite being an intermediary in bacterial nitrification and denitrification pathways. It is very toxic to many freshwater vertebrates and invertebrates (Doblander, 1996).

Nitrite is a natural component of the nitrogen cycle in ecosystems, and its presence in the environment is a potential problem due to its well-documented toxicity to animals (Lewis & Morris, 1986; Jensen, 2003). Increased nitrite concentrations cause major problems in intensive cultivation of commercial fish species and ornamental fish (Svobodova et al., 2005).

Table 3. Physico-chemical parameters of water recorded in Strâmba trout farm

Parameters		Month			
		May	June	July	August
pH	X±SD	7.28±0.08	7.51±0.03	6.68±0.09	6.88±0.38
	V%	1%	0%	1%	6%
Temperature (°C)	X±SD	11.57±0.04	15.34±0.68	15.29±0.37	16.48±0.53
	V%	0%	4%	2%	3%
Resistivity (Ω/cm)	X±SD	5597.67±115.78	4775.67±21.5	5576.67±82.92	5977.67±469.68
	V%	2%	0%	1%	8%
Conductivity (µS/cm)	X±SD	178.67±4.04	209.33±1.53	179.67±2.52	167.67±12.7
	V%	2%	1%	1%	8%
TSD (mg/l)	X±SD	0.09±0.002	0.11±0.003	0.09±0.003	0.08±0.01
	V%	2%	2%	3%	8%
Salinity (PSU)	X±SD	0.08±0.01	0.1±0	0.09±0.01	0.08±0.01
	V%	7%	0%	7%	8%
Dissolved oxygen (mg/L)	X±SD	10.08±0.2	9.21±0.21	9.95±0.11	9.22±0.25
	V%	2%	2%	1%	3%
Nitrites - NO_2 (mg/dm^3)	X±SD	0.05±0.09	0.008±0.003	0.01±0.006	0.12±0.01
	V%	159%	35%	43%	7%
Nitrates - NO_3 (mg/dm^3)	X±SD	6.24±0.06	12.97±0.32	3.26±0.36	3.01±0.003
	V%	16%	2%	11%	22%
Ammonia - NH_3 (mg/l)	X±SD	0.003±0.001	0.003±0.001	0.005±0.002	0.03±0.02
	V%	21%	27%	35%	71%

* X±SD – Mean ± Standard Deviation; V% - Coefficient of variation; TSD – Total dissolved solids

A major problem associated with the recirculation of water in a salmonid farm is the potential accumulation of ammonia and nitrite, which urges us to study the possible interactive effects of these pollutants. In a study of the Rainbow trout species (Vedel et al., 1998), when the desired concentrations of nitrites and ammonia were obtained by the addition of dissolved $NaNO_2$ and NH_4NO_3, the combined exposure to nitrites and ammonia resulted in high mortality to high exposure concentrations (600 µM NO_2^- și 18 µM NH_3). Higher oxygen levels in water at lower temperatures and lower fish metabolic rate at lower temperatures could make nitrite a less potent toxin at lower water temperatures (Jensen, 2003).

The bivariate Pearson correlation produces a sample correlation coefficient that measures the strength and direction of the linear relationships between pairs of variables.

I was analysed the correlations between the water parameters in all four consecutive

months and between the three fish farms. All relationships are positive, but not all are statistically significant. The strongest correlation between the farms is encountered in the case of Strâmba and Șoimul de Jos trout farms resulting in a significance threshold of 1%. This can also be seen from the collected data, where it can see that the parameters values are very close to each other. These strong correlations are the result of the close proximity of the two farms and due to their geomorphological conditions because there are located in areas volcanic mountains. Geographical location and landscape are the main elements that directly influence the climatic and meteorological properties of the area. In Strâmba and Șoimul de Jos trout farms, the distance between the springs and the water emplacement of the farm is relatively short, 5 km (Strâmba) and 4 km (Șoimul de Jos). Fiad farm is supplied by the Sălăuţa River, which has a length of 20 km from the spring to the emplacement of the farm.

CONCLUSIONS

Although salmonids, in general, are capable of tolerating wide variations of physical and environmental variables, tolerance intervals may be disregarded under certain circumstances in salmonid units. In particular, the maximum summer temperatures may exceed the critical thermal tolerance of the species (temperatures above 22-26°C, dissolved oxygen under 8-8.5 mg/l, lower or higher pH values than normal), which can cause high mortalities among the fishes (especially in the earlier stages of development).

Thus, as a recommendation, in the case of setting up a salmonid farm, at least the annual minimum and maximum values of temperature, pH, and dissolved oxygen of water should be evaluated before the actual construction is carried out, in order to be sure that the water quality ensure the salmonid welfare.

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REFERENCES

Ashley, P. J. (2007). Fish welfare: current issues in aquaculture. *Appl Anim Behav Sci*, 104, 199-235. doi:10.1016/j.applanim.2006.09.001

Brune, T. (2005). Aquaculture and Water Quality. Baton Rouge, Los Angeles: *The World Aquaculture Society* publisher.

Cocan, D., Popescu, F., Laţiu, C., Uiuiu, P., Coroian, A., Răducu, C., O. Coroian, C., Mireșan, V., Kokkinakis, A., Constantinescu, R. (2018). Effects of thermal stress on hematological and metabolic profiles in brown bullhead, *Ameiurus nebulosus* (LESUEUR, 1819). *AgroLife Scientific Journal*, 7(1), 33-41.

Delfosse, C., Bienboire-Frosini, C., Chabaud, C., Lafont-Lecuelle, C., Cozzi, A., & Pageat, P. (2016). Using physiological and zootechnical profiles to evaluate welfare in farmed rainbow trout *Oncorhynchus mykiss* (Walbaum) under stressful conditions. *Aquaculture International*, 24(5), 1449-1457.

Diersing, Nancy (2009). *Water Quality: Frequently Asked Questions*. Florida Brooks National Marine Sanctuary, Key West, FL.

Doblander, C. (1996). Metabolism and detoxification of nitrite by trout hepatocytes. *Biochimica et Biophysica Acta (BBA) - General Subjects*, 1289(2), 270–274. doi:10.1016/0304-4165(95)00166-2

Hay, J., Hayes, J., Young, R. (2006). Water Quality Guidelines to protect trout fisheries values. Report prepared for Horizons Regional Council. Cawthron report N. 1205.

Jensen, F.B. (2003). Nitrite disrupts multiple physiological functions in aquatic animals. *Comparative Biochemistry and Physiology*, Part A, 135, 9-24.

Lawrence, D. J., Stewart-Koster, B., Olden J. D., Ruesch, A. S., Torgensen, C. E., Lawler, J. J., Butcher, D. P., Crown J. K. (2014). The interactive effects of climate change, riparian management, and a nonnative predator on stream-rearing salmon. *Ecological Applications*, 24, 895-912.

Lewis, W. M., Morris, D. P. (1986). Toxicity of nitrite to fish: A Review. *Transactions of the American Fisheries Society*, 115, 183-195.

Light, T. S., Licht, S., Bevilacqua, A. C., Morash, K. R. (2005). The Fundamental Conductivity and Resistivity of Water. *Electrochemical and Solid-State Letters*, 8(1), E16. doi:10.1149/1.1836121

Nicolae, C. G., Sava, S. C., Marin, M. P., Pogurschi, E., Sava, B. A. (2017). Innovative solutions for removing nitrogen compounds from water of recirculating aquaculture systems using clinoptilolite natural zeolites. *Current Trends in Natural Sciences*, 6(11), 105-109.

Page, L. M., Burr, B. M. (2011). *A field guide to freshwater fishes of North America north of Mexico*. Boston : Houghton Mifflin Harcourt, 663 p.

Relic, R. R., Hristov, S. V., Vucinic, M. M., Poleksic, V. D., Zoran, Z., Markovic, Z. Z. (2010). Principles of

fish welfare assessment in farm rearing conditions. *Journal of Agricultural Sciences* 55(3), 273-282.

Stanković, D., Crivelli, A. J., Snoj, A. (2015). Rainbow Trout in Europe: Introduction, Naturalization, and Impacts. Reviews in *Fisheries Science & Aquaculture*, 23(1), 39-71. doi:10.1080/23308249.2015.1024825.

Svobodova, Z., Machova, J., Poleszczuk, G., Huda, J., Hamackova, J., Kroupova, H. (2005). Nitrite poisoning of fish in aquaculture facilities with water-recirculating systems: three case studies. *Acta Veterinaria Brno*, 74, 129-137.

Uiuiu, P., Cocan D., Constantinescu, R., Laţiu C., Popescu, F., El Mahdy, C., Ihuţ, A., Sava, A., Răducu C., Mireşan, V. (2019). Body thermal response to environment temperature in Rainbow trout (*Oncorhynchus mykiss*) during the summer season. *Scientific Papers. Series D. Animal Science*. LXII (1), 431-438.

Vedel, M., Rocher, J., Knoop, M., Vedel, F. (1998). Evidence of radial-axial motion couplings in an rf stored ion cloud. *Appl. Phys*, *B*. 66, 191-196.

Volpato, G. L., Giaquinto, P. C, Fernandes-De-Castilho, M., Baretto, R. E., Gonçalvesde-Freitas, E. (2009). Animal welfare: From concepts to reality. *Oecologia Brasiliensis* 13: 5-15.

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Alosa immaculata Bennet, 1835: A SHORT REVIEW OF THE SPECIES AND ITS BIOLOGY

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Abstract

Currently, in the continental and marine waters of the Earth, there are over 33,400 species of fish. Over time, naturalists, and specialists in ichthyology have proposed several variants of systematic classification of fish. Thanks to new methods and advanced scientific research equipment, 300-500 new species are discovered every year. This explains the fact that in just 20 years (1998-2017), 7,436 species were described. In 2004, the scientific organization Integrated Taxonomic Information System published ITIS Report, in which a complete taxonomic hierarchy of the genus Alosa is presented. Alosa immaculata (Pontic shad), is an important fish species in the ecological system of the Danube-Delta-Black Sea, with a significant economic value due to the high catches and to the nutritional qualities of meat. The knowledge of the essential elements regarding the biology and the exploitation of the species contributes to ensuring the necessary information for the conservation of the species and the management of the stocks. The current level of knowledge on the biology of the Pontic shad is known from the researches of the Romanian and Russian specialists from the years 1960-1970, but in the last 30 years, numerous changes have occurred in the environmental and exploitation conditions of the species. In this context, the aim of this paper is to present as updated information as possible regarding the biology and exploitation of the Pontic shad, under the new environmental conditions, to meet the current objective of species conservation in the requirements of the sustainable exploitation of the stocks.

Key words*: Alosa immaculata (Pontic shad), biology, stocks.*

INTRODUCTION

The Danube River covers a length of 2,857 km. Human activities have significantly influenced the flow of the Danube through flood prevention, navigation activities. The most important dams in the Danube at a distance of 943 km from the river, the Iron Gate I, and the Iron Gate II have formed an accumulation lake, which in turn represents obstacles for migratory fish species and Pontic shad.

Besides the negative impact of the dams and the regulation of the river flow, the common stocks of Pontic shad in the region of the Lower Danube have been affected by overfishing and the pollution. Some of the obstacles encountered in the standard effective management of these fish stocks may be the lack of harmonization and coordination of management.

Clupeidae is one of the world's most commercially essential families of fish. Despite their importance, little is known about the phylogenetic relationships within the genus *Alosa*, resulting in systematic and taxonomic uncertainty, which may undermine the establishment of adequate conservation measures (Faria et al., 2006).

The genus Alosa is present in the Northern hemisphere of the Earth. Many of the species representing this genus are in the Atlantic, Mediterranean, Black, and Caspian. In the Danube River and the Black Sea, are found *Alosa immaculata* (Bennett, 1838) - Pontic shad, *Alosa tanaica* (Grimm, 1901) - Azov shad, synonymous with subspecies *Alosa caspia nordmanni* (Antipa, 1906), and *Alosa maeotica* (Grimm, 1901) - Black Sea shad (Năvodaru et al., 2014).

According to IUCN Red List 2008, *Alosa immaculata* is a vulnerable species, and the current threat of the species is overfishing, at sea, and in the rivers during the migration runs, which is causing a population decline of unknown levels (Figure 1).

Figure 1. The geographic range of *Alosa immaculata* (IUCN, Red List 2008; Freyhof & Kottelat, 2008)

In the European Union, according to the Annex II of the Habitats Directive 92/43/EEC, the Pontic shad is a protected species, requiring protection under the Natura 2000 Network and site management, which is under its ecological requirements. Also, the species is listed in Annex IV, which obliges the Member States to ensure that their exploitation is compatible with maintaining a favorable conservation status. At the national level, the species is protected by the GEO no. 57/2007.

Pontic shad is a commercially important fish of the Danube Delta and the countries of the Lower Danube Region (Romania, Ukraine, and Bulgaria) (Ciolac & Patriche, 2004).

In this context, the purpose of this paper is to review some aspects regarding the biology and current status of the *Alosa immaculata* population along the Danube River and the Black Sea.

Taxonomy. Distribution. Morphology. Ecology and biology of the *Alosa immaculata*

Pallas (1811) made the first reports of the Pontic shad, under the name of western European shad, *Clupeonela pilchardus*. In the year 1835, Bennett describes the species under the name of *Alosa immaculata* - Pontic shad. *Alosa immaculata* is an anadromous fish species that inhabit the Black and Azov Sea, at varying depths and great distances from the coast of Ukraine, Romania, and Bulgaria (Bănărescu, 1964).

The *Alosa immaculata* is a marine, migratory, anadromous species, migrate for spawning from the Black Sea to the Danube River, and the average weight is around 240 g and the total length of 31 cm (Lenhardt et al., 2016) (Figure 2).

Figure 2. *Alosa immaculata* (Photo original)

The reproduction of *Alosa immaculata* takes place in the Danube, up to 80 km, between Brăila and Călărași county. Still, in the past, reproductive shad migrate upriver up to 1000 km, reaching only the Iron Gates II (863.55 km). The current of the Danube carries larvae and fry of Pontic shad to the sea (Cristea and Cristea, 1958).

Sexual maturation occurs at the age of 3 and 4 years old, and only a few individuals spawn two seasons (Năvodaru & Năstase, 2014; Țiganov et al., 2016).

Usually, a Pontic shad spawns only once or twice in its lifetime (Ciolac, 2004). Most spawning in the Danube River occurs between 180 and 743 km upstream (Kolarov, 1985; Schmutz, 2006). Individuals of two years have a low contribution among breeders being represented by individuals with growth and maturation accelerated. Also, fish with ages between 5 and 7 years are rarely found during migration (Năvodaru, 1997).

The migration is dependent on water temperature, and according to some authors begins in February - March when water temperatures reach 5-6˚C, with the highest rate in April, at water temperatures of 9-13˚C (Pavlov, 1953; Năstase et al., 2018). Năvodaru, in 1996 and 1998, said that the *Alosa immaculata* migration in the Danube River begins in spring at water temperatures of 3-7.5˚C, peaks in April and May between 9-17˚C, and is finished in June and July when water temperatures reach 22-26˚C.

Another critical factor that affects the entry of the *Alosa immaculata* into the Danube is turbidity and water level. According to Năvodaru (1997), higher water turbidity, and a high water level in the Danube slow down fish migration.

The sex structure of the migration population can vary depending on the seasonality and migration period. Several studies reported female dominance with the aging and a decrease of the males (Năvodaru, 1997; Năvodaru and Năstase, 2014; Țiganov et al., 2016).

Generally, the duration of the Danube shad migration is estimated at 100-150 days (Table 1).

Table 1. Size structure of *Alosa immaculata* in 2009, 2013, 2014 and 2016

Catch area/year	Catch period	Total weight (g)	Total length (cm)	The age of the fish caught	Sex ratio (M/F)	References
Danube River, 2009	April - June	100-400 g Average 276.72 g	24-39 cm Average 31.11 cm	-	-	Ibănescu et al., 2016
Black Sea coast, 2013	Spring season (March, April), summer (June, July) and autumn (September)	163-442 g Average 248 g	25.2-31.2 cm Average 28.2 cm	2 years -16% 3 years - 42% 4 years - 37% 5 years - 5 %	0.62, females dominant	Țiganov et al., 2016
Mouth of Danube River, 2014	January – May	163- 422 g Average 289 g	25.3 – 35.7 g Average 31.1 cm	3 years – 46% 4 years – 50% 5 years - 4 %	0.55, 64% - females, 36% of males	Năvodaru and Năstase, 2014
Mouth of Danube River, 2016	February – August	102-435 Average 236.8	22.7-36 Average 28.7 cm	Dominance 3-4 years	0.51, females dominant	Năstase et al., 2016

Generally, food consists of 70-75% of adult fish (Engraulis, Clupeonella, Sprattus) in the sea and Cyprinids in the Danube River, the rest being shellfish (Crangon, Upogebia, Idotheia), and other organisms according to their abundance and availability (Bănărescu, 1964; Cautiș et al., 1957).

Alosa immaculata has an elongated body, compressed laterally, and the mouth is terminal, broad, slightly oblique upwards. Well developed adipose eyelid, leaving a narrow, elliptical-shaped vertical opening. Laterally compressed abdomen from the tip of the snout to the base of anal. The hull is evident,

especially between ventral and anal. The dorsal fin is located towards the middle of the body, and the anal fin is located far behind. The backside of the body is colored in blue-green, silvery flanks, and sometimes white and sometimes darker head, fins are colorless (Antipa, 1909; Bănărescu, 1964).
Biological peculiarities of the species make it vulnerable to different threats, but the major ones are overfishing and loss of spawning grounds.
In the context of fishery management that takes ecological and ecosystem considerations, protected areas are highly relevant.
Recent data on shads stocks, distribution, population parameters, and genetics in the Black Sea area urgently are needed for the species conservation and management issues.

MATERIALS AND METHODS

The Danube is the most important stream in Romania. The Danube sector under study is of particular importance for fish populations, as it is a central wetland type (Ibănescu et al., 2016).
Several representative areas for each habitat type were chosen for fish sampling. The evidence comes from scientific and commercial fishing.
In order to study the biological parameters of the pontic shad species from the collected samples, samples were taken, which were analyzed in the laboratory.
Special fishing gear was used for Danube Pontic shad: driftnets and suitable boats operated by fishermen. The material was collected from different parts of the Danube, in the spring season (March, April), summer (June, July) and autumn (September) (Țiganov et al., 2016).
The main physiological parameters recorded for each individual were: total length, individual mass, age, and sex. Weight was determined in grams. The age was determined by reading the annual growth rings on the scales. The sex of each individual was determined by dissection.
The study of the species *Alosa immaculata* consisted in an ichthyological study of all the samples collected during the studied period, using both the samples collected from the experimental fishing and from the commercial fishing.

RESULTS AND DISCUSSIONS

In Romania, *Alosa immaculata* is a fish with high economic and socio-cultural value for the communities. Due to the highest nutritional quality of *Alosa immaculata* (has a big content of lipids 18-22% and a higher content of soluble vitamins), this fish is usually consumed in the Lent by the Christian population.
The fishery has a commercial value of about 1.5 million euros, with annual average catches of 200-500 tons. According to Năvodaru and Năstase (2014), *Alosa immaculata* has a cyclical evolution of catches, with minimums or maximums at 10-11 years, during the period 1960-1998, the minimum absolute was 200 t, and the maximum of 2,400 t.
Between years 2007 and 2010, according to data provided from FAO (2016) statistics regarding the catches in the Danube and the Black Sea, it is observed a dramatic decrease in 2014, of the level of catches in the Black Sea to approximately of 2 tons, while catches from the Danube in the last years are maintained around 400 tons per year (Figure 3).
Unfortunately, as much as the interest for the exploitation of the species, it is higher, as well as the danger of the drastic decline of the Danube and Sea populations Black is bigger. Also, the dramatic fall of Pontic shad migration in the Danube river for reproduction is a real issue that evidently should concern interested persons and involved institutions, companies, and organizations from both commercial and ecological point of view.
In this context, there it is recommended some management measures for the conservation of the *Alosa immaculata*, such as the introduction of bans, for example closing of certain fishing areas and seasons or protection of fish by regulating fishing methods and instruments for the Black Sea and Danube (Țiganov et al., 2016).
Knowledge of the essential elements of the biology and exploitation of the species contributes to ensuring necessary informatio for species conservation and management of stocks.

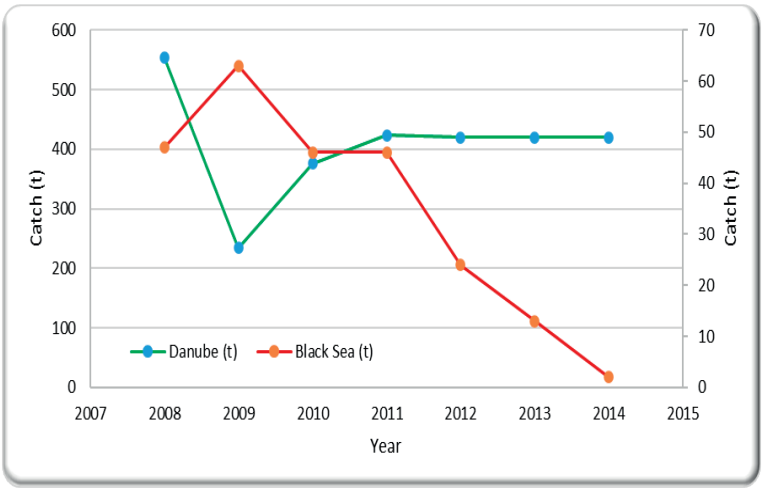

Figure 3. The level of catches of *Alosa immaculata* (Source FAO Statistics, 2016)

Control of fish stocks, including shad, is achieved by the interaction of three key compartments species - environmental - exploitation.

For better management, further investigation and more collaboration among countries in the Lower Danube River Region is needed: monitoring of stocks, studies on factors that influence change in shares, molecular genetic investigation of migrants, determination and protection of spawning and nursery places in the Danube River and its floodplains and delta as well as the costal shelf of the Black Sea.

CONCLUSIONS

Even if we consider the adverse changes of some ecological aspects related to environmental factors such as lower water level, water temperature, and pollution that could affect the success of the Danube shad reproduction. The most crucial cause of the decreasing of the stocks is the overfishing, mainly in Danube Delta area, which provides about 90% of total amount of capture in Danube River.

There are visible decreases in the Pontic shad population. The research is carried out only occasionally on the territory of Romania and Bulgaria. Changes resulting from human intervention in river systems affect migratory fish. Dams and river engineering structures will affect a species such as *Alosa immaculata* because their breeding areas have located in these river systems, as well as the adverse effects on the species that recur in the lower parts of this system are not very clear.

The monitoring of the migrating population of *Alosa immaculata* into the Danube River deserves further empirical study. Additional research is needed to quantify any changes in the number of individuals and their biometric variables and to validate if an upward trend in abundance is happening. The continuation of the study should provide the evidence necessary to determine possible changes in the conservation state of this species of community interest.

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REFERENCES

Antipa, G. (1909). *The ichthyological fauna of Romania*, XVI. Bucharest, RO: Vasile Adamachi Fund Publications, Romanian Academy Publishing House (In Romanian).

Bănărescu, P. (1964). *Pisces-Osteichthyes R.P.R. Fauna*, XVIII. Bucharest, RO: R.P.R. Academy Publishing House (In Romanian).

Cautiș, I., Iliescu, M., Maximov, A. (1957). Caspialose fund problem form Black Sea. *Bul ICP*, XV, 47-56.

Ciolac, A. (2004). Migration of fishes in Romanian Danube River. *Applied Ecology and Environmental Research*, 2(1), 143-163.

Ciolac, A., Patriche, N. (2004). Structure of Danube Shad (*Alosa pontica*, Eichwald 1838.) spawner flocks migrating for reproduction in Danube River. *Appl Ecol Env Res.*, 2(2), 53-58.

Cristea, A., Cristea, E. (1958). Some resulted obtained from prolificity of the Pontic shad (*Alosa pontica*). *Bul ICP*, XVII (3), 78-85.

FAO (2016). Fishery and Aquaculture Statistics. *FAO yearbook,* 125(7), Part 1.

Faria, R., Weiss, S., Alexandrino, P. (2006). A molecular phylogenetic perspective on the evolutionary history of *Alosa* spp. (Clupeidae). *Molecular phylogenetic and evolution,* 40, 298-304.

Freyhof, J., Kottelat, M. (2008). *Alosa immaculata.* The IUCN Red List of Threatened Species 2008: e.T907A13093654. Retrieved March 12, 2020, from https://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T907A13093654.en.

GEO no. 57/2007 *on the regime of natural protected areas, the conservation of natural habitats, wild flora and fauna.*

Habitats Directive 92/43/EEC *Council Directive 92/43/EEC on the conservation of natural habitats and of wild Fauna and flora*.

Ibănescu, D., Popescu, A., Nica, A. (2016). Estimation of growth and mortality parameters of the Pontic shad (*Alosa immaculata* Bennett, 1835) in Romanian section of the Danube river. *Lucrări Ştiinţifice-Seria Zootehnie*, 285-289.

Kolarov, P. P. (1985). *Biological characteristics and population dynamic of anadromous fish specie.* PhD thesis, Varna: Institute for Fish Resources.

Lenhardt, M., Višnjić-Jeftić, Ž., Navodaru, I., Jarić, I., Vassilev, M., Gačić, Z., Nikčević, M. (2012). *Fish stock management cooperation in the Lower Danube region: A case study of sturgeons and Pontic shad*. In: Lagutov V. (eds) Environmental Security in Watersheds: The Sea of Azov. NATO Science for Peace and Security Series C: Environmental Security, Dordrecht, NL: Springer Publishing House.

Lenhardt, M., Navodaru, I., Vassilev, M., Kalauzi, A., Regner, S., Višnjić-Jeftić, Ž., Tošić, K., Smederevac-Lalić, M. (2016). Model of the Pontic shad *Alosa immaculata* (Bennet, 1835) and anchovy *Engraulis encrasicolus* (Linnaeus, 1758) catch in the Danube river and Black Sea for the period 1920-2008. *Acta zool. bulg.*, 68(4), 557-561.

Năstase, A., Năvodaru, I., Cernisencu, I., Ţiganov, G., Popa, L. (2018). Pontic shad (*Alosa immaculata*) migrating upstream the Danube river and larval drift downstream to the Black Sea in 2016. *Scientific Annals of the Danube Delta Institute*, 23, 57-68.

Năvodaru, I. (1996). *Exploitation of Alosa Pontica in the Danube Delta, Romania.* - In: Co W x i. G. (Ed.): *Stock Assessment in Inland* Fisheries, 448-453. Oxford, UK: Fishing News Books Publishing House.

Năvodaru, I. (1997). *The evolution of the Pontic shad effectives under conditions of ecological of the river and their maintenance measures.* PhD Thesis, "Dunărea de Jos" University of Galaţi.

Năvodaru, I. (1998). Migraţia scrumbiei de Dunăre (*Alosa pontica*) şi factorii de mediu. *Analele Ştiinţifice ale Institutului Delta Dunării-1997*, VI(1), 149-152.

Năvodaru, I., Năstase, A. (2014). *New data on pontic shad (Alosa immaculata Bennet 1835) migration and drifting larvae in Danube River,* Deltaica, 3: 48 pp. In: Torok, L. ed., Noi date asupra prezenţei marilor peşti migratory anadromi în Marea Neagră - Zona marină a Rezervaţiei Biosferei Delta Dunării (New data on presence of the great anadroumous migratory fishes in Black Sea - marine zone of Danube Delta Biosphere Reserve), ISSN 2286–0789; doi: 10.14590/DDI02.D03.

Pallas, P., S. (1811). *Zoographia Rosso-Asiatica, sistens omnium animalium in extenso Imperio Rossico et adjancetibus, domicilia, mores et descriptiones, anatomen atque icones plurimorum,* III, Petropoli: Academiae scientarium impress Publishing House.

Pavlov, P. I. (1953). *Biologhiceskaia I promislovaia harakteristika nerestovo stada dunaiskoi seldi.* In: Dunaiska seldi i biologhiceskie osnovi eio promisla, 118-174. Kiev, USSR: Acad. Nauk. Publishing House.

Schmutz, S. (2006). *Assessment of the potential transboundary effects of the construction of the 270 Bystre Deep Water Navigation Channel on fish and fisheries*. Report to the ESPOO Inquiry Commission Vienna, 56.

Ţiganov, G., Năvodaru, I., Cernişencu, I., Năstase, A., Maximov, V., Oprea, L. (2016). Preliminary data on the studies *Alosa immaculata* in Romania marine waters. *Scientific Annals of the Danube Delta Institute*, 22, 141-148.

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EFFECTS OF SHORT-TERM STARVATION AND DIFFERENT DIETARY PROTEIN LEVEL ON LEUKOCYTE REACTION IN CULTURED RAINBOW TROUT *Oncorhynchus mykiss* (Walbaum, 1792)

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Abstract

Study of the physiological and haematological characteristics of cultured fish represents an important tool in monitoring environmental quality, physiological status and the health condition of fish. The purpose of this paper was to evaluate the leukocyte reaction of rainbow trout after applying short periods of starvation (2 days and 4 days) and re- feeding with different dietary protein levels. Six experimental variants in duplicate were created, as follows: two control groups, feed daily, ad libitum, with commercial pellets containing 41% crude protein (D41) and 50% crude protein (D50); two groups starved for 2 days (D2) and then fed with commercial pellets with 41% crude protein (D2/41), respectively 50% crude protein (D2/50) and two groups starved for 4 days (D4) and then fed with commercial pellets with 41% crude protein (D4/41), respectively 50% crude protein (D4/50). In order to determine the leukogram and absolute number of leukocytes, blood samples were taken, at the beginning and at the end of the experiment, in order to make smears that were coloured with May-Grunewald Giemsa panoptic method. Regarding the leukogram of rainbow trout it can be observed an insignificant decrease ($p>0.05$) of the relative number of lymphocytes, respectively an insignificant increase ($p>0.05$) of the relative number of neutrophils, while the case of the relative number monocytes, it was observed a significant decrease ($p<0.05$) in fish starved for 2 days. The absolute number of leukocytes ($\times 10^3$ cells/µl bloods) registered no statistically significant changes ($p>0.05$) between the experimental groups, while the absolute number of monocytes showed a significant decrease for the fish starved for 2 days. However, it can be concluded that application of short periods of starvation did not affect the immune defence system of the rainbow trout fish.

Key words*: leukocyte reaction, protein level, rainbow trout, starvation.*

INTRODUCTION

Both in natural conditions and in aquaculture, fish can experience periods of fasting (Barcellos et al., 2010; Rahmati et al., 2019). In aquaculture, starvation of fish could occur due to an inadequate diet or feeding protocols (López-Olmeda et al., 2012), or can be used as a feed management strategy to reduce the feeding cost (Blanquet & Oliva, 2010; Xiao et al., 2013) or to solve some water quality problems caused by overfeeding the fish (Turano et al., 2008).

According to FAO 2009, rainbow trout is a fast-growing fish species which have become highly economically important both globally and more specifically in Europe. Recently, the modern trout farming practices are targeted towards optimizing feed conversion by using high-energy, high-fat, low-protein diets, but such practices are not always acceptable to the consumers. In this context, starvation of fish is can also be practiced in order to hold back stocks to regulate supply in accordance with consumer demand and to reduce excess lipid levels.

There are many studies who demonstrated that after some period of starvation or exposure to unfavourable conditions such as low temperature, low oxygen, and reproductive effort, fish can experience an accelerated growth rate, a phenomenon called compensatory growth (Tian and Qin, 2003; Ali et al., 2003; Adakli and Tasbozan, 2015).

Generally, these starvation periods might induce some hormonal and biochemical changes at fish. The primary responses to stress can be described as the activation of the neuro-endocrine system through the release of stress-related hormones (cathecolamines and cortisol) in the blood, while secondary responses include hematological and biochemical changes.

Also, during starvation, the muscle glycogen is reduced, and fat or protein are mobilized aspect which can lead to impair fish meat quality (Barcellos et al., 2010; Sigholt et al., 1997; Thomas et al., 1999).

Blood parameters are commonly used as a suitable tool for clinical diagnosis, particularly in assessing the health and nutritional status of fish and can be used to evaluate the effect of starvation periods on fish welfare.

Information regarding the effects of feeding and starvation on haematological values of rainbow trout is available but there was no information about the effects of these factors on the hematopoietic system. There are some authors who say that even short periods of starvation might weaken the immunological system of fish (Caruso et al., 2010; Caruso et al., 2011; Shoemaker et al., 2003).

In this context, the aim of the present study was to evaluate the leukocyte reaction of rainbow trout after applying short periods of starvation (2 days and 4 days) and refeeding with different dietary protein levels (41% and 50%).

MATERIALS AND METHODS

Blood samples and analysis. Fish (initial weight 111.93±15.76 g; initial length 21.35±1.04 cm) were provided from a growth experiment, which lasted for 46 days. The experiment was carried out in the facility of the "Dunărea de Jos" University from Galați, Faculty of Food Science and Engineering, Romania.

Six treatments with duplicate were assigned, as follows: two control groups, feed daily, *ad libitum*, with commercial pellets containing 41% crude protein (D41) and 50% crude protein (D50); two groups starved for 2 days (D2) and then fed with commercial pellets with 41% crude protein (D2/41), respectively 50% crude protein (D2/50) and two groups starved for 4 days (D4) and then fed with commercial pellets with 41% crude protein (D4/41), respectively 50% crude protein (D4/50). The biochemical composition of the feed is presented in Table 1. At the end of the experiment, about 1 ml of blood was taken by caudal venous puncture, from 5 fish/on each experimental variant and blood smears were prepared and stained using the methods of Giemsa and Pappenheim (Wintrobe, 1967). The blood smears were used for microscopic observations. In total, we analyse 120 blood smears. The relative proportion of each type of white blood cells was obtained by microscopic examination of 200 leukocytes on blood smears. The absolute number of circulating blood leukocytes and thrombocytes were determinate in relation to 1000 erythrocytes and converted to unit blood volume.

Table 1. Biochemical composition of experimental diets

Ingredients	Composition	Diet41	Diet50
Crude protein	%	41	50
Crude fats	%	12	20
Crude fiber	%	3	0.7
Crude ash	%	6.5	8
Phosphorus	%	0.9	1.2
Vitamin A	UI	10000	6000
Vitamin D3	UI	1250	1200
Digestible energy	MJ/kg	14.2	19.7
Ingredients: Fish meal, fish oil, haemoglobin, full-fat soybean, soybean oil, wheat gluten, sunflower flour, wheat, and wheat products.			

Statistical analysis. Values obtained for the percentage and the absolute numbers of different types of white blood cells were presented as mean and standard deviation.

The obtained data were subjected to analysis of variance (ANOVA) to test the effect on the two factors. Duncan's multiple range test was used as a post hoc test to compare means at $p<0.05$. the SPSS version 21 software was used**.**

RESULTS AND DISCUSSIONS

The objective of this study was to examine welfare aspects, including the response to stress due to starvation (cycles of two days and four days) and refeeding with different dietary protein levels (41% and 50%).

Usually, in aquaculture fish are exposed to periods of starvation or restricted feed intakes. In these periods, the fish covers the energy requirements on the expense of body stores of nutrients, situation that can lead to the alteration of the physiological state in time (Lie and Huse, 1992).

Microscopic examination of blood smears coloured with MGG, did not show morphologic changes among leukocytes. Following microscopic examination of blood smears,

stained by the MGG method, it was observed that lymphocytes dominated in comparison with the other types of leukocytes, being present in a very large number (Figures 1-3).

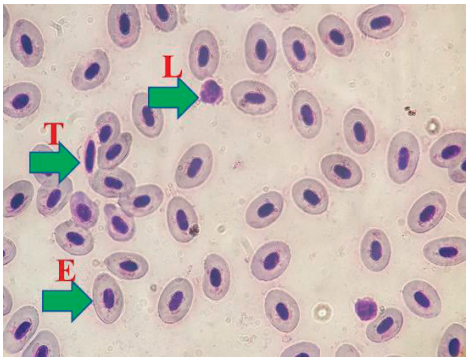

Figure 1. Morphology of circulating blood cell of the rainbow trout (L-lymphocytes, T-thrombocytes, E-Erythrocytes, 10 oc x 100 ob)

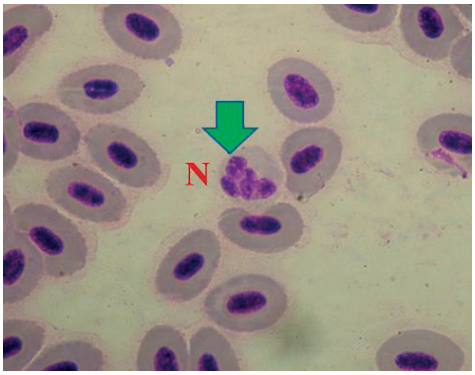

Figure 2. Morphology of circulating blood cell of the rainbow trout MGG staining (N-neutrophil, 10 oc x 100 ob)

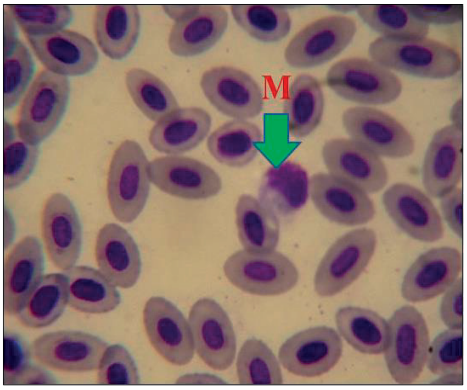

Figure 3. Morphology of circulating blood cell of the rainbow trout MGG staining (M- monocyte, 10 oc x 100 ob)

No eosinophils or basophils were found in fish from this study.

Compared with control groups (D41 and D50) the relative number of lymphocytes, showed an insignificant decrease ($p>0.05$) in variants starved for two and four days respectively. However, a decrease of these values was observed with the increase of the starvation period (Figure 4).

Regarding the average percentage value of monocytes, there is a non-uniform evolution. In fact, statistical analysis showed that the relative number monocytes registered a significant decrease ($p<0.05$) in fish starved for 2 days. Thus, if in the case of fish from control groups and those starved in 4-day cycles, a slightly higher value was observed (D41 - 0.67±0.12%; D50 - 0.50±0.14%; D4/41 - 0.83±0.16; D4/50 - 0.75±0.17%) for fish starved for 2-day cycles, the percentage of monocytes showed a significant decreases to 0.30% (D2/41 - 0.30± 0.11%; D2/50 - 0.30±0.13%) (Figure 4).

Regarding the average percentage of neutrophils those shows an insignificant increase ($p>0.05$) with the increase of starvation period (Figure 4).

Concerning the absolute number of cells reported ($\times 10^3$ cells/µl blood), it can be observed that they are dominant comparing to other types of leukocytes (Table 2).

The number lymphocytes, neutrophils, and monocytes are important indicators of fish health and one of the main parts of the body's non-specific immune system (Ahmadifar et al., 2009). In our study, ANOVA analysis showed no statistical ($p>0.05$) differences for the values of absolute number of leukocytes, lymphocytes, neutrophils or thrombocytes for the fish across all treatments.

The absolute number of leucocytes was not influenced ($p>0.05$) by the protein content of the administrated feed. However, lower values were observed in the case of 41% crude protein. Also, it can be observed that the absolute number of leucocytes registered an insignificant decrease with the increasing of the starvation period.

According to some authors, after applying of short stress period it can be observed a diminishing of the leukocyte count- leukopenia (Falcon et al., 2008). The authors explain this reaction is due to the release of corticosteroids

and catecholamines. These hormones favour haemoconcentration, due to the increased interstitial pressure that promotes an increase in fluid passage to the interstitial space (Allen and Patterson, 1995). Thus, the leukocytes are redistributed from the blood vessels to the tissues, causing immunosuppression (Koser and Oliveira, 2011). Similar studies showed the reduction of the absolute number of leucocytes after short-term starvation in the case of *Huso huso* (Morshedi et al., 2011). Also, Rios et al. (2011) reported leukopenia (lymphocytopenia) and thrombocytopenia in starved *H. malabaricus.*

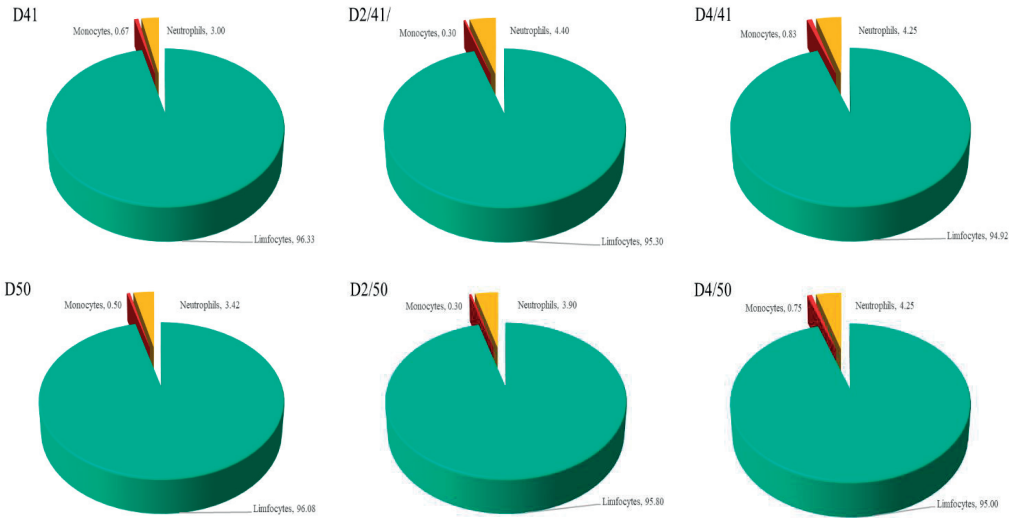

Figure 4. The leukogram (%) of rainbow trout at the experimental variants

Table 2. Changes in absolute values of the leucocyte series at the end of the experimental period

Experimental variants	Means ± SD ($\times 10^3$ cells/µl blood)				
	Leucocytes	Lymphocytes	Monocytes	Neutrophils	Thrombocytes
D_{41}	67.36±16.19	64.99±16.16	0.41±0.01	1.96±1.07	5.31±1.20
$D_{2/41}$	63.54±15.36	60.71±15.36	0.15±0.04	2.67±0.27	4.20±1.45
$D_{4/41}$	61.64±12.51	58.50±17.08	0.50±0.03	2.64±0.85	3.40±1.29
D_{50}	78.41±13.68	75.49±13.76	0.49±0.08	2.64±1.31	7.28±1.35
$D_{2/50}$	70.70±12.99	67.74±12.45	0.19±0.04	2.77±0.94	6.11±1.27
$D_{4/50}$	63.65±17.79	60.80±17.20	0.47±0.03	2.70±1.61	5.35±1.09

Also, the absolute number of lymphocytes at the end of the experimental period was not influenced by the protein content from the fed ($p>0.05$) or by the period of starvation. However, slightly lower values were observed in fish fed with 41% crude protein, as well as in fish starved in 2 and 4 days**.**

Fish monocytes are involved in a specific immunity as phagocytic cells and in the specific immune response as antigen-presenting and immunomodulating cells.

Regarding the absolute number of monocytes, it was observed significant decrease ($p<0.05$) in fish starved for 2 days (D2/41 - 0.15±0.04 $\times 10^3$ cells/µl, D2/50 - 0.19±0.04$\times 10^3$ cells/µl), while in the case of fish starved in 4-day cycles, the absolute number of monocytes registered no significant differences ($p>0,05$) compared to the fish fed daily (D4/41 - 0.50 ± 0.03 cells/µl, D4/50 - 0.47 ± 0.03 cell/µl).

The content of crude protein from feed did not influence the absolute number of monocytes.

By comparing the absolute number of neutrophils released in the blood, statistically insignificant differences are observed ($p>0.05$), the number being higher in the case of the

variant were fish were starved in comparation with the control groups. Slightly higher values were observed in fish fed with 50% PB feed, but no significant differences were recorded ($p>0.05$).

Fish thrombocytes are involved in blood clotting, phagocytosis, and other possible immunologic functions. In our study the absolute number of thrombocytes, did not reveal significant differences ($p>0.05$), but higher values were recorded in the variants were fish were fed with 50% crude protein.

Regarding the influence of starvation period on absolute number of thrombocytes it was observed a reduction with the increase of starvation period.

The physiological effects of starvation on fish welfare may vary considerably in relation to fish species and age, as well as to the length of the period of starvation. Although in our study, the leukocyte reaction was not significantly influenced by the starvation periods applied, further studies are needed to elucidate all the mechanisms involved.

CONCLUSIONS

The results of present study indicate that applying of short period of starvation on rainbow trout and refeeding with two different levels of protein (41% and 50%) induce no significant changes in terms of leukocyte reaction.

However, starvation caused a slight reduction of absolute number of leukocytes and lymphocytes, which is a response of fish organism caused by stress. These data show that innate immune mechanisms in fish are insensitive to dietary protein level but may be compromised if the starvation is applied for a longer period.

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REFERENCES

Adakli, A., Tasbozan, O. (2015). The effects of different cycles of starvation and refeeding on growth and body composition on European seabass (*Dicentrarchus labrax*). *Turkish Journal of Fisheries and Aquatic Sciences*, 15, 419-427.

Ahmadifar, E., Jalali, M. A., Sudagar, M., Azari, T., Mohammadi, Z. A. (2009). Effects of Aquavac Ergosan on growth performance, survival and haematological factors in beluga (*Huso huso*) juvenile. *Gorgan Journal of Agriculture and Natural Resources*, 16, 72-80.

Ali, M., Nicieza, A., Wootton, R. J. (2003). Compensatory growth in fishes: a response to growth depression. *Fish and fisheries*, 4, 147-190.

Allen, M., Patterson, S. (1995). Haemoconcentration and stress: a review of physiological mechanisms and relevance for cardiovascular disease risk. *Biology Psychology*, 41(1), 1-27.

Barcellos, L. J. G., Marqueze, A., Trapp, M., Quevedo, R. M., Ferreira, D. (2010). The effects of fasting on cortisol, blood glucose and liver and muscle glycogen in adult jundia *Rhamdia quelen*. *Aquaculture*, 30, 231-236.

Blanquet, I., Oliva T. A. (2010). Effect of feed restriction on the growth performance of turbot (*Scophthalmus maximus* L.) juveniles under commercial rearing conditions. *Aquaculture Research*, 41, 1255-1260.

Caruso, G., Maricchiolo, G., Micale, V., Genovese, L, Caruso, R., Denaro, M. G. (2010). Physiological responses to starvation in the European eel (*Anguilla anguilla*), effects on haematological, biochemical, non-specific immune parameters and skin structures. *Fish Physiology and Biochemistry*, 36, 71-83.

Caruso, G., Denaro, M. G., Caruso, R., Mancari, F., Genovese, L., Maricchiolo, G. (2011). Response to short term starvation of growth, haematological, biochemical and non-specific immune parameters in European sea bass (*Dicentrarchus labrax*) and blackspot sea bream (*Pagellus bogaraveo*). *Marine Environmental Research*, 72, 46-52.

Falcon, D. R., Barros, M. M., Pezzato, L. E., Narváez, W. V., Guimarães, I. G. (2008). Leucograma da tilápia-do-nilo arraçoada com dietas suplementadas com níveis de vitamina C e lipídeo submetidas a estresse por baixa temperatura. *Ciência Animal, Santa Maria*, 9(3), 543-551.

FAO. (2009). FAO FISHSTAT PLUS 2008 Database. Rome, Food and Agriculture Organization of the United Nations.

Koser, L., Oliveira, L. M. B. (2011). A Influência das alterações endócrinas e do estresse durante o ciclo menstrual sobre o periodonto. *Revista de Ciências Médicas e Biológicas, Salvador*, 10(3), 284-289.

Lie, Ø., and Huse, I. (1992). The effect of starvation on the composition of Atlantic salmon (*Salmo salar*). *Fiskeridirektoratets Skrifter, Serie Ernæring*, 5(1), 11-16.

López-Olmeda. J. F., Noble, C., Sánchez-Vázquez, F. J. (2012). Does feeding time affect fish welfare? *Fish Physiology and Biochemistry*, 38, 143-152.

Morshedi, V., Ashouri, G., Kochanian, P., Yavari, V., Bahmani, M., Pourdehghani, M., Yazdani, M. A. Porali, F. H. R., Azodi, M. (2011). Effects of short-term starvation on hematological parameters in cultured juvenile Beluga. *Journal of Veterinary Research*, 66, 363-368.

Rahmati, F., Falahatkar, B., Khara, H. (2019). Effects of various feeding and starvation strategies on growth, hematological and biochemical parameters, and body composition of Caspian brown trout (*Salmo caspius* Kessler 1877) parr, *Iranian Journal of Fisheries Sciences*, 18(3), 418-427.

Rios, F. S., Carvalho, C. S., Pinheiro, G. H. D., Donatti L., Fernandes M. N., and Rantin F. T. (2011). Utilization of endogenous reserves and effects of starvation on the health of *Prochilodus lineatus* (Prochilodontidae). *Environmental Biology of Fishes*, 91, 87-94.

Sigholt, T., Erikson, U., Rustad, T., Johansen, S., Nordtvedt, T., and Seland, A. (1997). Handling stress and storage temperature affect meat quality of farmed-raised Atlantic salmon (*Salmo salar). Journal of Food Science*, 62(4), 898-905.

Shoemaker, C. A., Klesius, P.H., Lim, C., Yildirim, M. (2003). Feed deprivation of channel catfish, *Ictalurus punctatus (Rafinesque*), influences organosomatic indices, chemical composition and susceptibility to Flavobacterium columnare. *Journal Fish Diseases,* 26, 553-561.

Tian, X. and Qin, J.G. (2004). Effects of previous ration restriction on compensatory growth in barramundi *Lates calcarifer. Aquaculture*, 235, 273-283.

Turano, M. J., Russell, J. B., & Harry, V. D. (2008). Effects of cyclic feeding on compensatory growth of hybrid striped bass (*Morone chrusops x M. saxitilis*) food fish and water quality in production ponds. *Aquaculture Research*, 39, 1514-1523.

Thomas, P.M., Pankhurst, N.W. and Bremner, H.A., (1999). The effect of stress and exercise on post-mortem biochemistry of Atlantic salmon and rainbow trout. *Journal of Fish Biology*, 54(6), 1177-1196.

Wintrobe M. M., (1967). *Clinical Haematology*. London, GB: Henry Kimpton Publishing House.

Xiao, J. X., Zhou, F., Yin, N., Zhou, J., Gao, S., Li, H., Shao, Q. J., and Xu, J. (2013). Compensatory growth of juvenile black sea bream, *Acanthopagrus schlegelii*, with cyclical feed deprivation and refeeding. *Aquaculture Research*, 44, 1045-1057.

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THE EFFECT OF FOOD TYPE (NATURAL VS. FORMULATED DIET) ON GROWTH PERFORMANCE AND COLORATION OF JUVENILE JAPANESE ORNAMENTAL CARP (Koi, *Cyprinus carpio* L.)

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Abstract

Fish shape, size and coloration are important quality indicators for determining the market value of ornamental fish species. Therefore, ornamental carp rearing technologies must target proper values of these quality indicators, while maximizing fish growth performance and profitability. The aim of present study is to evaluate the influence of administrated food type (natural diet - V1 8.8% protein vs. formulated diet - V2 55% protein) on growth performance and coloration of Japanese ornamental carp, while maintain the feed operational cost. The average specific growth rate indicates a superior fish production at V2 (formulated diet), compared to V1 (natural diet) experimental variant, while the average food conversion ratio (FCR) indicates better values for V2 (1 g feed/g biomass gain), compared to V_1 (4.5 g feed/g biomass gain). The protein efficiency ratio (PER) registered higher values at V1 (2.52), compared to V_2 (1.8), most probably due to protein input limited by feed operational cost restriction. The administration of the live food indicates a better fish coloration, compared with V_2, where artificial feed was administrated. Therefore, natural diet can improve coloration of juvenile ornamental carp.

Key words*: ornamental carp, live food, pellets, rearing, recirculation system.*

INTRODUCTION

Globally, the ornamental fish sector is growing and their production and trade is a profitable activity in aquaculture industry. Over 1 billion ornamental fish are traded globally each year (Hana et al., 2014).

Koi carp (*Cyprinus carpio* koi) is a popular and economically valuable ornamental fish, as koi industry has spread worldwide (Hongjian et al., 2015). Within the past few decades, the commercial production of koi has emerged as a major segment of the fish industry and koi carp (especially high-quality individuals) trade plays a major role in meeting a growing worldwide demand (Feng et al., 2019).

Koi (*Cyprinus carpio* L.) is a subspecies cultivated as an expensive, beautiful, and colourful pet fish for personal pleasure or competitive show, especially in Japan but also worldwide (Ping et al., 2018). The koi carp is characterized as an economically important species of ornamental freshwater fish (Raj et al., 2015).

Colour of ornamental fishes is an essential prerequisite for the quality as they fetch a higher price in the commercial market and is considered as an important quality attribute of the fish for consumer acceptability. Ornamental carp (koi) are characterized by a wide diversity of colours and colour patterns. More than 100 different types of coloration have been developed for these fish species, which are valued as pets (Xiangjun et al., 2012). Colour production in fish is due mostly to food (Elsah et al., 2018).

There are different source of carotenoids such as natural and synthetic origin which have been used to enhance the coloration of the fishes. The different synthetic carotenoids (β-carotenoids, canthaxanthin, zeaxanthin and astaxanthin) and natural carotenoids (such as plant materials, bacteria, algae, crustaceans, microalgae etc.) are used as colour enhancer. However, the high price of synthetic carotenoid forces the researcher to explore the natural sources and their application as colour enhancer (Manas et al., 2017).

Feed is the highest cost of production, having the greatest influence on profit, which is why the correct choice of feed is of crucial importance. Therefore, the aim of present study is to evaluate the influence of administrated food type (natural diet vs. formulated diet) on growth performance and coloration of Japanese ornamental carp, while maintain the feed operational cost.

MATERIALS AND METHODS

The experiment was conducted at the aquaculture pilot station of "Dunarea de Jos" University of Galati, Faculty of Food Science and Engineering, in four rearing units, equipped with individual water conditioning modules.

Thus, for biological, chemical and mechanical filtration, each rearing unit was connected to a Hagen AquaClear power filter, while for maintaining the oxygen concentration of technological water within optimum limits, a Resun Air Pump (1.6 L/min) was used. A daily water exchange rate of 40% was applied in order to assure optimum growth conditions for the biological material.

The biological material consists in 240 exemplars of koi carp, with mean body weight of 2.14 ± 0.015 g and mean body length of 5.02 ± 0.08 cm, acclimated for 10 days before the beginning of the experimental period. The exemplars were equally distributed in four rearing units, in order to assure two experimental variants, in duplicate (V1 - fish feed with natural diet, respectively V2 - fish feed with formulated diet).

The exemplars from V2 were adapted from natural to formulated diet during the acclimatization period (10 days before the beginning of the experimental period).

In V_1 experimental variant, the natural fish diet consists in *Tubifex tubifex*. The biochemical composition of *T. tubifex* carcass (% w/w basis), according to the producer, is presented in Table1.

Also, similar results of *T. tubifex* biochemical composition are reported by Voican and Radulescu, in 1979, and Mandall et al., in 2018 (Table 1). A daily feeding rate of 7% from total fish body weight (BW) was applied.

The feed was administrated two times per day, in the morning and late in the afternoon.

Table 1. Biochemical composition of *T. tubifex*

Parameter	Units	Values reported by the producer, for natural feed used in V1	Values reported by Mandal et al., 2018	Values reported by Voican and Radulescu, 1979
Protein	% w/w basis	8.8	4.02-6.38	8.8
Crude lipid	% w/w basis	2.8	0.85-3.02	3
Ash	% w/w basis	4.34	2.43-2.98	1

The evaluation of biomass gain was performed both at the end and at the middle of the 30 days experimental period, in order to o adjust the diet.

In establishing the feeding rates and respectively, the total feed protein input, the economical aspect was considered.

Thus, the desideratum of maintain the same feed operational cost for both experimental variants resulted in different protein input by natural, respectively by formulated administrated feed quantity.

At the end of feeding trial, the fish biomass growth performance was evaluated by considering the main parameters: weight gain percentage, specific growth rate (SGR), survival rate, feed conversion ratio (FCR) and protein efficiency ratio.

In V2 experimental variant, the fish were manually fed, four times / day, with formulated diet. The biochemical composition of the formulated diet is presented (Table 2). A daily feeding rate of 7% was applied.

Table 2. Biochemical composition of pellets

Parameter	Units	Value
Crude protein	%	55
Crude fat	%	16
Ash	%	10
Fiber	%	0.6
Phosphor	%	1.45
Vit. A	UI	14000
Vit D_3	UI	2300
Vit E	Mg	250
Vit C	Mg	500

The growth performance parameters were calculated according to Nuwansi et al. (2019), as follows:

(1) SGR (% day^{-1}) = ($\log_e$ Final weight - $\log_e$ Initial weight)/no. of days) * 100
(2) FCR = Feed Given (Dry Weight in g)/Net weight gain (Wet Weight in g)
(3) PER = Net Weight Gain (Wet Weight in g)/Protein Feed (g)
The temperature, pH and dissolved oxygen were monitored daily. The following equipment was used to measure the water quality parameters: oxygen concentration and temperature were measured by using the WTW Oxi 315 I, while the pH meter WTW, model pH 340 determined the pH value.
The evaluation of koi carp color is made by comparing the photo with a color ranking which varies in from 1 to 7 points (1 is the lowest color intensity). Color was judged by test panels of 10 persons, randomly recruited from the students. The treatments were not revealed to the individuals who were asked to rank the fish according to intensity of color. Score were subjected to statistical analysis (ANOVA test).

RESULTS AND DISCUSSIONS

The monitored water quality parameters were within the tolerable limits for ornamental carp rearing. Water temperature ranged from 23ºC to 26ºC at both experimental variants, with no statistically significant difference ($p>0.05$); pH ranged between 7.54-7.98 at V1 and between 6.57-7.72 at V2 experimental variant (Figure 1); dissolved oxygen (DO) ranged between 4.1-5.3 mg/L at V_1 and 4.1-5.1 mg/L at V2 experimental variant (Figures 1 and 2).

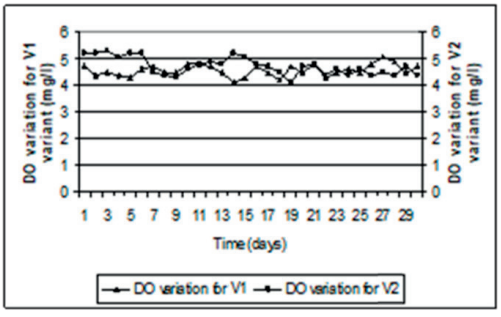

Figure 1. The dynamics of do for both experimental variants

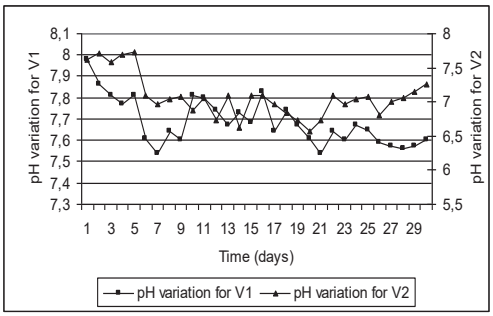

Figure 2. The dynamics of ph for both experimental variants

Colour is one of the most important quality criteria which determine the market value of koi carp. The ornamental carp colours are determinate by the distribution of specifically cells named chromatophores that are situated in the epidermis and in the dermal superior tissue. They are rich in pigments and can be situated in the skin layer or immediately below the scales. A chromatophore is a branched cell, within which the color pigment can be moved. The pigment spreads throughout the entire cell (which gives the koi exemplar the color of the cell) or it is concentrated in one small spot in the center (resulting in the background color, usually pale or dark).
They vary in size, density and superimposition of these cells or the complete absence of one or more basic colours can produce colours or patterns that characterize koi carp or other fish. Dark pigment (melanophore) and red-orange pigment (xanthophore) combine, in order to produce dark and bright colours in carp. The distribution of these pigments is affected by a number of different factors including: water quality, background color, administrated diet or temperature.
Ornamental carp (koi) are characterized by a wide diversity of colours and colour patterns. More than 100 different types of coloration have been developed for these fish, which are valued as pets.
However, colour production in fish is due mostly to food. In conditions of captivity, the type of food is restricted, while various types of food are used in aquaculture, from processed dry food to small aquatic animals. Also, fish growth is affected by several environmental factor such as temperature (Sarvendra et al.,

2018), photoperiod and food availability, technological factors and also, genetical factors.

Regarding colors maintenance, in the case of present experiment, after only 30 experimental days, it has observed an evident decrease of color for koi carp exemplars which were fed only with formulated diet, fact that can indicate a degeneration of pigments who gives the specifically colors. The colors were more vivant in variant which were fed with natural diet, formed with *T. tubifex*. The conclusions are based on the registered results, after processing the scores resulted after ranking the fish exemplars from both experimental variants according to the intensity of their tegument color. Thus, the exemplars reared in V1 experimental variant registered an average score of 7±1.05 points, while the exemplars from V2 registered an average color intensity ranking score of 3.3±0.94 points. The results indicates are statistically significant ($p<0.05$) and confirms the importance of natural feed administration for maintaining and improving koi carp color of tegument.

Growth performance and feed utilization of the experimental biomass of koi carp, fed with different experimental diets are presented in Table 3.

Table 3. The growth parameters for both variants

Parameters/Variant	V1 - natural feed	V2 - formulated feed
Mean initial weight (g)	2.14	2.3
Mean final weight (g)	3.13	7.26
Weight gain (g)	0.99	4.96
Weight gain (%)	46	316
Survival (%)	100	100
GR	0.03	0.16
SGR (% BW/day)	1.26	3.83
FCR	4.5	1
PER	2.52	1.8

Thus, it can be observed that fish biomass reared by applying the feeding regime and the specific diet administrated in case of V2 assures better growth parameters, compared to V1 experimental variant. The average specific growth rate indicates a superior fish production at V2 (3.83 %BW/day), compared to V1 (1.26 % BW/day) experimental variant. Also, from the perspective of feeding strategy efficiency, the average food conversion ratio (FCR) indicates significantly better values for V2 experimental variant (1 g feed/g biomass gain), compared to V1 (4.5 g feed/g biomass gain). However, the protein efficiency ratio (PER) registered higher values at V1 experimental variant (2.52), compared to V2 (1.8), most probably due to feeding diet and feeding regime applied, revealing the ability of fish organism to utilize better the proteins provided by natural feed, compared to those provided by formulated feed input.

In V1 variant, food restriction, due to the purpose of maintaining a similar feed operational cost between both variants, had significant effects on growth performance of ornamental carp. This can explain the inferior results registered in term of growth performance for fish biomass fed with natural diet formed by *T. tubifex*.

Also, referring to technological water quality parameters, it can be observed that the water treatment units were less performed at V1, compared to V2. Thus, lower values of water pH and DO are recorded in V1 experimental variant, compared to V2, fact which may indicate a possible higher organic load for the experimental variant where natural diet was administrated, compared to the variant where formulated diet was used. However, the production system performs well in case of both variants, fact which reveals its ability to support more intense feeding regimes than those applied in present experiment. It has been revealed that the excess feeding increases the organic load, principally carbon and nitrogen (mainly as total ammonia nitrogen). As aerobic conditions need to be maintained for fish growth and welfare, a higher organic load is expected to cause enhanced oxygen demand for its treatment (oxidation). The increasing oxygen demand leads to higher energy consumption for aeration, increasing system operational cost and a larger environmental footprint (Uri et al., 2020). Thus, in order to achieve high economical performance, it is recommended to use aquaculture production systems close to their production capacity limits.

The registered survival rate of 100% confirms that both feeding regime, as well as the production system, performed properly for

assuring optimal conditions for rearing koi crap in the tested development stage.

Optimization of dietary protein level in the diets of *Cyprinus carpio* is important factor because the cost of feed, which is considered to have a large share in relation to total variable costs of the aquaculture economic activity, is largely influenced by source, quality and level of protein. Optimization of dietary protein available for somatic growth is necessary for an efficient cost-effective use of feed. Generally, an increase of protein level in fish diet improves fish production, but proportionally increases feed cost (Lee & Sang-Min, 2005).

Feed and feeding activity are the most critical factors to be considered for profitable aquaculture. Fish farmer spends a significant amount of their total production cost on feed, which is considered as the most expensive item in aquaculture. For successful aquaculture practices, it is essential to determine the minimum level of protein that can assure the maximum growth of fish biomass, while achieving the best operational costs.

Rearing temperature and diets are considered the main growth limiting factors in fish. In the livestock industry, feed plays an important role as it accounts for approximately 60-80% of the production cost, depending on breed and growth and reproduction stage of the fish (Uyeh et al., 2018).

Generally, feed formulation is done by specifying the nutritional requirements as rigid constraints and an algorithm attempts to find a feasible cost-effective formulation.

Inadequate protein in feed causes reduced growth, but when excess is given in a diet, the additional protein is transformed into energy by direct oxidation of amino acids, which leads to increased production costs and additional nitrogen waste. Poor dietary protein and energy levels and their ratios will result in decreased fish performance, increased production cost and deterioration of water quality resulting from wasted food.

CONCLUSIONS

From an economic point of view, appropriate feeding strategy is fundamental for the success of ornamental carp culture.

The growth of the fish from V1 experimental variant was seriously affected by the economical constraints, related to the high cost of the *T. tubifex*, which limits the daily feeding ratio. It is essential to provide the best diet at the least possible cost in order to cut down operational costs and subsequently increase profit, while maintain proper growth rate for biological material.

Thus, the growth performance parameters indicate better results for the experimental variant where formulated feed was administrated. This can be related to lower cost of formulated feed, compared to natural feed formed by *T. tubifex*. This situation generated a higher protein input in V2 experimental variant, due to higher feed quantity administrated, while respecting the constrains related to the use of identical amount of feed operational cost for both tested variants.

However, by analyzing the PER value from both experimental variants, it can be concluded that the protein provided by natural food is better assimilated by koi carp biomass compared to the protein provided by formulated feed.

Also, since koi carp is considered an ornamental fish species and its aspect and color mainly reflects its market value, the color examination must be considered as an important indicator in choosing the proper diet from those tested in present experiment. Thus, better results in terms of color are registered when using natural diet, compared to formulated diet.

Therefore, for ornamental koi carp nutrition, in order to maintain and improve the color of the tegument throughout the entire production cycle, it is recommended to use natural diets. However, formulated diets which contains colors additives can have a precise and predictable effect, although constrains related to feed operational costs may occur.

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REFERENCES

Elsah, A. U., Marco, P., Franco, A., Jorge, L. F. (2018). The Effect of Live Food on the Coloration and Growth in Guppy Fish, *Poecilia reticulate*. *Agricultural Sciences*, 9, 171-179.

Feng, H., Yingying, L., Qing, W., Gaoxue, W., Bin, Z., Yingying, W., Weiwei, Z., Jiyuan, Y., Chun, L., Sven, M. B., Cunbin, S. (2019). Carbon nanotube-based DNA vaccine against koi herpesvirus given by intramuscular injection. *Fish & Shellfish Immunology*, 98, 810-818.

Hana, D., Iva, K. V., Piackova, T., Vesely, A., Cizek, M. D. (2014). Ornamental fish as a source of plasmid-mediated quinolone resistance genes and antibiotic resistance plasmids. *Veterinary Microbiology,* 171 (3-4), 413-421.

Hongjian, G., Haiwen, H., Atsushi, S., Hajime, H., Miki, N., Tomonori, S. (2015). Ulcer disease prophylaxis in koi carp by bath immersion with chicken egg yolk containing anti-*Aeromonas salmonicida* IgY. *Research in Veterinary Science*, 99, 82-86.

Lee, O. K. and Sang-Min, L. (2005). Effects of the dietary protein and lipid levels on growth and body composition of bagrid catfish, *Pseudobagrus fulvidraco*. *Aquaculture*, 243, 323-329.

Manas, K.M., Deepjyoti, B., Nandeesha, TL, Sahoo, S., Adarsh, B.K., Sikendra K. (2017). Effect of dietary natural carotenoid sources on colour enhancement of Koi carp, Cyprinus carpio L. *International Journal of Fisheries and Aquatic Studies*, 5(4), 340-345.

Mandall, R. N., Kar, S., Chakrabarti, P. P., Chattopadhyay, D. N., Paul, B. N., Adhikari, S., Maity, J., Pillai, B. R. (2018). Production of tubifex - a new dimension of aquaculture in feeding juvenile fish. *Aquaculture Asia*, 22(3), 19-32.

Nuwansi, K.K.T., Verma, A.K., Rathore, G. C., Prakash Chandrakant, M.H. Prabhath, G.P.W.A. (2019). Utilization of phytoremediated aquaculture wastewater for production of koi carp (*Cyprinus carpio* var. koi) and gotukola (*Centella asiatica*) in an aquaponics. *Aquaculture,* 507, 361-369.

Ping, O., Ruixue, Y., Junjie, C., Kaiyu, W. Yi G., Weiming, L., Xiaoli, H., Defang, C., Jing, F., Zhengli, C. Li T., Lizi, Y. (2018). First detection of carp edema virus in association with cyprinid herpesvirus 3 in cultured ornamental koi, *Cyprinus carpio* L., in China. *Aquaculture*, 490, 162-168.

Raj, K., Swaminathana, T. R., Rahul, G. K., Arathi, D., Basheera, V. S., Jenab, J. K. (2015). Mass mortality in ornamental fish, *Cyprinus carpio* koi caused by a bacterial pathogen, *Proteus hauseri*. *Acta Tropica*, 149, 128-134.

Sarvendra, K., Sahu, N. P., Ranjan, A. (2018). Feeding de-oiled rice bran (DORB) to Rohu, *Labeo rohita*: Effect of varying dietary protein and lipid level on growth, body composition, and insulin like growth factor (IGF) expression. *Aquaculture*, 492, 59-66.

Uri, Y., Adrian, B., Itamar, G., Amit, G. (2020). Potential environmental impact resulting from biased fish sampling in intensive aquaculture operations. *Science of The Total Environment*, 707, 1-6.

Uyeh, D., Dooyuma, R., Mallipeddib, T., Pamulapatib, T., Parka, J., Kima, S., Wooa, Y. H. (2018). Interactive livestock feed ration optimization using evolutionary algorithms. *Computers and Electronics in Agriculture*, 155, 1-11.

Voican, V., Radulescu, I. (1979). *Practical aquaristic*. Bucuresti, RO: Ceres Publishing House (In Romanian).

Xiangjun, S. Yu C., Yuantu, Y., Zhihong, M., Yongjun, L., Tieliang, L. Na J., Wei, X., Lin, L. (2012). The effect of dietary pigments on the coloration of Japanese ornamental carp (koi, *Cyprinus carpio* L.). *Aquaculture*, 342-343, 62-68.

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THE INFLUENCE OF FEEDING RATE ON GROWTH PERFORMANCE OF *Acipenser stellatus* (Pallas, 1771) REARED IN INTENSIVE CONDITIONS

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Abstract

In order to assure high financial sustainability of intensive sturgeon aquaculture, a proper feeding rate must be identified for different stages of growth. Therefore, the present study aims to evaluate the effect of two different feeding rates (F1 - 1% biomass weight BW, respectively F2 - 2% BW) on growth performance of A. stellatus, with a mean individual biomass of 201.7±32.82 g, reared in intensive conditions. The F2 experimental variant register, overall, a better feeding efficiency, revealed by feed conversion rate (FCR) average value (1.02 g feed/g biomass gain), compared to F1 (1.28 g feed/g biomass gain). Also, the sturgeon exemplars from F2 experimental variant manifested, overall, a superior ability of using feed protein for growth (higher average protein efficiency ratio - PER: 2.39 g/g), compared to F1 (PER: 1.95 g/g). The feeding rate applied at F2 assures the maximization of sturgeon production quantity, emphasized by the higher overall values of average specific growth rate (SGR: 1.74% BW/day at F2, compared to 0.78% BW/day at F1). The biomass and length coefficients of variation (CVw, respectively CV_L) indicate, for both experimental variants, a high homogeneity degree of stellate sturgeon experimental population (CVw: 16.51% at F1 and 12.53% at F2, respectively CV_L: 5.29% at F1 and 4.43% at F2, both registered at the end of the experiment). Therefore, it is recommended to apply 2% feeding rate in order to assure a better efficiency of sturgeons rearing technology, during the analyzed growth stage.

Key words*: FCR, feeding rate, intensive aquaculture, PER, stellate sturgeon.*

INTRODUCTION

The need of ensuring a global sustainable development, on medium and long term, of fisheries and aquaculture is obvious.

Thus, increasing the sustainability of aquaculture production systems is absolutely necessary, as they play a dual role in achieving the aforementioned goal, as follows: ensuring the necessary fish production for human consumption and contributing, actively, to the activities within the repopulation programmes.

This hypothesis is confirmed also by Memiș et al. (2008), addressing specifically to sturgeon's aquaculture, in order to emphasize the potential of this economic activity to contribute to the conservation of wild declined populations, through restocking and by providing a consistent sturgeons products supply, without exploiting wild population.

As Cristea et al. (2002) mentioned, in aquaculture, production systems are classified according to a multitude of technical, technological and ecological criteria.

Among the various types of production systems, recirculating aquaculture systems (RAS) offer the possibility of a rigorous control of the technological process (Cristea et al., 2002; Timmons et al., 2018) throughout the entire production cycle, aiming to ensure optimal growing conditions for differed fish species.

The high operational cost of fish produced in recirculating systems can causes a multitude of technical and financial problems, which must be solved in real time, in order to ensure their RAS competitiveness (Engle, 2010). Thus, the increase of technological maturity, involving rearing various fish species in different development stages, in RAS, is necessary.

Sturgeons are fish species which are suitable to be reared in RAS conditions, as they are considered of having a high market value. However, in order to maximize the profitability of sturgeon's aquaculture by optimizing the variable costs, proper feeding technologies must be applied for each development stage.
According to Dorojan et al. (2014), stellate sturgeon is considered one of the most studied species in terms of super-intensive growth. Also, Dicu et al. (2013) confirmed that among scientific studies which target stellate sturgeon rearing technology, there is little information regarding the nutritional requirements for different development stages.
Also, Petrea et al. (2019) stated that the establishing of a proper feeding rate for a certain development stage is important in order to maintain proper rearing conditions and to assure an efficient feeding management.
Thus, the present study aims to evaluate the effect of two different feeding rates (F1 - 1% biomass weight BW, respectively F2 - 2% BW) on growth performance of *A. stellatus*, with a mean individual biomass of 201.7±32.82 g, reared in intensive conditions.

MATERIALS AND METHODS

The description of RAS Pilot Station
The present study was conducted in RAS pilot station within "Dunărea de Jos" University of Galați, during a 46 days experimental period. The experimental intensive production system was designed and configurated according to the indications presented by Cristea (2008). The detailed designed of the aquaculture intensive production system is described in Figure 1.

Technological indicators
The analysed technological indicators were as follows: *Individual biomass gain*: IBG = (Bf)-(Bi)/fish number [g/fish], with Bf - final fish biomass; Bi - initial fish biomass (1); *Relative growth rate*: RGR = ((Bf-Bi)/t)/Bi [g/g/day], with Bf - final fish biomass; Bi - initial fish biomass, t - duration of the experiment (2); *Specific growth rate*: SGR = 100 x (ln Bf - ln Bi)/t [% fish biomass/day], with Bf - final fish biomass, Bi - initial fish biomass, t - duration of the experiment (3); *Feed conversion ratio*: FCR = F/IBG [kg feed intake/kg fish biomass gain], with F - feed intake, FBG - individual biomass gain (4); *Protein efficiency ratio*: PER = IBG/(F*CP/100) [kg/kg], with FBG - individual biomass gain, F - feed intake, CP - crude protein (5); *Condition factor*: K=W/L-3x100, with, W - body weight, L - body length (6); *Variation coefficient*: CVw/L = (Dev. St./Avg w/L) x 100 [%], with Dev. St. - standard deviation, Avgw/L - fish body weight/length (7).

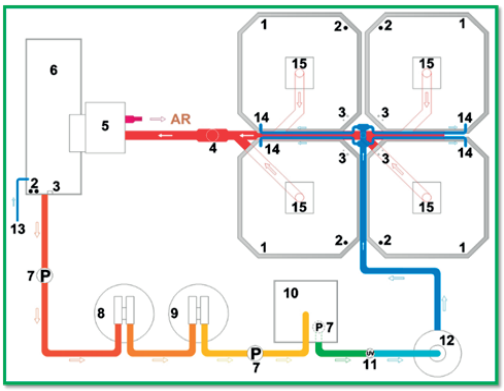

Figure 1. The design of RAS pilot station: rearing units - No. 1; nitrogen compounds sensors - No. 2; water level sensors - No. 3; RAS outlet structure - No. 4; mechanical drum filter - No. 5; sump - No. 6; pumps - No. 7; sand filter - No. 8; activated charcoal filter - No. 9; biological trickling filtration unit - No. 10; sterilization UV filter - No. 11; oxygenation unit - No. 12; automatically fresh water inlet No. 13; rearing units water inlet/outlet structure - No. 14, 15 (Petrea et al., 2019)

Technological indicators
The analysed technological indicators were as follows: *Individual biomass gain*: IBG = (Bf)-(Bi)/ fish number [g/fish], with Bf - final fish biomass; Bi - initial fish biomass (1); *Relative growth rate*: RGR = ((Bf-Bi)/t)/Bi [g/g/day], with Bf - final fish biomass; Bi - initial fish biomass, t - duration of the experiment (2); *Specific growth rate*: SGR = 100 x (ln Bf - ln Bi)/t [% fish biomass/day], with Bf - final fish biomass, Bi - initial fish biomass, t - duration of the experiment (3); *Feed conversion ratio*: FCR = F/IBG [kg feed intake/kg fish biomass gain], with F - feed intake, FBG - individual biomass gain (4); *Protein efficiency ratio*: PER = IBG/(F*CP/100) [kg/kg], with FBG - individual biomass gain, F - feed intake, CP - crude protein (5); *Condition factor*: K=W/L-3x100, with, W - body weight, L - body length

(6); *Variation coefficient*: CVw/L = (Dev. St./Avg w/L) x 100 [%], with Dev. St. - standard deviation, Avgw/L - fish body weight/length (7).

Biological material and experimental design

The fish biomass composed of stellate sturgeon specimens (242.6±38.7 g), which are the subject of the present study, was equally distributed within the four rearing units.

Two feeding rates were tested (F1 - 1% BW, respectively F2 - 2% BW), in replicate.

Feed was administrated by using automatic feeders. Intermediary biometric and biomass measurements were made in order to upgrade the daily administrated feed quantity.

Water quality assessment

The daily water quality parameters (temperature, dissolved oxygen (DO) and pH) were measured using portable sensors - HQ40d Portable, Multi-Parameter (HACH).

The nitrogen compounds ($N\text{-}NH_4$, $N\text{-}NO_2$, $N\text{-}NO_3$), phosphorus (P_2O_5), chemical oxygen demand (COD), percentage removal of BOD5 and turbidity were measured twice per week.

The spectrophotometric method, using Merk kits for spectroquant photometer, Nova 400, was used for determining the nitrogen compounds, phosphorus (P_2O_5) and chemical oxygen demand (COD) concentrations in the technological water.

Also, the percentage removal of BOD5 from technological water was determined by applying Winkler`s method on a Velp IP54 analyzer.

The spectrophotometric method was applied, by using a turbidimeter VELP, TB1, for the determinations of technological water turbidity.

Statistical methods

The software IBM SPSS Statistics 20 for Windows was used for the statistical analysis presented in present paper.

The T test (α=0.05) was applied in order to identify the statistical differences between treatments, after the Kolmogorov-Smirnov normality test was performed. The ANOVA test (post-hoc Duncan test) was performed in order to compare variants.

RESULTS AND DISCUSSIONS

Water quality parameters

The technological water quality parameters registered proper concentrations for rearing stellate sturgeon in the analysed development stage (Table 1). Thus, the nitrogen compounds, as well as phosphorus, registered a higher average concentration for both F2 duplicate trials (*B3*: 0.28±0.08 mg L^{-1} $N\text{-}NH_4$, 0.13±0.04 mg L^{-1} $N\text{-}NO_2$, 91.83±11.6 mg L^{-1} $N\text{-}NO_3$, 25.11±6.9 mg L^{-1} P_2O_5; *B4*: 0.30±0.11 mg L^{-1} $N\text{-}NH_4$, 0.11±0.05 mg L^{-1} $N\text{-}NO_2$, 87.39±16.1 mg L^{-1} $N\text{-}NO_3$, 23.94±5.1 mg L^{-1} P_2O_5), compared to F1 duplicate trials (*B1*: 0.21±0.09 mg L^{-1} $N\text{-}NH_4$, 0.09±0.03 mg L^{-1} $N\text{-}NO_2$, 79.26±18.6 mg L^{-1} $N\text{-}NO_3$, 21.32±6.2 mg L^{-1} P_2O_5; *B2*: 0.22±0.07 mg L^{-1} $N\text{-}NH_4$, 0.08±0.02 mg L^{-1} $N\text{-}NO_2$, 74.74±14.9 mg L^{-1} $N\text{-}NO_3$, 19.77±5.3 mg L^{-1} P_2O_5) (Table 1). This may be due to the different feeding rate applied, respectively different quantity of feed input.

The difference between F1 and F2 experimental variants in terms of water quality parameters may indicate the need of practicing a higher recirculation flow for the RAS Pilot Station, in order to prevent fast accumulation of nitrogen compounds, as well as phosphorus, in the technological water, at the level of the rearing units, thus, decreasing the hydraulic retention time.

However, the low pH values (6.18±0.54 upH at B3, respectively 6.15±0.51 upH at B4, compared to 6.32±0.43 upH at B1, respectively 6.37±0.48 upH at B2) correlated with superior nitrogen compounds and phosphorus concentration, recorded in B3 and B4, compared to B1 and B2, may indicate a higher organic matter accumulation at the level of F2 experimental variant rearing units, compared to F1 (Table 1). This situation is confirmed by the results registered in terms of DO (lower concentration of DO registered in *F2*: 7.42±0.88 mg L^{-1} at B3, respectively 7.49±1.09 mg L^{-1} at B4, compared to *F1*: 7.76±0.62 mg L^{-1} at B1, respectively 7.63±0.56 mg L^{-1} at B2), BOD5 removal percentage and COD concentration (higher values registered in *F2*: 64.95±16.85 % BOD 5 at B3, 67.68±15.86% BOD5 at B4, 81.07±22.79 mg L^{-1} COD at B3, 79.81±24.93 mg L^{-1} COD at B4, compared to *F1*: 56.96±14.78 % BOD 5 at B1, 53.73±12.84

% BOD5 at B2, 68.45±14.84 mg L^{-1} COD at B1, 73.12±16.04 mg L^{-1} COD at B2) (Table 1).

Stellate sturgeon growth performance indicators

No mortalities were registered during the experimental trial, therefore confirming the proper functionality of RAS Pilot Station.

The average specific growth rate indicates a superior fish production at F2, both after 23 days (intermediary stage of experimental period) - 1.77 %BW/day and after 46 days (final stage of experimental period) - 1.72 %BW/day, compared to F1 experimental variant (0.87 %BW/day, respectively 0.65% BW/day) (Table 2.).

From the perspective of feeding strategy efficiency, the average food conversion ratio (FCR) indicates similar values at the intermediary stage of the experimental period (1.09 g feed/g biomass gain at F1, respectively 1.01 g feed/g biomass gain at F2), while at the end of the experimental trial F2 register significantly better results (1.04 g feed/g biomass gain), compared to F1 (1.47 g feed/g biomass gain) (Table 2).

The average protein efficiency ratio (PER) registered higher values in the first part of the experimental period, for both F1 and F2, compared to the last part of experimental trial (Table 2).

However, although F2 registered better average PER values in both experimental stages (2.43 g/g, respectively 2.36 g/g), compared to F1 (2.25 g/g, respectively 1.66 g/g), the differences between the experimental variants are more obvious in the last part of the experimental period.

Therefore, the PER results revealed a superior ability of fish organism reared in F2 to utilize proteins, which positively affects growth rate, compared to F1 (Table 2).

This situation is correlated with the superior results registered for RGR in F2 experimental variant, compared to F1 (Table 2).

Table 1. Water quality parameters

WATER QUALITY PARAMETER	B1	B2	B3	B4
N-NH_4 (mg L^{-1})	0.21±0.09	0.22±0.07	0.28±0.08	0.30±0.11
N-NO_2 (mg L^{-1})	0.09±0.03	0.08±0.02	0.13±0.04	0.11±0.05
N-NO_3 (mg L^{-1})	79.26±18.6	74.74±14.9	91.83±11.6	87.39±16.1
P_2O_5 (mg L^{-1})	21.32±6.2	19.77±5.3	25.11±6.9	23.94±5.1
pH	6.32±0.43	6.37±0.48	6.18±0.54	6.15±0.51
Turbidity (NTU)	4.88±0.38	4.68±0.49	5.33±0.52	5.19±0.42
BOD5 (%)	56.96±14.78	53.73±12.84	64.95±16.85	67.68±15.86
DO (mg L^{-1})	7.76±0.62	7.63±0.56	7.42±0.88	7.49±1.09
Temperature (°C)	22.82±0.44	22.83±0.46	22.90±0.39	23.17±0.41
COD (mg L^{-1})	68.45±14.84	73.12±16.04	81.07±22.79	79.81±24.93

Table 2. Growth performance indicators for each of the experimental variants

TECHNOLOGICAL INDICATOR	EXPERIMENTAL PERIOD	EXPERIMENTAL VARIANTS			
		F1		F2	
		B1 (1%)	B2 (1%)	B3(2%)	B4(2%)
Experimental period (days)	*Initial - Intermediary*	13	13	13	13
	Intermediary - Final	13	13	13	13
Survival (%)	*Initial*	100	100	100	100
	Intermediary	100	100	100	100
	Final	100	100	100	100
Individual average biomass (g/fish)	*Initial*	240.0	243.1	246.5	240.7
	St. Dev. Initial	32.34	45.53	35.08	41.89
	Intermediary	268.6	272.4	309.3	303.7
	St.dev. Intermediary	34.86	53.81	38.53	37.62
	Final	291.5	297.3	381.0	385.7
	St. Dev. Final	36.50	60.96	46.57	49.50
Individual average length (cm/fish)	*Initial*	46.6	46.7	47.1	46.2
	St. Dev.Initial	2.54	3.43	2.76	3.15
	Final	48.9	48.5	50.4	50.8
	St. Dev. Final	2.28	2.88	2.25	2.23
Fish stocking density (kg/m^2)	*Initial*	3.27	3.31	3.36	3.28
	Intermediary	3.66	3.71	4.21	4.13
	Final	3.97	4.05	5.19	5.25
Individual biomass gain (g/fish)	*Initial - Intermediary*	28.6	29.3	62.8	63.0
	Intermediary - Final	22.9	24.9	71.8	82.0

Fish stocking density gain (kg/m²)	*Initial - Intermediary*	0.4	0.4	0.9	0.9
	Intermediary - Final	0.3	0.3	1.0	1.1
Relative growth rate (g/g/day)	*Initial - Intermediary*	0.0092	0.0093	0.0196	0.0201
	Intermediary - Final	0.0066	0.0070	0.0179	0.0208
Feed protein (%)		41	41	41	41
Daily feeding ratio (% BW)		1	1	2	2
Specific growth rate - SGR (% BW/day)	*Initial-Intermediary*	0.87	0.88	1.74	1.79
	Intermediary - Final	0.63	0.67	1.61	1.84
Individual total length gain (% BL/day)	*Initial - Final*	2.36	1.87	3.30	4.59
Feed conversion ratio - FCR (g feed / g biomass gain)	*Initial - Intermediary*	1.09	1.08	1.02	0.99
	Intermediary - Final	1.52	1.42	1.12	0.96
Protein efficiency ratio - PER (g/g)	*Initial - Intermediary*	2.23	2.26	2.39	2.46
	Intermediary - Final	1.60	1.71	2.18	2.53
Weight variation coefficient - CVw (%)	*Initial*	13.47	18.73	14.23	17.40
	Intermediary	12.98	19.75	12.46	12.39
	Final	12.52	20.50	12.22	12.83
Length variation coefficient - CV_L (%)	Initial	5.46	7.36	5.85	6.82
	Final	4.66	5.93	4.47	4.40

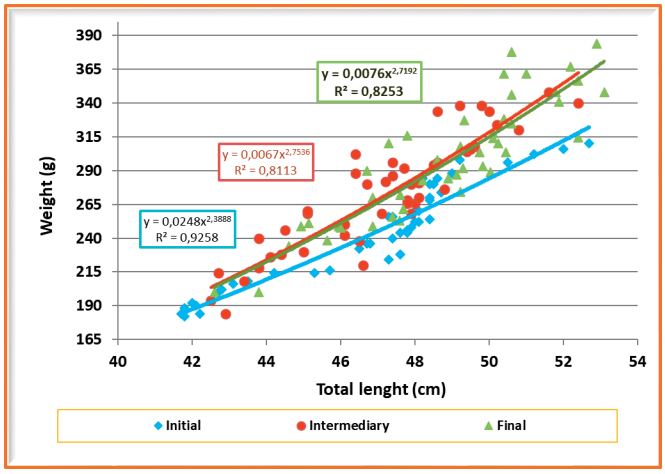

Figure 2. Total Length-Weight relation for F1 biomass, during the experimental period

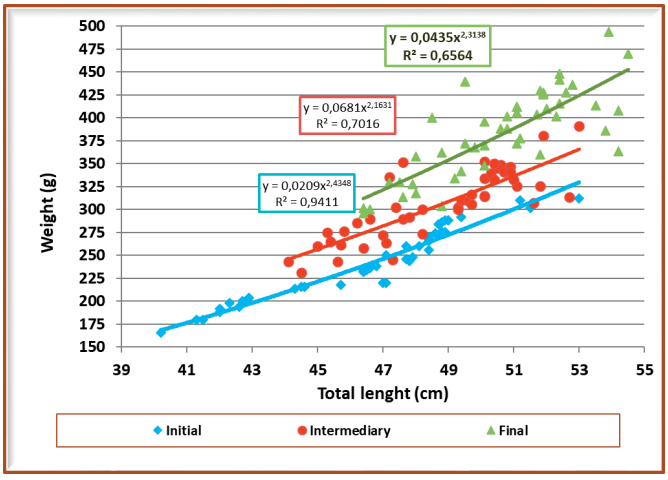

Figure 3. Total Length-Weight relation for F2 biomass, during the experimental period

Table 3. Growth performance indicators of stellate sturgeons reared in RAS, during similar development stage, reported by different authors

Reference	Stellate sturgeon average biomass (g)	SGR (%BW/day)	FCR (g feed / g biomass gain)	PER (g/g)
Dorojan et al., 2015	188.33-201.03	1.68-1.81	1.01-1.13	1.93-2.14
Dorojan et al., 2014	121.21-122.78	0.78-0.79	1.46-1.48	1.4-1.43
Dicu et al., 2013	204 ± 8	2.32-2.52	> 2	2.33-2.60
Petrea et al., 2019	396.87-456.37	0.65-1.15	1.5-1.61	1.53-1.67

By analyzing the variation coefficients, it can be stated that both experimental variants had registered a high homogeneity degree among the specimens (Table 2). However, higher values of average weight variation coefficient, registered at F1 in both experimental stages (16.37% at the intermediary stage, respectively 16.51% at the final stage of the experiment), compared to F2 (12.42% at the intermediary stage, respectively 12.53% at the final stage of the experiment) reveals a high competition for feed among F1 fish specimens, compared to F2 stellate sturgeons.

This can be due to the feeding management applied (feeding rate of 1% BW/day at F1, compared to 2% BW/day at F2).

The hypothesis is also confirmed if analysing the registered values for length variation coefficient. Thus, higher values of average length variation coefficient, registered at F1 in both experimental stages (6.41% at the intermediary stage, respectively 5.29 % at the final stage of the experiment), compared to F2 (6.34 % at the intermediary stage, respectively 4.43 % at the final stage of the experiment).

As mentioned by Petrea et al. (2019), the condition status of biological material was evaluated by using the allometric condition factor F ($F = \frac{W}{L^b}$, where b = allometric exponent, experimentally determined).

It can be observed that allometric exponent "b" has its values under three units in both intermediary and final stages of the experimental period, for both F1 and F2 experimental trials, fact that indicates a faster growth in length rather than weight (Figures 2 and 3).

Also, the K condition factor registered lower values at the end of the trial at F1 (0.249 for B1, respectively 0.260 for B2), compared to F2 (0.298 for B1, respectively 0.294 for B2).

By analysing the data reported by different authors, related to growth performance indicators of stellate sturgeons reared in RAS during similar development stage, it can be stated that the SGR results recorded in present study are similar to those reported by Dorojan et al. (2014) (0.78-0.79% BW/day) and Petrea et al. (2019) (0.65-1.15% BW/day) and lower compared to the results reported by Dorojan et al. (2015) (1.68-1.81% BW/day) and Dicu et al. (2013) (2.32-2.52% BW/day) (Table 3).

Also, the FCR and PER results recorded in present study are similar to those reported by Dorojan et al. (2015) (FCR: 1.01-1.13 g feed/g biomass gain; PER: 1.93-2.14 g/g) and better compared to the results reported by Dorojan et al. (2014) (FCR: 1.46-1.48 g feed/g biomass gain; PER: 1.40-1.43 g/g), Dicu et al. (2013) (FCR: over 2 g feed/g biomass gain; PER: 2.33-2.60 g/g) and Petrea et al. (2019) (FCR: 1.50-1.61 g feed/g biomass gain; PER: 1.53-1.67 g/g).

However, the growth performance is strongly related to the technical performance of RAS production system.

CONCLUSIONS

As a conclusion, it can be stated that during the analyzed stellate sturgeon development stage, better production and cost efficiency and achieved if 2% BW feeding rate is applied.

Also, the intensive production system is suitable for maintaining water quality parameters into an optimal range for rearing stellate sturgeons during the analysed development stage, even if a 2% BW feeding rate is applied.

It is recommended, in future similar studies, to extend the experimental period in order to have a better view related to the influence of feeding rate on stellate sturgeon growth performance.

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REFERENCES

Cristea, V. (2008). *Scientific partnerships to develop a pilot recirculating system for the promotion and implementation of innovative technologies for superintensive aquaculture*. Granted by: Ministry of Education and Research, National Authority for Scientific Research, Program CEEX, Module I, AGRAL.

Cristea, V., Ceapă, C., Grecu I. (2002). *Engineering of recirculating systems in aquaculture*. Bucharest, RO: Didactic and Pedagogic Publishing House.

Dicu., M. D., Cristea, V., Dediu, L., Maereanu, M., Petrea, S. M. (2013). The influence of feeding frequency on growth performance and meat quality of *A. stellatus* (Pallas, 1771) species, reared in a recirculating aquaculture system. *Lucrări Ştiinţifice – Seria Zootehnie*, 60, 193-199.

Dorojan, G. O., Cristea, V., Placintă, S., Petrea, S. M. (2014). The influence of some phytobiotics (Thyme, Seabuckthorn) on growth performance of stellate sturgeon (*A. stellatu*s, Pallas, 1771) in an industrial recirculating aquaculture system. *Scientific Papers. Animal Science and Biotechnologies,* 47(1), 205-210.

Dorojan, G. O., Cristea, V., Cretu, M., Coadă, M. T., Dediu, L., Grecu, I., Placintă, S. (2015). Effect of thyme (*Thymus vulgaris*) and vitamin E on growth performance and body composition of *Acipenser stellatus* juveniles. *AACL Bioflux*, 8(2), 195-202.

Engle, C. (2010). *Aquaculture Economics and Financing: Management and Analysis 1st Edition*. New Jersey, USA: Wiley-Blackwell Publishing House.

Memiş, D., Ercan, E., Çelikkale, M. S., Timur, M., Zarkua, Z. (2009). Growth and survival rate of russian sturgeon (*Acipenser gueldenstaedtii*) larvae from fertilized eggs to artificial feeding. *Turkish Journal of Fisheries and Aquatic Sciences,* 9, 47-52.

Petrea, S. M., Mogodan, A., Simionov, I. A., Cristea, V. (2019). Effect of feeding rate on growth performance of *A. stellatus* (Pallas, 1771) reared in a recirculating aquaculture system. *Scientific Papers. Animal Science Series. Lucrări Ştiinţifice - Seria Zootehnie*, 72, 252-257.

Timmons, M. B., Guerdat, T., Vinci, B. J. (2018). *Recirculating Aquaculture 4th Edition*. New York, USA: Ithaca Publishing Company LLC.

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WATER QUALITY DURING THE VEGETATION PERIOD IN A STURGEON CAGE FARM

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Abstract

A complex characterization of the physicochemical parameters of the water was performed in a sturgeon cage farm located in a dam in Southeastern Bulgaria during the different stages of the vegetation period. Water monitoring included key indicators for fish farming: temperature, dissolved oxygen, oxygen saturation, electrical conductivity, total hardness, pH, permanganate oxidability, biochemical oxygen demand over five days; ammonium nitrogen; nitrate nitrogen, orthophosphate content. There were significant negative correlation relationships: water temperature with dissolved oxygen levels (Rp = -0.702; P <0.05) and nitrate nitrogen (Rp = -0.867; P<0.01). Positive significant correlations were found between the electrical conductivity of the water with the water hardness (Rp = 0.636; P <0.05); water hardness with dissolved oxygen level (Rp = 0.855; P<0.01) and water saturation with oxygen (Rp = 0.958; P <0.001); dissolved oxygen and nitrate nitrogen in water (Rp = 0.647; P<0.05); phosphates with ammonium nitrogen levels (Rp = 0.598; P<0.05).

Key words*: aquaculture, characteristics of water, correlation relationships, sturgeon.*

INTRODUCTION

In recent years, sturgeon farming has developed rapidly in a number of countries, with Bulgaria one of them (Bronzi et al., 2019). Bulgarian production is mainly represented by industrial cage farms located in large reservoirs (Nikolova, 2019).

Industrial aquaculture in general can only develop if it provides good conditions for the cultivated species and does not have a negative impact on the environment. A number of studies have demonstrated the effects of cage farms on the reservoirs in which they are located (Dochin & Stoyneva, 2014; Dochin, 2015). Among the dominant species in the reservoirs with cages, the occurrence of cyanoprokaryotes, a potential producer of toxins, has been identified (Dochin, 2019; Dochin and Iliev, 2019).

European Commission policy on water protection is a priority, with particular emphasis on ensuring that aquaculture production does not adversely affect the ecosystems in which it is localized (Viella, 2007). Knowledge of the dynamics of individual aquatic indicators and the interrelationships between them is at the heart of sustainable aquaculture, which ensures optimal conditions for the cultivated species and is environmentally friendly (Nikolova, 2013). In developing approaches to increase the sustainability of aquaculture, and its important component - 'environmental sustainability', one of the most important tasks is to keep the reservoirs in good 'healthy' and productive condition (Nikolova, 2020). The water quality is crucial for the bio-friendly growing of hydrobionts.

We present in this paper the results of our monitoring studies of the water during the growing season, at a sturgeon cage farm located in a large reservoir in Southeastern Bulgaria.

MATERIALS AND METHODS

The study was conducted during the vegetation period (April - December incl.) on a cage sturgeon farm located in Kurdzhali Reservoir. According to its type, the reservoir refers to large and deep ones. Its area is 16.07 km², the volume is 532.9 x 10^6 m³. The reservoir is located in South-East Bulgaria, at 41°37’ N latitude and 25°20’ E longitude. It falls into the South Bulgarian climate zone, East Rhodope climate region.

Russian sturgeon (*Acipenser gueldenstaedtii*); Beluga (*Huso huso*); Siberian sturgeon (*Acipenser baeri*); Sterlet (*Acipenser ruthenus*); Stellate sturgeon (*Acipenser stellatus*); hybrids between species are grown on the farm. Feeding has been performed with a commercial granulated sturgeon feed.

To study temperature (TW, °C), dissolved oxygen (DO, $mg.dm^{-3}$) and oxygen saturation (DOS, %), *in situ* daily measurements were performed with an Elke Sensor MJ2000 Marvet junior oximeter.

For a full *ex situ* analysis of the water, samples were taken on a monthly basis and were immediately transported to a laboratory. A total of 9 samples were analysed. The analyses were performed in the laboratory complex of the Agricultural University - Plovdiv, accredited in accordance with the BSS (Bulgarian State Standard) EN ISO 17025/2018. The analysis included basic indicators for fish farms: conductivity (EL, $\mu S.cm^{-1}$) - determined according to BSS EN 27888: 2002; total water hardness (Ht, mg $eqv.dm^{-3}$) - according to BSS 3775: 1987; pH - according to BSS 3424: 1981; oxidability by $KMnO_4$ (OP, $mgO_2.dm^{-3}$) - by analytical method (BSS 17.1.4.16:1979, ISO synchronized); biochemical oxygen demand over five days (BOD_5, $mg.dm^{-3}$) - BSS 17.1.4.07:1978; ammonium nitrogen ($N\text{-}NH_4^+$, $mg.dm^{-3}$) - according to BSS ISO 7150-1: 2000; nitrate nitrogen ($N\text{-}NO_3^-$, $mg.dm^{-3}$) - BSS ISO 7890-3: 1998; orthophosphate content ($P\text{-}PO_4$, $mg.dm^{-3}$) - according to BSS 7210: 1983).

For statistical processing the IBM SPSS Stasistics 21 was used.

RESULTS AND DISCUSSIONS

Our study shows that, as a whole, the water parameters were within the limits of technological standards for fish farming, in the cultivation of thermophilic freshwater fish species. The average water temperature during Spring varied from 12.47 to 17.10°C (Table 1).

As the vegetation period progressed, it gradually increased from 22.04°C to the maximum for the studied period of 26.06°C in August. At the beginning of Autumn (September) the average temperature drops to 24.49°C and at the end of the period (November) to 16.08°C (Table 2).

Table 1. Results of the complete water analysis in the spring-summer period

Indicators	Months				
	IV	V	VI	VII	VIII
TW, ^0C	12.47	17.10	22.04	25.17	26.06
EL, $\mu S.cm^{-1}$	300	300	450	180	160
Ht, mg $eqv.dm^{-3}$	3.95	3.96	3.82	1.32	1.60
pH	7.35	6.79	7.31	8.80	7.42
DO, $mg.dm^{-3}$	9.86	8.99	7.04	5.25	4.75
DOS, %	128.9	129.5	112.1	88.5	81.2
$N\text{-}NH_4$, mg. dm^{-3}	0.22	0.17	0.15	0.27	0.14
$N\text{-}NO_3$, mg. dm^{-3}	1.50	1.40	1.30	1.1	1.00
$P\text{-}PO_4$, mg. dm^{-3}	0.04	0.17	0.08	0.05	0.06
OP, mgO_2. dm^{-3}	4.16	4.00	2.93	3.44	4.90
BOD_5 , mg. dm^{-3}	4.22	1.29	3.82	3.03	9.10

Table 2. Results of the complete water analysis in the autumn-winter period

Indicators	Months			
	IX	X	XI	XII
TW, ^0C	24.49	20.44	16.08	10.60
EL, $\mu S.cm^{-1}$	320	320	175.5	159.5
Ht, mg $eqv.dm^{-3}$	1.50	1.46	1.50	1.50
pH	8.15	6.88	6.81	7.50
DO, $mg.dm^{-3}$	4.67	5.30	5.72	7.05
DOS, %	77.7	81.8	80.8	88.3
$N\text{-}NH_4$, mg. dm^{-3}	0.96	0.22	0.21	0.17
$N\text{-}NO_3$, mg. dm^{-3}	1.00	1.50	1.30	1.60
$P\text{-}PO_4$, mg. dm^{-3}	0.18	0.11	0.08	0.08
OP, mgO_2. dm^{-3}	3.40	3.63	3.22	4.66
BOD_5 , mg. dm^{-3}	1.36	1.00	0.95	2.03

In terms of temperature, each type of fish has an area of ecological and physiological optimum when all life processes are best coordinated, with potential growth opportunities determined by the total heat that the fish receive throughout the year, especially during the fattening period (Golovanov, 2011). The author emphasizes that only in the gradient of the environmental factors (temperature, pH etc.), when hydrobionts have a choice, the conditions for maximum effective nutrition, growth and development can be ensured. There is no such opportunity in cages, which is why the adequacy of the conditions in which the fish are forced to exist is particularly important.

The results we have obtained show that the water temperature in the farm we study provides good conditions for growth. The vegetation period is prolonged and even at the beginning of Winter (December) the average temperature was above 10°C.

The maximum average temperature is at the upper limit indicated for sturgeon. Golovanov and Golovanova (2015) indicate that sturgeon optimal temperature for growth is lower than

thermophilic eurythermal carp species, but is higher than cold-water stenothermal trout fish. According to the authors, the area for the ecological and physiological optimum for sturgeons is in the range of 18-26°C, while for the older, the optimum range is lower than for the younger ones. When sturgeon species are fattening, the water temperature is recommended to be 19 to 24°C (Vasilieva et al., 2006).

Electrical conductivity characterizes the concentration of dissolved salts in water reservoirs (Woynarovich et al., 2010). The indicator gives an idea of the level of mineralization and trophicity, as higher nutrient concentration would have higher values of electrical conductivity (Kutty, 1987). In our study we found significant dynamics of the indicator during the different stages of the vegetation period. EL in Spring is 300 $\mu S.cm^{-1}$, in Summer - 160 to 450 $\mu S.cm^{-1}$, in Autumn - 175.5 to 320, and in Early Winter - 159.5 $\mu S.cm^{-1}$.

In a study by Traykov (2005) of the trophicity of the same reservoir in which the farm studied by us is located, the maximum electrical conductivity values - 223.5 µS/cm were established at the end of the vegetation period and the minimum in Spring - 110.6 µS/cm. The average electrical conductance levels found in one of the later studies of the same reservoir are in the range of 272-343.2 $\mu S.cm^{-1}$ (Dochin, 2015). Comparison of the values indicated for the whole reservoir with the average electrical conductance in our study (262.8 $\mu S.cm^{-1}$) shows relatively low levels in the cage farm.

The recommended water hardness values for sturgeon farms are 6-8 mg/l (Chebanov and Galich, 2013). In large reservoirs, hardness can change significantly throughout the year. In our study, the indicator ranged from 1.32 to 3.96 mg $eqv.dm^{-3}$ during the spring-summer period and from 1.46 to 1.50 mg $eqv.dm^{-3}$ during the Autumn and Winter months.

Wurts and Durborov (1992) state that the water hardness in fish ponds is relatively stable and usually changes within weeks or months, while the pH concentration may change daily.

For all hydrobionts, the favorable pH values are close to neutral. When keeping thermophilic fish species, pH values in the range of 6.5-8.5 are considered optimal (Grigorjev and Sedova, 2008). For sturgeon species, water with a pH of 7-8 (Chebanov and Galich, 2013) is considered appropriate, and higher values of 7.5-8.5 (Vasilieva et al., 2006) are allowed when grown in cage farms. In our study, for most of the spring-summer period, the pH of the water was within technological limits. Only in July the value of the indicator was above the optimum upper limit, reaching 8.8, but at the next control normal levels were found. During the autumn-winter period the pH is within the optimum range for the farmed fish species.

Sturgeon species belong to the group of fish with relatively high requirements for oxygen content in water (6-7 mg/l), but are able to live at lower values (5-6 mg/l) (Ivanov, 1988). For optimal DO when growing sturgeon in cages, Vasilieva et al. (2006) indicate values of 8-9 $mg.dm^{-3}$. The authors cite 5 $mg.dm^{-3}$ dissolved oxygen as the minimum concentration for the sturgeon. According to Chebanov and Galich (2013) the lower limit for DO is 4 $mg.l^{-1}$. In the sturgeon farm we studied, at the beginning of the Summer, the amount of DO was within the technological standards, falling below 6 $mg.l^{-1}$ in July. The period below the optimum values was reported from July to September, and it should be noted that the minimum limit was above 4 $mg.l^{-1}$.

The water saturation with oxygen was in the optimum range, throughout the observation period. During the spring-summer period, DOS ranges from 81.2 to 128.5% and in the autumn-winter period from 77.7 to 88.3. Todorov and Ivancheva (1992) indicate for optimal fish growth waters with DOS over 70% and for life over 35-40%. According to Moorings (1991), the minimum optimal DOS level for sturgeons is 60%.

Nitrogen and phosphorus are biogenic elements important for reservoir productivity, but at high concentrations lead to degradation of aquatic ecosystems. The nitrogen forms in aquaculture ponds depend on the way the fish are fed, the individual characteristics of the pond, the aeration, etc. (Bhatnagar and Devi, 2013). In our study, ammonium nitrogen varied from 0.14 (August) to 0.27 $mg.dm^{-3}$ (July) during the spring-summer period, and in the autumn-winter one from 0.17 (December) to 0.96 $mg.dm^{-3}$ (September). In September, the amount of ammonium nitrogen exceeded 0.5

mg.l^{-1}, cited by Chebanov and Galich (2013) as optimal for sturgeon farming, but below the 1 mg.l^{-1} specified by Kozlov (1998) as the norm for fish farming ponds.

An important indicator in monitoring is nitrates, which are an end product of nitrification in reservoirs and can have a negative effect on fish at concentrations higher than 100 mg/l (Bregnballe, 2015). Generally, in fish ponds, the nitrate content should be in the range of 0.2 to 2.0 mg/l with a tolerable limit of 3.0 mg/l (Kozlov, 1998). In our study during the spring-summer period, the amount of nitrates ranged from 1 to 1.50 mg.dm^{-3}, and during the autumn-winter period from 1 to 1.60 mg.dm^{-3}.

The phosphate rate in fish farming ponds is 0.2 mg/l at a tolerable limit of 2 mg/l (Kozlov, 1998). Water with phosphate content of not more than 0.3 mg/l is considered to be optimal for sturgeon growing by Chebanov and Galich (2013). In our study, phosphate levels are well below this limit. During the spring-summer period, the indicator varied from 0.04 to 0.17 mg.dm^{-3} and during autumn-winter one from 0.08 to 0.11 mg.dm^{-3}.

Technological standards also include the level of oxidability. The permanganate oxidability of water in sturgeon farms should not exceed 10 mgO_2.dm^{-3} (Vasilieva et al., 2006; Chebanov & Galich, 2013). In the spring-summer period the indicator ranges from 2.93 to 4.90, and in the autumn-winter - from 3.22 to 4.66 mgO_2.dm^{-3}.

Another indicator related to the amount of organic matter in water is BOD_5. No more than 10 mg/l BOD_5 is recommended for fish ponds (Grigorjev and Sedova, 2008). Chebanov and Galich (2013) indicate the value of 2 mgO_2/l as optimal for sturgeon species. Chattopadhyay et al. (1988) as a result of experiments have found that the optimal range for BOD_5 in fish farming is 10 -20 mg/l.

In our study, the mean BOD_5 in the spring-summer season was 4.3 mg.dm^{-3}, and in the autumn-winter - 1.34 mg.dm^{-3}, the variation being broad. In the spring-summer values range from 1.29 to 9.10 mg.dm^{-3}, and in the autumn-winter - from 0.95 to 2.03 mg.dm^{-3}. The maximum value was set in August when the water temperature increased to the maximum values during the vegetation period and the DO level dropped below 5 mg.dm^{-3}. At the same time, it should be noted that the levels of permanganate oxidability and pH in August were within technological limits.

The correlation analysis we performed shows complex relationships between the studied water indicators (Table 3). Wurts and Durborov (1992) noted that the interaction between the different chemical components determines the water quality in the fish farming ponds, and most of the water characteristics are not constant.

We found significant negative correlations of water temperature with dissolved oxygen levels (Rp = -0.702; P <0.05) and nitrate nitrogen (Rp = -0.867; P <0.01). A negative relationship between water temperature and nitrate content has been reported by Dochin et al. (2015). At the same time, the negative relationship between water temperature and phosphate content discovered by the authors does not correspond with our results.

In our study, temperature correlates negatively with hardness, water saturation with oxygen and permanganate oxidability, and positively with electrical conductance, pH, ammonium nitrogen, phosphates and BOD_5, but the dependencies are insignificant.

Wurts and Durborov (1992) state that a number of water characteristics, among which pH and total water hardness are interrelated and can determine reservoir productivity, oxygen content etc.

Table 3. Correlations between different water indicators

Variables	El	Ht	pH	DO	DOS	N-NH_4	N-NO_3	P-PO_4	OP	BOD_5
TW	0.119	-0.307	0.472	-0.702*	-0.415	0.338	-0.867**	0.136	-0.263	0.370
El		0.636*	-0.198	0.293	0.467	0.181	0.122	0.346	-0.557	-0.203
Ht			-0.369	0.855**	0.958***	-0.286	0.337	0.047	-0.089	0.060
pH				-0.378	-0.292	0.464	-0.553	-0.158	-0.095	0.145
DO					0.935***	-0.378	0.647*	-0.078	0.163	-0.102
DOS						-0.350	0.429	-0.012	0.025	0.006
N-NH_4							-0.493	0.598*	-0.289	-0.296
N-NO_3								-0.123	0.150	-0.422
P-PO_4									-0.206	-0.525
OP										0.538

*Correlation is significant at the * 0.05, ** 0.01, *** 0.001*

Water hardness in our study correlates negatively with pH.

Positive significant correlations were found between total water hardness with conductivity (Rp = 0.636; P <0.05), dissolved oxygen level (Rp = 0.855; P <0.01) and water saturation with oxygen (Rp = 0.958; P <0.001). A high and significant positive correlation was discovered between dissolved oxygen and nitrate nitrogen (Rp = 0.647; P <0.05). Phosphates significantly correlated only with ammonium nitrogen levels, with a positive relationship (Rp = 0.598; P <0.05).

The results of our study are in line with those of many authors. Thus, Traykov et al. (2010), in a study of a number of reservoirs in Bulgaria, found a high negative correlation of total phosphorus with the level of dissolved oxygen in water and pH, while the relationship of phosphorus with electrical conductance and total nitrogen was positive.

Zhen et al. (2019), in aquaculture ponds, have discovered a high, significant, negative correlation between nitrate content and dissolved oxygen in water. The authors indicate that 99.3% of the nitrate behavior can be explained and predicted by dissolved oxygen. Luo et al. (2019) report negative correlations between water temperature and dissolved oxygen level, between oxygen and ammonium nitrogen, and a positive correlation between water temperature and ammonium nitrogen. In their study, Bhatnagar and Devi (2012) found a negative correlation between DO and BOD_5 and between DO and ortho-phosphate.

CONCLUSIONS

The conducted study in a sturgeon cage farm showed that generally water parameters were within the limits of technological standards for fish farming, in the cultivation of thermophilic freshwater fish species.

During the vegetation period, significant dynamics of individual indicators were observed. Water temperature provides good conditions for growth. The vegetation period is prolonged and even at the beginning of Winter the average temperature was above 10°C.

A single increase in pH above the upper optimal limit was observed in July. A period with lower than optimal oxygen values was reported from July to September, and it should be noted that the minimum values were above the sturgeon limits.

The oxygen saturation of water was in optimal range throughout the studied period. There are no deviations from the water requirements for thermophilic fish species related to nitrogen and phosphorus; oxidability and biochemical oxygen demand.

Significant negative correlations of water temperature were found with dissolved oxygen levels and nitrate nitrogen. Positive significant correlations were discovered between total water hardness with electrical conductivity, dissolved oxygen level and water saturation with oxygen. A high and significant positive correlation was discovered between oxygen dissolved in water and nitrate nitrogen. Phosphates significantly correlated only with ammonium nitrogen levels, with a positive relationship.

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REFERENCES

Bhatnagar, A., Devi, P. (2012). Application of correlation and regression analysis in assessing lentic water quality: a case study at Brahmsarovar Kurukshetra, India. *International Journal of Environmental Science*, 3(2), 813-820.

Bregnballe, J. (2015). A Guide to Recirculation Aquaculture An introduction to the new environmentally friendly and highly productive closed fish farming systems. FAO and EUROFISH, 95 p.

Bronzi, P., Chebanov, M., Michaels, J.T., Wei, Q., Rosenthal, H., Gessner, J. (2019). Sturgeon meat and caviar production: Global update 2017. *Journal of Applied Ichthyology*, 35(1), 257-266.

Chattopadhyay, G.N., Saha, P.K., Ghosh, A., Karmakar, H.C. (1988). A study on optimum BOD levels for fish culture in wastewater ponds. *Biological Wastes*, 25(2), 79-85.

Chebanov, M.S., Galich, E.V. (2013). Sturgeon Hatchery Manual. FAO. Ankara. 303 p.

Dochin, K. (2015). Seasonal dynamics and species composition of the phytoplankton in Kardzhali and Dospat reservoirs. PhD Thesis, Sofia University "St. Kliment Ohridski", 201 p.

Dochin, K. (2019). Phytoplankton functional and morphological groups in large reservoirs used for

aquaculture in Bulgaria. *Bulgarian Journal of Agricultural Science*, 25(1), 166-176.

Dochin, K., Iliev, I. (2019). Functional classification of phytoplankton in Kardzhali Reservoir (Southeast Bulgaria). *Bulgarian Journal of Agricultural Science*, 25(2), 385–395.

Dochin, K., Stoyneva, M. (2014). Effect of long-term cage fish-farming on the phytoplankton biodiversity in two large Bulgarian reservoirs. *Berichte des naturwissenschaftlich-medizinischen Vereins in Innsbruck*, 99, 49-96.

Dochin, K., Kuneva, V., Hadjinikolova, L., Iliev, I. (2015). Correlations between the environmental factors and phytoplankton in Kardzhali and Dospat reservoirs. *Bulgarian Journal of Animal Husbandry*, LII (2), 52-62.

Golovanov, V. (2011). Temperature as an optimization factor in the fish life. Natural conditions and experiment. In: Proceeding of the 1st conference „Current state of biological resources of inland water bodies“, 12-16 October 2011, Borok, Russia, Vol. 1, 169-180 (Ru).

Golovanov, V.K., Golovanova, I.L. (2015). Temperature optimum and upper temperature limit of sturgeon fish species. *ATSU Vestnik, Fisheries husbandry*, 3, 110-118 (Ru).

Grigorjev, S.S., Sedova, N.A. (2008). Industrial fish farming. Part I. Biological basis and main directions of fish farming by industrial methods. Petropavlovsk - Kamchatskij, KamchatGTU, 186 p (Ru).

Kozlov, V.I. (1998). *Fish farmers guide*. Moscow, RU: VNIRO publishing house, 342 p (Ru).

Kutty, M.N. (1987). Site selection for aquaculture. Chemical features of water. http://www.fao.org/3/AC183E/AC183E00.htm#TOC.

Luo, Y., Cheng, Y. Li D., Gao, Z., Wang, L., Wu, J. Jiang, D. (2019). Annual Variation of Water Quality in Aquaculture Ponds for *Apostichopus japonicus*. *Asian Agricultural Research*; Cranston 11(11), 25-27. DOI:10.1901/j.enki.issn1943-990.2019.11.006.

Nikolova, L. (2013). *Organic aquaculture*. Academic Publishing House of Aricultural University, 89 p. (Bg).

Nikolova, L. (2019). Modern aspects of sturgeon culture in republic of Bulgaria. Conference proceedings. NACEE (Network of Aquaculture Centers in Central-Eastern Europe) International Scientific and Practical Conference. Mukachevo, Ukraine, May 22, 2019, 43-48.

Nikolova, L. (2020). *Improving the sustainability of aquaculture*. Plovdiv, BG: Academic Publishing House of the Agricultural University, 148 p.

Privezencev, Ju.A. (1991). *Intensive fish farming*. Moscow, RU: Agropromizdat, 368 p.

Todorov, M., Ivancheva, E. (1992). *Manual for works in fish-farming*. BG: Zemizdat, 147 p.

Traykov, I. (2005): Factors influencing the trophic state of Kurdzhali Reservoir. PhD Thesis, University of Sofia “St. Kliment Ohridski”. 189 p. (Bg).

Traykov, I., Tosheva, A., Uzunova, E. (2010). Physicochemical characteristics of dam lakes in Southwestern Bulgaria. *Bulgarian Journal of Agricultural Science*, 16(3), 350-357.

Vasilieva, L.M., Yakovleva, A.P., Scherbatova, T.G., Petrushina, T.N., Tyapugin, V.V., Kitanov, A.A., Arkhangelskiy, V.V., Sudakova, N.V., Astafieva, S.S., Fedoseyeva, E.A. (2006). Technologies and biological norms for sturgeon farming in the VI th climate fish farming area /Ed. N.V. Sudakova/M.: VNIRO Publishing. 100 p (Ru).

Viella, S. (2007). Manual on Effluent Treatment in Aquaculture: Science and Practice. *Aquaetreat - Improvement and Innovation of Aquaculture Effluent Treatment Technology*, 162 p.

Woynarovich, A., Moth-Poulsen, T., Péteri, A. (2010). Carp polyculture in Central and Eastern Europe, the Caucasus and Central Asia:a manual. FAO Fisheries and Aquaculture Technical Paper. No. 554. Rome, FAO. 2010. 73 p.

Wurts, W.A., Durborov, R. (1992). Interactions of pH, Carbon Dioxide, Alkalinity and Hardness in Fish Ponds. *SRAC Publication*, 464, 1-4.

Zhen, C. M., Ling, T. C., Amin, M. F. M., Onsanit, S., Rasat, M. S. M., Jamaludin, M. H., Abdullah, N. H., Ahmad, M. I., Wong, K. N. S. W. S. (2019). The Relationship between Dissolved Oxygen and Nitrate in Aquaculture Pond: Integrated Sensor System. *International Journal of Innovative Technology and Exploring Engineering* (IJITEE): 8(12S2), 432-436.

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THE PHYSIOLOGICAL STATUS OF STURGEON HYBRIDS FED WITH HERBAL SUPPLEMENTS IN RECIRCULATING AQUACULTURE SYSTEM

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Abstract

Sturgeon hybrids are an important romanian aquaculture species raised for meet and caviar. Knowing the beneficial properties of medicinal herbal supplements, the objectives of present study was to evaluate the effects of sea buckthorn and thyme extract on haematological parameters in juvenile sturgeon hybrids reared in recirculating aquaculture system. The juvenile hybrids were distributed in following groups: (1) Control, untreated fish, (2) fish fed with 1% Sea buckthorn, (3) fish fed with 1% thyme/kg feed and (4) fish fed with mix 0.5% Sea buckthorn and 0.5% thyme. The blood sampling was analyzed to: RBCc, WBCc, Ht, Hb, MCV, MCH, and MCHC. The supplementation of the diet of the hybrid resulted in the improvement of the physiological state, registering differences, depending on the period of administration of phytobiotics as well as the specific action of each type of plant extract. This study provides new information for the use of medicinal herbs as supplementation to sturgeon hybrids diet; their use can increase disease resistance by improving the physiological status.

Key words: *haematological parameters, sturgeon hybrid, sea buckthorn, thyme herbal extract.*

INTRODUCTION

The sturgeons of the Black Sea and Danube River have been highly endangered due to overfishing, loss of natural habit and water pollution. In Romania the growth of sturgeons in a controlled system started 20 years ago to produce meat and caviar but also to reduce of pressure on natural wild sturgeon population. As many animal species, hybrid sturgeons are bred to get the best characteristics from both parents.

The important objective in aquaculture is to keep on fish health as well as to enhance fish performance. The intense use of antibiotics for disease control has affected the growth of fish species because it cause to antibiotic and chemical resistance (MacMillan, 2001). One of the most popular methods of controlling diseases in aquaculture is prophylactic administration of natural plant extract or essential oils by improvement the defence mechanism of fish (Citarasu, 2010).

World Health Organization (WHO) encourages supplemented diets incorporated with medicinal herbs or plants which minimize the use of chemicals in fish diet (Dada, 2015). Sea buckthorn contains many natural antioxidants in all of its parts like as sterols, tannins, vitamins, and minerals (Kumar et al., 2013). Its leaves, stems, tubers, roots as well as blossom contain a high content of ascorbic acid (vitamin C), and also carotenoids, polyphenols, flavonoids, tocopherols, alkaloids, chlorophyll derivates, amino acids and amines (Christaki, 2012).

Thyme essential oil has two major components: as percentages of the total content are the phenols, carvacrol and thymol that represent the main antioxidant components. The thyme oil has the most pronounced antimicrobial activity compared to other oils used in aquaculture (Kateryna et al., 2012).

In sturgeon culture, herbs such as *Allium cepa, Rosa canina, Aloe vera, Allium sativum, Camellia sinensis* among others have been reported to enhance appetite, immune responses and survival rate (Akrami et al., 2015; Dadras et al., 2016; Sharif et al., 2017; Lee et al., 2014; Ebrahimi et al., 2017).

Several researches have reported the beneficial effects of dietary supplemented with sea-buckthorn and thyme oil extract on different fish species but there is no documented

evidence about the effect of this oil extracts on sturgeon hybrid which is a cross between a *Acipenser gueldenstaedtii* female x *Acipenser ruthenus* male (Kucukgul et al., 2013; Yilmaz et al., 2012).
Knowing the beneficial properties of sea buckthorn and thyme oil, the aim of this study was to evaluate the effects of sea-buckthorn and thyme oil extract on the haematological responses in sturgeon hybrid to promote alternative drug to chemotherapeutics in aquaculture.

MATERIALS AND METHODS

Experimental design

The juvenile hybrid sturgeon (298.73 ± 0.7 g) from the sturgeon farm of S.C. Danube Reasearch Consulting (Tulcea, Romania), obtained by cross between a *Acipenser gueldenstaedtii* female x *Acipenser ruthenus* male, were used for this experiment.
Fish were acclimatized for 2 weeks prior to beginning the experiment, during this period they were fed with Coppens. After the acclimation period, fish were randomly distributed into 12 fiberglass tanks, in four treatment groups including V1-CNT–Control, untreated fish, fed with normal diet, V2-SB- fish fed with 1% Sea buckthorn (*Hippophae rhamnoides)/*kg feed, V3-TH- fish fed with 1% Thyme (*Thymus vulgaris*)/kg feed and SB+TH- fish fed with 0.5% Sea buckthorn and 0.5% Thyme/kg feed.

Isolation of Essential Oils and preparing diet

The oils were isolated from dried materials by extractions which carried out in a pilot-plant supercritical carbon dioxide extractor (Natex, Prozesstechnologie GesmbH, Austria, Fabr. no. 10-023/2011). The essential oils used in the study were added to feed via pulverization method. The diets were dried in drying stove for 24 hours at 85°C. Feed was given three times a day at 8:00, 14:00 and 20:00 at a rate of 2% body weight.

Haematology assay

In the experiment, six fish per group on the 6 and 12 week, at 24 h after feeding, were used for blood sampling, for the determination of haematological analysis. Blood samples (1.5 ml) were collected from the caudal vein of individual fish using a heparinized 2 ml syringe and were analyzed with routine method used in fish hematology (Svobodova et al., 1991).
Red blood cells (RBC, 10^6 mL^{-1}), hematocrit (Hct, %) and hemoglobin (Hb, g dL^{-1}) were determined by using the method by Blaxhall and Daisley, in 1973. The hematological indices of mean cell hemoglobin concentration (MCHC), mean cell haemoglobin (MCH) and mean cell volume (MCV) were calculated. For each fish two blood smears were immediately dried, fixed and colored with May-Grünwald Giemsa panoptic method. The types of leukocytes were determined based on identification characters listed by Svobodova et al. (1991). Absolute number of circulating blood leukocytes and thrombocytes were determined in relation to 1000 erythrocytes in haemograms stained with panoptic method MGG and converted to unit blood volume.

Statistical analysis

Data were subjected to statistical analysis using the SPSS software ver. 18. The results were submitted to the variance analysis (ANOVA) ($p<0.05$) and Tukey test used to determine the significant differences between haematological parameters ($p<0.05$).

RESULTS AND DISCUSSIONS

After 6 weeks of treatment, statistical analysis of data showed that there were no significant differences ($P>0.05$) of haematological indices compared to the control group.
At the end of the 12 week, the RBC levels and Ht significantly increased ($P<0.05$) in fish fed with 0.5% sea buckthorn and 0.5% thyme when compared to control. The herbal extract did not change Hb concentration ($P>0.05$) at the end of both experimental periods (Table 1).
Blood smears from the experimental groups did not reveal any swelling, shrinkage or other deformations in blood cells.
Haematological indices, including WBCs, RBCs, Hb, Hct, MCHC, MCV and MCH, are used as important diagnostic tools to assess the health and physiological status of fish (Fazio et al., 2013). According to some authors the addition of different herbal extract in fish food had significant influence on some of

haematological parameters of different fish species (Gabriel et al., 2015; Güllü et al., 2016; Quezada-Rodríguez et al., 2016; Yilmaz and Ergün, 2012).

The erythrocyte constants can provide information on the size, shape, and hemoglobin loading of red blood cells, thus reflecting their degree of function. The statistically insignificant differences ($p>0.05$) of the VEM, HEM and CHEM values recorded in the three feeding variants compared to the V1-control indicate a good physiological state of the hybrids (Table 1).

Table 1. Hematological profiles of juvenile sturgeon hybrid after feeding with sea-buckthorn and thyme oil extract for 6 and 12 weeks (mean ± SD)

Sampling time	Percent oil in feed	RBC (10^6 mL^{-1})	Htc (%)	Hb (g dL^{-1})	MCV (fL)	MCH (pg)	MCHC (g dL^{-1})
After 6 weeks	CNT	0.85±0.12	23.79±2.00	8.32±0.47	285.56±55.97	98.67±10.62	35.13±4.39
	1 SB	0.89±0.09	23.64±1.71	8.26±0.62	265.56±20.53	92.51±8.09	34.90±2.84
	1 THY	0.88±0.08	23.38±3.17	7.89±1.44	265.82±22.62	90.73±8.19	34.40±5.74
	0.5 SB + 0.5 THY	0.91±0.10	24.29±2.96	7.88±0.78	267.92±32.18	87.11±8.43	32.93±5.25
After 12 weeks	CNT	0.82±0.08	23.35±0.84	8.18±0.39	285.48±17.57	99.99±5.39	35.05±0.72
	1 SB	0.9±0.11	23.55±1.01	8.32±0.58	263.32±21.62	92.78±5.68	35.31±1.48
	1 THY	0.91±1.09	23.63±0.98	8.37±0.67	262.21±19.83	92.67±7.51	35.39±2.07
	0.5 SB + 0.5 THY	1.05±0.13*	25.04±1.04*	8.83±0.48	241.8±20.48	85.17±6.45	35.26±0.79

The improvement of the values of the main haematological indicators (RBCc and Htc) in V4 variant may be due to the synergistic effect of some compounds extracted from thyme (carvacrol and tymol) respectively from sea buckthorn (vitamins C and E, flavonoids, carotenoids). The improvement of these hematological indices can signify the ability of herbal extracts to stimulate erythropoiesis, thus increasing the capability of oxygen transport and reinforcement of defense mechanisms against physiological stress.

In folk medicine, sea buckthorn has been used since ancient times to treat anemia due to the high content of vitamin C, which together with vitamins A, E and folic acid exerts immunomodulatory properties. The evaluation of the leukocyte profile may reflect the state of the general immune system of the fish (Ellis, 1977).

Numerous studies have shown the beneficial effect of feed additive with different plant extracts in improving the general physiological state of the fish as a result of their immunostimulatory action by increasing the total number of leukocytes (Jian et al., 2004; Nya et al., 2009; Abdel-Tawwab et al., 2010; Harikrishnan et al., 2010).

On the other hand, several studies in *Cyprinus carpio*, *Oncorhynchus mykiss*, *Oreochromis* sp. reported that some herbal extracts presented lower hematological indices and to a certain extent cause anemia in fish especially at higher dosage (Pakravan et al., 2011; Haghighi et al., 2014; Gabriel et al., 2015).

Therefore, optimization of herbal extracts based not only on growth and feed utilization parameters but also on blood parameters such hematology is essential for aquaculture (Gabriel, 2019).

Microscopic blood smears analysis led to the identification of the following categories of leukocytes: lymphocytes 87.54-94.48% (the fish's leukocyte system is of lymphocytic type), monocytes 0.96-2.16%, neutrophyles 2.66-9.59% and eozinophyles 0.63-3.56%. Basophilic granulocyte leukocytes have not been identified on blood smears.

The cytological study of blood smears provides important diagnostic information, reflecting the physiological state of the fish (Docan, 2014). In this study, significant increases have been

reported in the leukocyte and eozinophyle cells in V2 group, respectively monocyte and neutrophyle cells in V4 group, after 6 weeks feeding (Table 2).

Table 2. Leukocyte profiles of juvenile sturgeon hybrid after feeding with sea-buckthorn and thyme oil extract (mean ± SD)

Sampling time	Percent oil in feed	Leukocyte ($x10^3$ cel/µl)	Limphocyte ($x10^3$ cel/µl)	Monocyte ($x10^3$ cel/µl)	Neutrophyle ($x10^3$ cel/µl)	Eozinophyle ($x10^3$ cel/µl)
After 6 weeks	CNT	61.33±5.86	55.89±4.93	0.78±0.14	2.96±0.34	1.7±0.82
	1 SB	67.71±8.17*	61.53±7.02	0.75±0.15	5.01±0.93*	0.41±0.11
	1 THY	64.87±11.07	60.59±11.71	0.68±0.29	3.24±0.86	0.37±0.07
	0.5 SB + 0.5 THY	64.41±10.21	56.7±9.74	1.34±0.25*	5.89±0.46*	0.47±0.19
After 12 weeks	CNT	58.13±5.18	54.76±4.77	0.91±0.11	1.92±0.33	0.58±0.13
	1 SB	62.65±7.86	59.04±6.12	0.65±0.14	2.59±0.39*	0.42±0.09
	1 THY	62.55±9.91	58.93±9.09	0.63±0.10	1.88±0.11	1.04±0.18*
	0.5 SB + 0.5 THY	64.93±2.02*	60.92±2.70	0.88±0.23	1.97±0.24	1.09±0.21*

At the end of 12 weeks feeding significant increases have been reported in the leukocyte and eozinophyle cells in V4 group and neutrophyle cells in V2 group. These elevations determined in the haematological indicators support the findings obtained by the investigators concerning the subject.

After 6 weeks of experiment, was observed a significant increase ($p<0.05$) with 10.4% of the total number of leukocytes in the case of V2 group compared with V1 group (Table 2). This increasing tendency of the total number of leukocytes compared to the control group is maintained at the end of the 12 weeks, but only in the mixed diet group the total number of leukocytes increased significantly ($p<0.05$) by 11.70% compared to the V1 group.

The increases in lymphocytes, neutrophyles and eozinophyles, which are the basic elements of the defense system, showed the effect of sea buckthorn and thyme herbal extract in body defenses and this was also confirmed in the other studies.

Considering that a reduction in the number of leukocytes could be the consequence of nutritional errors, the results obtained in this study suggest that the plant extracts have contributed to the improvement of the physiological state of the hybrids from groups V2, V4 (Cain et al., 2003).

The leukocytes are important indicators of the nonspecific defence reaction, the increase of the total number of leukocytes registered at the end of the experimental period in V4 group, suggests the beneficial effect, combined, their immunomodulatory (Pedro et al., 2005).

Leukocytes perform major functions in the body which, via monocytes, are involved in triggering the immune response (Bektas and Ayik, 2009) and neutrophyles contribute to the activation of nonspecific immunity.

Scientists have intensified efforts to exploit natural products such as herbs in developing alternative dietary supplements that enhance growth performance, health and immune system of cultured fish, as these products are inexpensive, safer, effective, and can be easily prepared and are biodegradable (Syahidah et al., 2015).

CONCLUSIONS

In this study, the effects *of sea buckthorn and thyme oil extract* administered as a mixture with the feed, on the hematological profile in sturgeon hybrid were detected. The supplementation of the diet of the hybrid resulted in the improvement of the physiological state, registering differences, depending on the period of administration of phytobiotics as well as the specific action of each type of plant extract.

The analysis of the blood metabolic profile highlights the synergistic action of the herbal

extract on the physiological state of the hybrids: intensification of the haematopoiesis (increase of the RBCc and Htc in V4 group) and improvement of the non-specific defences (increase of WBCc).

Conclusively, in this study the use of sea buckthorn and thyme extract herbal as feed supplements on sturgeon hybrid, can improving the physiological status and immunity by stimulating the production of blood cells and other hematological indices.

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REFERENCES

Abdel-Tawwab, M., Ahmad, M. H., Seden, M. E. A., Sakr S. F. M. (2010). Use of green tea, *Camellia sinensis* L., in practical diet for growth and protection of Nile tilapia, *Oreochromis niloticus*, against *Aeromonas hydrophila* infection. *Jounal of World Aquaculture Societe*, 41, 203-213.

Akrami, R., Gharaei, A., Mansour, M. R., Galeshi, A. (2015). Effects of dietary onion (*Allium cepa*) powder on growth, innate immune response and hematoebiochemical parameters of beluga (*Huso huso* Linnaeus, 1754) juvenile. *Fish. Shellfish Immunol.*, 45, 828-834.

Bektas, S., Ayik, O. (2009). Hematological parameters and erythrocyte osmotic fragility in rainbow trout, *Onchorhynchus mikiss*, experimentally infected with *Pseudomonas putina*. *Journal of Fisheries and Aquatic Science*, 4(5), 246-253

Blaxhall, P. C., Daisley, K. W. (1973). Routine haematological methods for use with fish blood. *Journal of Fish Biology*, 5, 771-781.

Cain, K., Grabowsk, I., Reilly J., Lytwyn M. (2003). Immunomoldulatory effects of a bacterial-derived - I,3-glucan administered to tilapia (*Oreochronis niloticus* L.) in a Spirulina - based diet. *Aquaculture Research*, 34, 1241-1251.

Christaki, E. (2012). *Hippophae rhamnoides* L. (sea buckthorn): A potential source of nutraceuticals. *Food Public Health*, 2, 69-72.

Citarasu, T. (2010). Herbal biomedicines: a new opportunity for aquaculture industry. *Aquaculture International*, 18, 403-414.

Dada, A. A. (2015). Improvement of tilapia (*Oreochromis niloticus*, Linnaeus, 1758) growth performance fed three commercial feed additives in diets. *Journal of Aquatic Resources Development*, 6, 325.

Dadras, H., Hayatbakhsh, M. R., Shelton, W. L., Golpour, A. (2016). Effects of dietary administration of *Rose hip* and *Safflower* on growth performance, haematological, biochemical parameters and innate immune response of Beluga, *Huso huso* (Linnaeus, 1758). *Fish and Shellfish Immunology*, 59, 109-114.

Docan, A. (2014). Fish hematology. Galati, RO: Galati University Press Publishing House (in Romanian).

Ebrahimi, V., Parviz, A., Hamid, S., Azarm, M., Hasanpour, S. (2017). Effects of dietary green tea (*Camellia sinensis* L) on acute stress responses in sturgeon hybrid (*Huso huso* ♂ × *Acipenser ruthenus* ♀). *Aquaculture Research*, 48(2), 618-623.

Ellis, A. E. (1977). The leukocytes of fish: a review. *Journal of Fish Biology*, 11, 453- 491

Fazio, F., Marafioti, S., Torre, A., Sanfilippo, M., Panzera, M., Faggio, C. (2013). Haematological and serum protein profiles of *Mugil cephalus*: effect of two different habitat. *Ichthyological Research*, 60, 36-42.

Gabriel, N. N., Qiang, J., He, J., Ma, X. Y., Kpundeh, M. D., Xu, P. (2015). Dietary Aloe vera supplementation on growth performance, some haemato-biochemical parameters and disease resistance against *Streptococcus iniae* in tilapia (GIFT). *Fish and Shellfish Immunology,* 44, 504–514.

Gabriel, N. N. (2019). Review on the progress in the role of herbal extracts in tilapia culture. *Cogent Food and Agriculture*, 5(1), 1-21.

Güllü, K., Acar, Ü., Kesbiç, O. S., Yılmaz, S., Ağdamar, S., Ergün, S., Türker, A. (2016). Beneficial effects of oral allspice, pimenta dioica powder supplementation on the hemato-immunological and serum biochemical responses of *Oreochromis mossambicus*. *Aquaculture Research*, 47(9), 2697-2704.

Haghighi, M., Rohani, M. S., Samadi, M., Tavoli, M. (2014). Study of effects *Aloe vera* extract supplemented feed on hematological and immunological indices of rainbow trout (*Oncorhynchus mykiss*). *International Journal of Advanced Biological and Biomedical Research*, 2, 2143-2154.

Harikrishnan, R., Balasundaram, C., Heo, M. S. (2010). Herbal supplementation diets on haematology and innate immunity in gold fish against *Aeromonas hydrophila*. *Fish and Shellfish Immunology*, 28, 354-361.

Jian, J., Wu, Z. (2004). Influence of traditional Chinese medicine on non-specific immunity of Jian Carp (*Cyprinus carpio* var. Jian). *Fish Shellfish Immunology*, 16, 185-191.

Kateryna, K., Mahendra, R. (2012). Antibacterial activity of *Thymus vulgaris* essential oil alone and in combination with other essential oils. *Bioscience*, 4 (2), 50-56.

Kucukgul, A., Altug, K., Durali, D., Mesut, U. (2013). Therapeutic effects of thyme (*Thymus vulgaris* Linneaus) and fennel (*Foeniculum vulgare* Miller) essential oils in infected rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Digest Journal of Nanomaterials and Biostructures*, 8(3), 1069-1078.

Kumar, M. S. Y., Tirpude, R. J., Maheshwari, D. T., Bansal, A., Misra, K. (2013). Antioxidant and antimicrobial properties of phenolic rich fraction of seabuckthorn (*Hippophae rhamniodes* L.) leaves *in vitro*. *Food Chemistry*, 141, 3443-3450.

Lee, D. H., Lim, S. R., Han, J. J., Lee, S. W., Ra, C. S., Kim, J. D. (2014). Effects of dietary garlic powder on growth, feed utilization and whole body composition changes in fingerling sterlet sturgeon, *Acipenser ruthenus*. *Asian-Australasian Journal of Animal Sciences*, 27(9), 1303-1310.

MacMillan, J.R. (2001). Aquaculture and Antibiotic Resistance: A Negligible Public Haelth Risk? *World Aquaculture*. 49, 51-68.

Nya, E., Austin, B. (2009). Use of dietary ginger, *Zingiber officinale* Roscoe, as an immunostimulant to control *Aeromonas hydrophila* infections in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Disease*, 32, 971-977.

Pakravan, S., Hajimoradloo, A., Ghorbani, R. (2011). Effect of dietary willow herb, *Epilobium hirsutum* extract on growth performance, body composition, haematological parameters and *Aeromonas hydrophila* challenge on common carp, *Cyprinus carpio*. *Journal of Aquaculture Research*, 43, 1-9.

Pedro, N., Guijarro, A. I., Patiño, M. A. L., Alvarez, R. M., Delgado, M. J. (2005). Daily and seasonal variations in hematological and blood biochemical parameters in the tench, *Tinca tinca* Linnaeus, 1758. *Journal of Aquaculture Research*, 36, 1185-1196.

Quezada-Rodríguez, D. R. P., Fajer-Ávila, E. J. (2016). The dietary effect of ulvan from *Ulva clathrata* on hematological-immunological parameters and growth of tilapia (*Oreochromis niloticus*). *Journal of Applied Phycology*, 1-9.

Svobodova, Z., Pravda, D., Palackova, J. (1991). Unified methods of haematological examination of fish, Methods No. 20, 31 p. *Research Institute of Fish Culture and Hydrobiology*, Vodnany, Czech Republic.

Sharif, R. M., Haghighi, M., Bazari, M. S. (2017). Study on nanoparticles of *Aloe vera* extract on growth performance, survival rate and body composition in Siberian sturgeon (*Acipenser baerii*). *Iranian Journal of Fisheries Sciences*, 16(2), 457-468.

Syahidah, A., Saad, C. R., Daud, H. M., Abdelhadi, Y. M. (2015). Status and potential of herbalapplications in aquaculture: a review. *Iranian Journal of Fisheries Sciences*, 14, 27-44.

Yılmaz, S., Ergün, S. (2012). Effects of garlic and ginger oils on hematological and biochemical variables of sea bass *Dicentrarchus labrax*. *Journal of Aquatic Animal Health*, 24(4), 219-224.

Yılmaz, S., Ergün, S., Çelik, E. Ş. (2012). Effects of herbal supplements on growth performance of sea bass (*Dicentrarchus labrax*): Change in body composition and some blood parameters. *Journal of BioScience and Biotechnology*, 1(3), 217-222.

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CURRENT STATE OF THE MOLLUSC POPULATIONS IN THE RAZIM-SINOE LAGOON SYSTEM

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Abstract

Following the changes in physical factors, the benthic fauna was affected by the decrease in water salinity. The aim of this paper is to identify the trend of changes in the mollusc populations of the most important lagoon system on the Romanian Black Sea coast. After a period of reduced concern for the knowledge of Razim-Sinoe Lagoon System ecological evolution, the studies and researches carried out by GeoEcoMar in 2002-2017 aimed at updating the qualitative and quantitative assessment of the structure of the mollusc populations in the four main lakes, as well as improving the knowledge of the structural and functional changes in these lakes. Currently, the population structure is composed of 14 living species among of the 52 found in the composition of the substrate as recent or subfossil shell debris. By comparing the biological data recorded between 2002 and 2017 with data from the 1970s, it became obvious that a series of euryhaline species and the most sensitive freshwater forms had disappeared. These species were gradually replaced by new freshwater stenobiotic forms, which are more resistant.

Key words*: Danube Delta, mollusk populations, NW Black Sea.*

INTRODUCTION

Coastal lagoons are particular ecosystems in the boundary between continents and the sea (Tagliapietra et al., 2009). The particular features of the coastal lagoons make them one of the most productive ecosystems in the world and especially interesting for humans, whom they provide with a wide variety of societal benefits (Knoppers, 1994; Kennish and Paerl, 2010). Human activity has profoundly altered the state of the ecosystems, contributing to the massive depletion of natural resources and affecting a major part of the services provided by the lagoons ecosystems such as Venice Lagoon in Italy (Solidoro et al., 2010); Man Menor in Spain (Velasco et al., 2018); Razim-Sinoe Lagoon in Romania (Gomoiu, 2009).

Razim-Sinoe Lagoon System, situated in the NW part of the Black Sea, is part of Danube Delta Biosphere Reserve ROSCI0065 (North Lat 44º 54' 6"; East Long 28º 55' 19"). Human interventions of the past century have brought a morphological and hydrographic changes affecting the hydrologic regime, hypsometric changes modifying bottom habitat parameters (depths and sediment structure), water chemistry structure and regime changes modifying mineralization, nutrient and pollutant loadings, and biological changes marked by eutrophication, loss of biodiversity, decreased bioproductivity, and impoverished fishery (Vadineanu et al., 1997; Gomoiu et al., 2007; Gomoiu et al., 2008; Gomoiu, 2009; Gómez-Baggethun et al., 2019). These changes have resulted into a complete change of the Lagoon specific ecosystems compared to its pristine state. Antipa (1894) mentions the Razim Lake as one of the places with the greatest fisheries on the Black Sea coasts and maybe, without exaggeration, the greatest ones in Europe. This Lagoon System has come into light more than 100 years ago as a result of the practical interest in fisheries.

In order to improve the biological productivity and fish production, large scale technical works have been done (cutting canals, recalibration of some canals through widening and deepening their cross-sections for increasing the water discharge from Sf. Gheorghe distributor, building dams and closing the links with the Black Sea).

Recent variations in salinity regime and the obvious tendency toward water freshening have been caused on the one hand by the gradual reduction of marine influence (partial closing

the natural links with the sea) and, on the other hand, by the increasing amount of freshwater from the Danube. These changes have left their mark on the population's structure of mollusc. Molluscs are common, highly visible, ecologically and commercially important at global scale as valuable resources.

Species assemblages of the Lagoon System have been studied for more than 90 years by Borcea (1926), Antipa (1941), Grossu (1962), Teodorescu-Leonte et al. (1956), Teodorescu-Leonte and Leonte (1969), Teodorescu-Leonte (1966; 1977). Despite the fact that from the point of view of distribution of the Pontocaspian mollusc species of the complex is well documented (Popa et al., 2009; Popa et al., 2010; Popa et al., 2012; Wesselingh et al., 2019; van de Velde et al., 2019); the information regarding structure and ecological assessment is scarce (Gomoiu et al., 2007; Gomoiu et al., 2008; Paraschiv et al., 2010a; Paraschiv et al., 2010b). In this paper, we improve the knowledge regarding to mollusc fauna and analyse how the changes in lake dynamics have affected its populations. This is an attempt to provide a baseline for future data collection, in support of conservation and restoration of lagoon ecosystem.

MATERIALS AND METHODS

Description of the area

The Razim-Sinoe lagoon system (RSLS) is situated on the north-western coast of the Black Sea, Romania. The surface area is 863.5 km^2 with a maximum depth of 3.5 m. It is the largest lagoon system of the whole Black Sea coast, located south of the Danube Delta. The main lakes are Razim with an area of 415 km^2, Goloviţa (118.7 km^2), Zmeica (54.6 km^2) and Sinoe (171.5 km^2) (Gâştescu, 1998). During the period that preceded the construction of the canals ensuring the connection with Dunavăţ and Dranov rivulets, the water of Razim lake used to have a salinity that was close to that of the Black Sea and even higher during certain periods (Antipa, 1916). During the 1924-1925 period it was variable, and in 1937 it reached the level of 0.5 g/l in Razim and 1.5 g/l in Goloviţa (Teodorescu-Leonte et al., 1956).

Currently, in the RSLS, the total salts content is ranged between 0.1-1.0 g/l, but it is not stabilized yet, being dependent on the regime of the Danube waters (Figure 1).

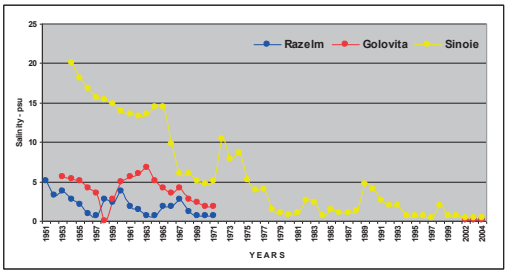

Figure 1. Evolution of salinity (psu) in the lakes from RSLS (period 1951-1956 after Teodorescu-Leonte et al., 1956; 1956-1968 - Teodorescu-Leonte, 1977; 1968-1971 - Breer, 1976; 1971-1998 - Lazar et al., 1995; Alexandrov et al., 1998; 2002-2004 - Dimitriu et al., 2008; Dinu et al., 2015)

According to Dimitriu et al. (2008), the superficial sediments of the Razim and Golovita lakes are dominated by silt and silty clays. The sandy sediments represent about 24% of the substrate of the Razim Lake, being present mainly in the southern sector. The substrate of Zmeica Lake is covered, to a large extent, by sandy sediments, with the exception of western extremity of it, where silty sediments appear. More than 60% of the substrate of Lake Sinoe is covered by silty sediments (silty clays ÷ silty sands).

Data collection and analysis

During several cruises of GeoEcoMar's R/V Istros performed in 2002-2017, 250 benthic samples (2002-2004: 239 samples, 2017: 11 samples) were collected by means of a van Veen-type grab (0.02 m^2) and Bacescu-type dredge (40 cm x 30 cm frame, with net's mesh size of 5 mm) (Figure 2). The samples were partly processed on-board (washing through 0.5 mm mesh size sieve), preserved with 4% formalin and stored for subsequent laboratory analyses.

The density and wet biomass data were represented at square meter (indv.m^{-2}, g.m^{-2}). Molluscs were weighed with shells.

The identification of molluscs was performed following the main key guide provided by Grossu (1956; 1962) and Jadin (1952). The species nomenclature was checked following the World Register of Marine Species portal (www.marinespecies.org).

Taxonomical references are based on the check-list of land and freshwater Gastropoda of

Europe (Bank, 2020) and Bivalvia (Araujo, 2020).

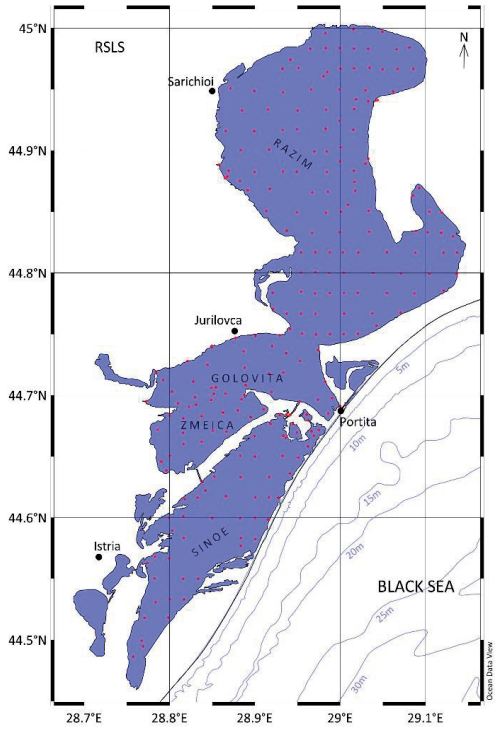

Figure 2. The Razim-Sinoe Lagoon System - map of stations performed in 2002-2004 period and 2017

The structure of the macrobenthic community was analysed in terms of species composition (S), population density (A), dominance (D), frequency (F), diversity and biomass and ecological significance index.

The research carried out in 2002-2004 period, was funded by the Romanian Ministry of Education and Research through the National Research Development and Innovation Programme CERES (Research Project Contract 156/2001), in the 2017 - by the Ministry of Research and Innovation - ANCSI - Core Program: PN16450104.

RESULTS AND DISCUSSIONS

Analyses of the 250 samples helped identify 52 species of molluscs (Appendices), out of which 14 species were found alive and form the present structure of the populations (*Acroloxus lacustris, Bithynia tentaculata, Ampullaceana balthica, Viviparus viviparus, Adacna fragilis* (possible *A. laeviuscula* Eichwald, 1829), *Hypanis (Adacna) plicata relicta, Anodonta cygnea, Corbicula fluminea, Dreissena polymorpha, Monodacna colorata, Monodacna pontica, Sphaerium corneum, Unio pictorum,* and *Unio tumidus*). The rest of the molluscs were found only as broken shells which made up the shell debris as sediment fraction in the substrate composition (Appendices). Most species are typical for freshwater environments and only five species have a Pontocaspian character (*D. polymorpha*, *A. fragilis, H. plicata relicta, M. colorata* and *M. pontica*).

The number of species in the sampling sites varies between zero and six, with a mean abundance of 246.1 indv.m^{-2} in density terms and 92.3 g.m^{-2} of biomass (Table 1).

Table 1. Ecological parameters for mollusc populations in the RSLS in 2002-2004 period

Lake	S	Density indv.m^{-2}	Biomass g.m^{-2}
Razim	9	856.2	216.3
Goloviţa	9	47.3	42.4
Zmeica	5	28	101.6
Sinoe	6	52.9	8.7
RSLS	14	246.1	92.3

As the ecological significance index reveals, the most important mollusc populations that inhabited the lagoon system benthic habitat were: *D. polymorpha* (A-398.4 indv.m^{-2} and 46.6 g.m^{-2}; F-20%), *A. cygnea* (A-6.3 indv.m^{-2} and 18.8 g.m^{-2}; F-11%), and *U. pictorum* (A-3.1 indv.m^{-2} and 20.9 g.m^{-2}; F-6%). *Dreissena polymorpha* was found in all lakes, either fixed on to *A. cygnaea*, *U. pictorum*, and *V. viviparus* shells or grapelike clustered with greatest abundance in front of Dunavăţ and Dranov channel (Figures 3 and 4). The freshwater species, *Anodonta cygnea* and *Unio pictorum*, incoming into the Razim-Sinoe lagoon, presented a "patchy" distribution. *Anodonta* prevail in the Razim Lake (11.51 indv.m^{-2}) and the *Unio* has been very abundant in Zmeica (14 indv.m^{-2}), in muddy and silty sediments rich in organic matter.

Overall high biomass values were recorded in Lake Razim in the mouth areas of the Dranov and Mustaca channel and around Popina Island, where a proper substrate type consist of mixed sediments (sand and mud) with large amount of shell debris used as secondary hard substrate or

settlement of juveniles of epibenthic species (Figure 4).

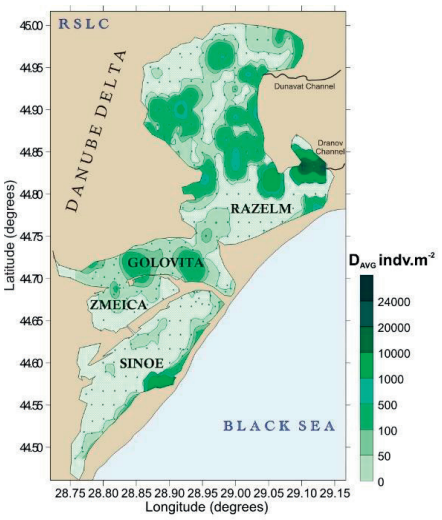

Figure 3. Average density distribution of mollusc populations in the RSLS in 2002-2004 period

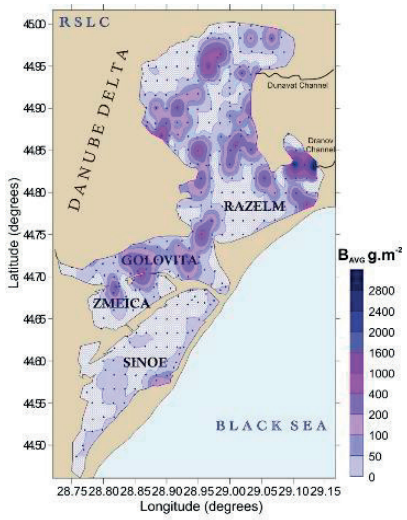

Figure 4. Average biomass distribution of mollusc populations in the RSLS in 2002-2004 period

Less abundant were *Adacna fragilis* and *H. plicata relicta* due to their ecological preferences for better-oxygenated sandy-mud sediments, which could explain their small number in the complex dominated by muddy substrata. In Sinoe Lake, these were found in the sandy area bordering the coastline in the eastern littoral zone, where they recorded the greatest densities (21.7 indv.m^{-2}) of the whole lagoon system. In general, *Adacna* species occur on sandy-mud substrate. In the Caspian Sea, they can tolerate salinities between 4-14 psu (Bogutskaya et al., 2013). *Monodacna colorata* inhabits muddy and sandy-mud substrate and has its optimum habitat between 0.03-4 psu, but can also tolerate higher salinities (Bogutskaya et al., 2013). Our results show that the freshwater species are found in more muddy-clay sediments, and Ponto-Caspian ones in the transition area between clay and sand.

We note the presence of invasive species of freshwater mollusc in the Razim Lake - the bivalve *Corbicula fluminea*, which came from the Danube, being limited to the mouth of the Dunavăţ Channel, where reached, in 2003, an average abundance of 1.57 indv.m^{-2}. The number of species considered under *Corbicula* genus is not yet known. We recognize two hyper variable species, *Corbicula fluminea* (Müller, 1774) and *Corbicula fluminalis* (Müller, 1774), although their taxonomical status is not clear yet. In the 20th century, *Corbicula* clams were introduced in North and South America, Europe and North Africa (Mouthon, 1981; Bij de Vaate, 1991; Arujo et al., 1993; Swinnen et al., 1998; Paunovic, 2007). In Romania it was first encountered at Berzasca, in the Porţile de Fier area in 1997 (Skolka and Gomoiu, 2001); two years later it was found downstream, at Vadu Oii (Bij De Vaate and Hulea, 2000). Currently, it inhabits the whole length of the Danube. Although negative effects of the introduction of Asian clams on industrial facilities have been documented for other recipient areas, such problems with *Corbicula* have not been reported for Romania and adjacent areas.

Other non-indigenous species, which invaded during the last decades the waters from Razim-Sinoe lagoon, are *Sinanodonta woodiana* (its dispersal history was established in several papers, Sárkány-Kiss (1986); Sárkány-Kiss et al., 2000; Sîrbu et al., 2006; Popa and Popa, 2006). In 2003, it found fresh valves of this species nearby of Popina Island.

The issue of ecological evolution has been a challenging subject for discussions and interpretations. Today, under the impact of global changes, the situation has become more and more complicated, uncertainties have

grown, and predictions are more difficult to make.

During the second half of the XXth century Razim-Sinoe lagoon has been seriously affected by human careless and destructive intervention, whether it had to do with the cutting of new water channels, blocking of inlets by engineering works, developing fishing ponds and agriculture polders or with the pollution of the Danube river due to sewage, industrial waste, pesticides and nutrients, reduction of flooding zones. Anthropogenic activities (closure of marine outlets, opening of channels connecting to the Danube) caused a salinity decrease of the lagoon system (Alexandrov et al., 1998; Bretcan et al., 2009; Romanescu and Cojocaru, 2010). At the beginning of the '50 of the last century the salinity of the lagoon represented the object of some systematic researches, in order to increase the fish productivity. Therefore, the molluscs' populations, from those periods, represented 44% of marine species, 25% of Ponto-Caspian relicts, 25% brackish water and 6% freshwater species (Teodorescu-Leonte et al., 1956). According to Teodorescu-Leonte (1966), the Ponto-Caspian relicts (especially *Adacna* and *Monodacna* genus) represent 28% the diet of the carp (*Cyprinus carpio*), 23% for the roach (*Rutilus rutilus*) and vimba bream (*Vimba vimba*), 13% for the gobies and 12% for the common bream (*Abramis brama*). As a result of this study, it appears that the *Adacna* and *Monodacna* genus, which recorded up to 300 kg/ha is an important source of fish feed.

After 1970, through the closure of the mouth Gura Portiţa, the freshwater input coming from the Danube increased. The period 1965-1977 is characterized by variations in salinity (Lazar et al., 1996) and the populations of molluscs were represented in Sinoe Lake by 12 species (33% - marine, 33% - brackishwater, 25% - Ponto-Caspian relicts and 9% freshwater).

In the period 1978-1982 there is a tendency of stability of mollusc populations. Related to this period, Lazar et al. (1996) found in Sinoe Lake 20 species: 50% - marine, 20% - brackish water, 20% - Ponto-Caspian relicts and 10% freshwater (Figure 5).

From 1982 to 1995, the few studies (Lazar et al., 1996; Mustata et al., 1996; Nicoara et al., 1995-1997) report a presence of small number of species of molluscs during the period of the accentuated process of water freshening in the lagoon (Figure 5). Most are Ponto-Caspian relicts (*A. fragilis, H. plicata relicta, D. polymorpha, M. colorata* and *M. pontica)* that have recorded in 1994 a biomass of 600 g.m^{-2} (Lazar et al., 1996). After 1995 these species appear sporadically and in an extremely small number.

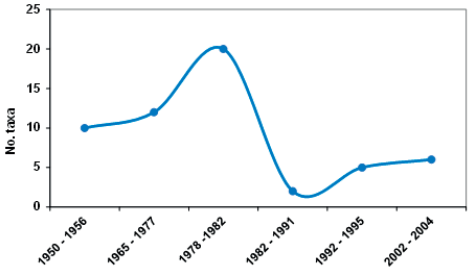

Figure 5. Diversity changes of the mollusc populations in Sinoe Lake

Today, Ponto-Caspian relicts in the Black Sea Basin are restricted to relatively small areas in lagoons, estuaries and deltas with salinity gradients along the coasts of Romania, Ukraine and Russia (Mordukhai-Boltovskoi, 1979; Anistratenko et al., 2011). The RSLS is a classic example of habitat for Ponto-Caspian species. Currently, these species are in decline, yet the causes of the decline are not fully known. Habitat destruction, pollution, poaching and invasive species (Popa et al., 2009; Paraschiv et al., 2010b; Zarbaliyeva et al., 2016). However, the molluscs' fauna is mostly dominated by freshwater species while Ponto-Caspian species reduced their distribution area. In these studies only *Hypanis plicata relicta* (A-3.1 indv.m^{-2}) was found in Sinoe Lake in the sandy area. In 2002-2004, *Adacna fragilis* and *Monodacna colorata* had isolated populations, reaching an average density of 1.6 indv.m^{-2} and 2.1 indv.m^{-2} in Razim, 5.2 indv. m^{-2} and 1.7 indv.m^{-2} in Goloviţa and 4.7 indv.m^{-2} and 6.2 indv.m^{-2} in Sinoe Lake, respectively. *Monodacna pontica* in the Sinoe Lake in 2004, was identified in the vicinity of the littoral sand bank with the highest densities of 21.7 indv.m^{-2}. Average density of it in the 2002-2004 period was 3.6 indv.m^{-2} in Razim and 12.4 indv.m^{-2} in Sinoe. However, in 2017 only *M. colorata* (A-2.5 indv.m^{-2}) was recorded

in Razim. Among all of the Limnocardiid species, the *Monodacna* genus species are the most common in the RSLS. Perhaps the small number of stations performed did not allow to surprise the real picture of the Ponto-Caspian species presence.

Following the changes in physical factors, the benthic fauna was greatly affected by the decrease in water salinity. The variations of the salinity regime, the obvious tendency towards water freshening in the last years, caused on the one hand by the gradual reduction of marine influence (partial closing of the natural links with the sea) and, on the other hand, the increasing amount of freshwater from the Danube, left their mark on the structure of benthic populations. The most important changes in the species composition structure of the lagoon fauna occurred in the populations of molluscs. Before 1956 over 65% of the mollusc populations consisted of marine and brackish water forms, 25% - Ponto-Caspian relicts and only 6% of them were freshwater ones (Teodorescu-Leonte et al., 1966). This proportion was completely changed, at the time when our researches were performed; thus, during 2002-2017, freshwater species became dominant representing 64% of the total, followed by 35% Ponto-Caspian relicts, which yet found favourable conditions for development in the lagoon, brackish and marine elements were not found (Appendices and Figure 6).

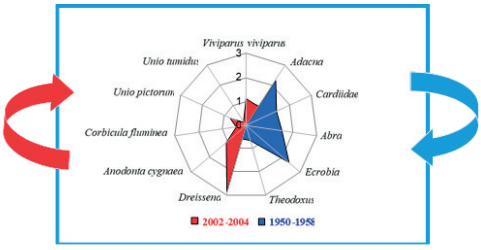

Figure 6. Shifting of the structure of the mollusc populations in Razim-Sinoe lagoon system from the `50s till present time

By comparing the present results concerning the moluscs fauna of Razim-Sinoe lagoon with the previously published data (Teodorescu-Leonte, 1977), we can affirm that some species have disappeared from the benthic community in the past years, especially the euryhaline species (Cardiidae, *Abra, Ecrobia*) and the more sensitive freshwater ones (*Theodoxus*). At the same time, there appeared new freshwater-stenobiotic, and more resistant forms (*Anodonta cygnea, Corbicula fluminea, Unio pictorum* etc.) (Figure 6).

CONCLUSIONS

In the 250 stations surveyed between 2002 and 2017 from RSLS, only 14 (4 - Gastropoda and 10 - Bivalvia) alive species forming the current structure of the populations out of 52 species identified in the substrate composition were found. Most species are typical for freshwater environments and only five species have a Ponto-Caspian character (*Dreissena polymorpha, Adacna fragilis, Hypanis plicata relicta, Monodacna colorata* and *Monodacna pontica*). After the ecological significance index, the most important mollusc populations were *Dreissena polymorpha, Anodonta cygnea* and *Unio pictorum.*

After analysing the molluscs data recorded between 2002 and 2017 with those from the 1970s, it became obvious that, euryhaline species and the most sensitive freshwater forms had disappeared. These species were gradually replaced by new freshwater stenobiotic forms, which are more resistant. Therefore, the gradual transition from a marine environment to a lacustrine one brought about the ecological succession of species in the lagoon system, which became an ecosystem consisting mainly of freshwater species.

Our results point to the necessity of a establishing a brackish water regime to support the conservation of mollusc populations (especially Ponto-Caspian species) and increasing the food variety of economically important fish species in the Razim-Sinoe Lagoon System.

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REFERENCES

Alexandrov, L., Bilal, I., Telembici, A., Serştiuc, D., Zaharia, T. (1998). Impact des chamgements hydrologiques des dernieres 25 annees dans la lagune Sinoe, sur l`evolution du zooplankton. *Cercetări marine*, IRCM, Constanţa, 31, 247-260.

Anistratenko, V. V., Khaliman, I. A., Anistratenko, O. Y. (2011). *Mollyuski Azovskogo morya*, Kiev UA: Naukova dumka (In Russian).

Antipa, G. (1894). *Razim Lake, the current state of the fishermen in it and the means of correction/Lacul Razim, starea actuala a pescariilor din el si mijloacele de indreptare*. Bucharest, RO: Imprimeria Statului Publishing House (In Romanian).

Antipa, G. (1916). *Fisheries and fishing in Romania/Pescaria si pescuitul in Romania*. Bucharest, RO: Acad. Romana Fond V. Adamachi 8(46) (In Romanian).

Antipa, G. (1941). *Black Sea, Oceanography, bionomy and general biology/Marea Neagra, Oceanografia, bionomia si biologia generala, vol.I*. Bucharest, RO: Acad. Romana Fond V. Adamachi 10(55) (In Romanian).

Araujo, R. (2020). Fauna Europaea: Mollusca, Bivalvia. Fauna Europaea version 2.6.2. Retrieved April 10, 2020 from http://www.faunaeur.org.

Arujo, R. D., Moreno, D., Ramos, M. A. (1993). The Asiatic clam *Corbicula fluminea* (Müller, 1774) in Europe. *American Malacological Bulletin*, 10(1), 39-49.

Bank, R., A. (2020). Fauna Europaea: Mollusca, Gastropoda. Fauna Europaea version 2.6.2. Retrieved March 10, 2020 from http://www.faunaeur.org.

Bij de Vaate, A. (1991). Colonization of the German part of the River Rhine by the Asiatic clam, *Corbicula fluminea* Muller, 1774 (Pelecypoda, Corbiculidae). *Bulletin Zoologisch Museum, Universiteit van Amsterdam*, 13(2), 13-16.

Bij de Vaate, A., Hulea, O. (2000). Range extension of the Asiatic clam *Corbicula fluminea* (Müller, 1774) in the River Danube: first record from Romania. *Lauterbornia*, 38, 23-26.

Bogutskaya, N. G., Kijashko, P. V., Naseka, A. M., Orlova, M. I. (2013). *Identification keys for fish and invertebrates. Volume 1: Fish and molluscs*. St. Petersburg – Moscow, RU: KMK Scientific Press Ltd. (In Russian).

Borcea, I. (1926). Quelques remarques sur les Adacnides et principalement des lacs Razelm. *Ann. Sci. Univ. Jassy*, 13(3-4), 449-473.

Bretcan, P., Murarescu, O., Samoila, E., Popescu, O. (2009). Water Management in the Razim-Sinoie Lacustrine Complex. in *International Symposium on Water Management and Hydraulic Engineering,* Ohrid, Macedonia, 791–802.

Gâştescu, P. (1998). Razim-Sinoie lake complex, Romania. In: Encyclopedia of Hydrology and Lakes. Encyclopedia of Earth Science. *Springer*, Dordrecht https://doi.org/10.1007/1-4020-4497-6_184.

Gómez-Baggethun, E., Tudor, M., Doroftei, M., Covaliov, S., Năstase, A., Onără, D., Cioacă, E. (2019). Changes in ecosystem services from wetland loss and restoration: an ecosystem assessment of the Danube Delta (1960-2010). *Ecosyst. Serv.,* 39, 1-17.

Gomoiu, M.-T., Begun, T., Teaca, A. (2007). Perspectives and uncertainties in the evolution of the lagoon ecosystems of the western coast of the Black Sea. *Joint Plenary Meeting and Field Trip of IGCP 521 "Black Sea-Mediterranean corridor during the last 30 ky: Sea-level change and human adaptation," and IGCP 481 "Dating Caspian Sea Level Change"* (September 8–17, 2007, Southern Branch of the Institute of Oceanology, Russian Academy of Sciences, and Demetra Beneficent Foundation, Gelendzhik, Russia, and Kerch, Ukraine), 72–74.

Gomoiu, M. T., Begun, T., Opreanu, P., Teaca, A. (2008). Present state of benthic ecosystem in Razelm-Sinoie Lagoon Complex (RSLC). *Limnological reports 37 Proceedings. 37th IAD Conference. The Danube River Basin in a changing World.* Chişinău, Moldova, 108-112.

Gomoiu, M. T. (2009). Ecological state of the Romanian Black Sea littoral lacustrine ecosystems. *EGU General Assembly 2009*, 11950.

Grossu, A. V. (1956). *Mollusca - Gastropoda Prosobranchia si Opistobranchia. Fauna Republicii Populare Române.* Bucharest, RO: Academia Romana, 3(2) (In Romanian).

Grossu, A. V. (1962). *Mollusca - Bivalvia. Fauna Republicii Populare Române*. Bucharest, RO: Academia Romana. 3(3) (In Romanian).

Grossu, A. V. (1973). Les Limnocardiides actuelles du Bassin Ponto-Caspique. *Inf. Soc. Belgique de Malacologie*, 2 (7-8), 123-152.

Jadin, V. I. (1952). Molluscs of fresh and brackish waters of the USSR. *Isdatelstvo An SSSR*, 1-376.

Dimitriu, R. G., Oaie, G., Gomoiu, M. T., Begun, T., Szobotka,S., Radan, S. C., Fulga, C. (2008). O caracterizare interdisciplinara starii geoecologice actuale a complexului lagunar Razelm-Sinoe la începutul secolului XXI. *Geo-Eco-Marina*, 14, 69-74.

Dinu, I., Umgiesser, G., Bajo, M., de Pascalis, F., Stănică, A., Pop, C., Dimitriu, R. G., Nichersu, I., Constantinescu, A. (2015). Modelling of the response of the Razelm-Sinoe lagoon system to physical forcing. *Geo-Eco-Marina*, 21, 5-18.

Kennish, M. J., Paerl, H. W. (2010). Coastal lagoons. Critical habitats of environmental change. *CRC Press, Boca Ratón*, 1-15.

Knoppers, B. (1994). *Aquatic primary production in coastal lagoons*. B. Kjerfve (Ed.), Coastal Lagoon Processes, Elsevier Oceanography series, 60, 243-286.

Lazar, A., Bilal, I., Serstiuc, D. (1996). Evoluţia biodiversităţii zoobentosului în lacul Sinoe (The evolution of biodiversity of zoobenthos in the lake Sinoie), *ASICPDD*, Tulcea, Romania, V(1).

Mordukhai-Boltovskoi, P. D. (1979). Composition and Distribution of Caspian Fauna in the Light of Modern Data. *Int. Rev. der gesamten Hydrobiol. und Hydrogr*., 64(1), 1-38.

Mouthon, J. (1981). Sur la presence en France et en Portugal de *Corbicula* (Bivalvi and Corbiculidae) onginaire d'Asia. *Basterie*, 45, 109-116.

Mustata, Gh., Nicoara, M., Trandafirescu, I., Popov, I., Ochea, M. (1996). Structure and dynamics of the lake Razim (DDBR), zooplankton and zoobenthic populations. *ASICPDD*, Tulcea, România, V(1).

Nicoara, M., Mustata, Gh., Popov, L., Ochea, M., Surugiu, V., Pălici, C. (1995-1997). Biodiversity study of the Lake Goloviţa regarding changes occured in zooplankton and benthic populations. *Analele Ştiinţifice ale Universităţii "Al. I. Cuza" Iaşi, s. Biologie animal,* XLI-XLIII, 7-15.

Paraschiv, G. M., Begun, T., Teaca, A., Bucur, M., Tofan, L. (2010a). New data about benthal populations of the Golovita and Zmeica lakes. *Journal of Environmental Protection and Ecology*, 11(1), 253–260.

Paraschiv, G. M., Tofan, L., Schroder, V., Bucur, M. (2010b). Analysis of zoobenthal communities from the Razim-Sinoe lagoon complex. *Journal of Environmental Protection and Ecology*, 11(1), 253-260.

Paunovic, M., Csányi, B., Knežević, S., Simić, V., Nenadić, D., Jakovčev-Todorović, D., Stojanović, B., Cakić, P. (2007). Distribution of Asian clams *Corbicula fluminea* (Müller, 1774) and *C. fluminalis* (Müller, 1774) in Serbia. *Aquatic Invasions*, 2(2), 99-106.

Popa, O. P., Popa, L. O. (2006). *Sinanodonta woodiana* (Lea, 1834), *Corbicula fluminea* (O. F. Müller, 1774), *Dreissena bugensis* (Andrusov, 1897) (Mollusca: Bivalvia): alien invasive species in Romanian fauna. *Travaux du Muséum National d'Histoire Naturelle „Grigore Antipa",* 49, 7-12.

Popa, O. P., Sarkany-Kiss, A., Kelemen, B. S., Iorgu, E. I., Murariu, D., Popa, L. O. (2009). Contributions to the knowledge of the present Limnocardiidae fauna (Mollusca, Bivalvia) from Romania. *Trav. du Muséum Natl. d'Histoire Nat. "Grigore Antipa"*, LII, 7-15.

Popa, O. P., Iorgu, E. I., Kelemen, B. S., Murariu, D., Popa, L. O. (2010). Morphometric analysis of some populations of Lymnocardiid species (Mollusca: Bivalvia) from Razelm Lake Complex (Romania). *Trav. du Muséum Natl. d'Histoire Nat. "Grigore Antipa"* 53(1), 13–19. https://doi:10.2478/v10191-010-0002-y.

Popa, L., Popa, O., Iorgu, E., Kelemen, B., Murariu, D. (2012). Molecular insights into the taxonomy of *Hypanis* (Bivalvia, Cardiidae, Lymnocardiinae) in the Black Sea lagoons. *Helgol Mar Res*, 66, 153–158. https://doi.org/10.1007/s10152-011-0256-1.

Romanescu, G., Cojocaru, I. (2010). Hydrogeological considerations on the western sector of the Danube Delta - A case study for the Caraorman and Sărăturile fluvial-marine levees (with similarities for the Letea levee), *Environ. Eng. Manag. J.,* 9(6), 795–806.

Sarkany-Kiss, A., (1986). *Anodonta woodiana* (Lea, 1834) a new species in Romania (Bivalvia, Unionacea). *Travaux du Muséum d'Histoire Naturelle "Grigore Antipa*", 28, 15-17.

Sarkany-Kiss, A., Sirbu, I., Hulea, O. (2000). Expansion of the adventive species *Anodonta woodiana* (Lea, 1834) (Mollusca, Bivalvia, Unionoidea) in central and eastern Europe. *Acta oecologica*, 7 (1-2), 49-57.

Solidoro, C., Bandelj, V., Bernardi, F. B., Camatti, E., Ciavatta, S., Cossarini, G., Facca, C., Franzoi, P., Libralato, S., Canu, D. M., Pastres, R., Pranovi, F., Raicevich, S., Socal, G., Sfriso, A., Sigovini, M., Tagliapietra, D., Torricelli, P. (2010). *Response of the Venice Lagoon Ecosystem to Natural and Anthropogenic Pressures over the Last 50 Years*. In Kennish, M. J., Paerl, H.W. (Eds.), Coastal Lagoons. Critical Habitats of Environmental Change, CRC Press, Boca Ratón, 483-511.

Skolka, M., Gomoiu, M.-T. (2001). Alien invertebrates species in Romanian waters. *Ovidius University, Annals of Natural Sciences, Biology - Ecology Series*, 5, 51-55.

Sirbu, I., Sarkany-Kiss, A., Sirbu, M., Benedek, A. M. (2006). The Unionidae from Transylvania and neighboring regions (Romania). *Heldia* 6, 151–160.

Swinnen, F., Leynen, R., Sablon, L., Duvivier, L., Vanmaele, R. (1998). The Asiatic clam *Corbicula* (Bivalvia: Corbiculidae) in Belgium. *Bulletin del l'Institute Royal des Sciences Naturelles de Belgique. Biologie*, 68, 47-53.

Tagliapietra, D., Sigovini, M., Ghirardini, A. V. (2009). A review of terms and definitions to categorise estuaries, lagoons and associated environments. *Mar. Freshw. Res.*, 60, 497–509. https://doi:10.1071/MF08088

Teodorescu-Leonte, R., Leonte, V., Matei, D., Soileanu, B. (1956). Observatii asupra complexului Razelm-Sinoe in perioada 1950-1952, *Analele ICPDD*, Tulcea, 1, 1-50.

Teodorescu-Leonte, R. (1966). Rezultatele cercetarilor asupra bazei trofice a complexului Razelm si perspectivele productiei piscicole in acest complex prin dirijarea poluarii. *Buletinul Institutului de Cercetări si Proiectari Piscicole*, 25 (1), 38–46.

Teodorescu-Leonte, R., Leonte, V. (1969). Variation des biocenoses benthiques du Complexe Razelm, en fonction des conditions de salinité. *Rapports de la Commission Internationale pour l'Étude Scientifique de la Mer Méditerranée. CIESM, Monaco*, 19 (5).

Teodorescu-Leonte, R. (1977). *Le complexe Razelm-Sinoe un systeme de lacs typiquement saumatres*. In Băcescu M., Pora E., (Eds.) 1977 – Biologie des eaux saumaitres de la mer Noire, 213-233.

Vadineanu, A., Cristofor, S., Ignat, G., Romanca, G., Ciubuc, C., Florescu, C. (1997). Changes and opportunities for integrated management of the Razim-Sinoe Lagoon System. *International Journal of Salt Lake Research,* 6, 135-144. https://doi.org/10.1023/A:1009077609504

van de Velde, S., Jorissen, E. L., Neubauer, T. A., Radan, S., Pavel, A. B., Stoica, M., Van Baak, C. G. C., Martínez G., A., Popa, L., de Stigter, H., Abels, H. A., Krijgsman, W., and Wesselingh, F. P. (2019). A conservation palaeobiological approach to assess faunal response of threatened biota under natural and anthropogenic environmental change. *Biogeosciences*, 16, 2423–2442. https://doi.org/10.5194/bg-16-2423-2019.

Velasco, A. M., Pérez-Ruzafa, A., Martínez-Paz, J. M., Marcos, C. (2018). Ecosystem services and main environmental risks in a coastal lagoon (Mar Menor, Murcia, SE Spain): the public perception. *J. Nat. Conserv.*, 43, 180-189.

Wesselingh, F. P., Neubauer, T. A., Anistratenko, V. V, Vinarski, M. V., Yanina, T., ter Poorten, J. J., Kijashko, P., Albrecht, C., Anistratenko, O. Yu., D'Hont, A., Frolov, P., Gándara, A. M., Gittenberger, A., Gogaladze, A., Karpinsky, M., Lattuada, M., Popa, L., Sands, A. F., van de Velde, S., Vandendorpe, J., Wilke, T. (2019). Mollusc species from the Pontocaspian region - an expert opinion list. *ZooKeys*, 827, 31-124. https://doi.org/10.3897/zookeys.827.31365.

www.marinespecies.org

Zarbaliyeva, T. S., Akhundov, M. M., Kasimov, A. M., Nadirov, S. N., Hycenova, G. G. (2016). The influence of invasive species on the Caspian Sea aboriginal fauna in the coastal waters of Azerbaijan. *Russ. J. Biol. Invasions,* 7(3), 227-236. http://doi:10.1134/S2075111716030139

APPENDICES

The mollusc species composition in the Razim-Sinoe Lagoon System (R-Razim, G-Goloviţa, Z-Zmeica, S-Sinoe) in 2002-2004 period and 2017 year

Crt. no.	Species	Shell debris		Living fauna			
		R	S	R	G	Z	S
	GASTROPODA						
1	*Acroloxus lacustris* (Linnaeus, 1758)				+		
2	*Ampullaceana balthica* (Linnaeus, 1758)						+
3	*Bithynia tentaculata* (Linnaeus, 1758)				+		
4	*Bittium reticulatum* (da Costa, 1778)		+				
5	*Caucasotachea vindobonensis* (C. Pfeiffer, 1828)	+					
6	*Chrysallida sp.*		+				
7	*Clathrocaspia gmelinii* (Clessin & W. Dybowski in W. Dybowski, 1887)	+					
8	*Clessiniola variabilis* (Eichwald, 1838)	+					
9	*Ecrobia maritima* (Milaschewitsch, 1916)		+				
10	*Ecrobia ventrosa* (Montagu, 1803)	+	+				
11	*Esperiana esperi* (Férussac, 1823)	+					
12	*Hydrobia acuta* (Draparnaud, 1805)	+					
13	*Lithoglyphus naticoides* (C. Pfeiffer, 1828)	+	+				
14	*Planorbarius corneus* (Linnaeus, 1758)	+					
15	*Potamopyrgus antipodarum* (Gray, 1843)	+	+				
16	*Pseudamnicola razelmiana* Grossu, 1986	+					
17	*Rapana venosa* (Valenciennes, 1846)		+				
18	*Retusa truncatula* (Bruguière, 1792)	+	+				
19	*Retusa variabilis* (Milaschewitsch, 1912)		+				
20	*Rissoa membranacea* (J. Adams, 1800)		+				
21	*Rissoa splendida* Eichwald, 1830		+				
22	*Theodoxus danubialis* (C. Pfeiffer, 1828)	+	+				
23	*Theodoxus fluviatilis* (Linnaeus, 1758)	+					
24	*Tritia neritea* (Linnaeus, 1758)	+					
25	*Tritia reticulata* (Linnaeus, 1758)		+				
26	*Valvata piscinalis* (O. F. Müller, 1774)	+	+				
27	*Viviparus viviparus* (Linnaeus, 1758)	+	+	+	+	+	
	BIVALVIA						
28	*Abra alba* (W. Wood, 1802)		+				
29	*Abra segmentum* (Récluz, 1843)	+	+				
30	*Adacna fragilis* Milaschewitsch, 1908	+	+	+	+	+	
31	*Anodonta cygnea* (Linnaeus, 1758)	+	+	+	+	+	+
32	*Cerastoderma glaucum* (Bruguière, 1789)	+	+				
33	*Cerastoderma sp.*	+					
34	*Corbicula fluminea* (O. F. Müller, 1774)			+			
35	*Dreissena polymorpha* (Pallas, 1771)	+	+	+	+	+	+
36	*Euglesa subtruncata* (Malm, 1855)	+					
37	*Hypanis (Adacna) plicata relicta* Milaschevich, 1916	+	+				+
38	*Hypanis dolosmiana* (Borcea, 1926)	+					
39	*Lentidium mediterraneum* (O. G. Costa, 1830)		+				
40	*Monodacna colorata* (Eichwald, 1829)	+	+	+	+		+
41	*Monodacna pontica* Eichwald, 1838	+	+	+			+
42	*Mya arenaria* Linnaeus, 1758		+				
43	*Mytilaster lineatus* (Gmelin, 1791)		+				
44	*Mytilus galloprovincialis* Lamarck, 1819		+				
45	*Parvicardium exiguum* (Gmelin, 1791)		+				
46	*Pseudanodonta complanata* (Rossmässler, 1835)	+					
47	*Sinanodonta woodiana* (I. Lea, 1834)	+					
48	*Sphaerium corneum* (Linnaeus, 1758)				+		
49	*Sphaerium rivicola* (Lamarck, 1818)	+					
50	*Spisula subtruncata* (da Costa, 1778)		+				
51	*Unio pictorum* (Linnaeus, 1758)	+	+	+	+	+	
52	*Unio tumidus* Philipsson, 1788			+			
	Total	**31**	**31**	**9**	**9**	**5**	**6**

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