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GENETICS AND BREEDING

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STUDY OF THE TECHNOLOGICAL CHARACTERS OF SILKWORMS (*Bombyx mori* L.) ON ARTIFICIAL DIET

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Abstract

The purpose of the research is to prolong the period of silkworm rearing during the summer and autumn season. The rearing was carried out in the months of July, August and September at the Experimental Station of Sericulture at the Agricultural University in Plovdiv, Bulgaria. Silkworms in the first, second and third instars were fed by applying artificial diet, and in the last two larval instars – on mulberry leaves from Morus alba L. The results obtained from the studied technological characters show that there is a decrease in the basic values of the cocoon weight and filament length. For other characters, the decrease is negligible. The main conclusion is that the rearing of silkworms during the summer months by using combination of feeding on artificial diet in the first three instars and on mulberry leaves in the fourth and fifth instars is successful and leads to the possibility for more employment of people and increasing of the income of the silkworm rearers.

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

INTRODUCTION

The specificity of the climatic conditions in Bulgaria allows the rearing of silkworms with mulberry leaves to be carried out during the half of the year – from the beginning of May to the middle of October (about five months). Accordingly, there is an existing scheme of silkworm eggs production, in which spring and late-spring rearing are carried out with eggs produced in the previous year, summer rearing – with treated with hydrochloric acid eggs from spring generation, and autumn rearing – with hydrochloric acid-treated eggs laid by silkworms of spring or summer generation.

At the same time, in subtropical countries, the rearing begins as early as March, while in most tropical countries, it occurs throughout the year. In the countries of the Southern Hemisphere, the seasons of silkworms rearing are reciprocal to those in Bulgaria.

In India and Thailand, some of the best coconut crops are harvested during the so-called "winter" season from December to February. In the last decade in countries such as Japan, Italy, USA, Canada, UK and others, year-round silkworms rearing with so-called "artificial food" has been developed.

The idea of developing artificial diet as a replacement of the fresh mulberry leaves originated decades ago out of a simple query "Why do silkworms feed only on mulberry leaves?". This question caused a number of studies on the composition of mulberry leaves and in particular on the compounds which attracts the silkworms. Globally, continuous efforts are being made to improve the performance of artificial diet through permutations and combinations. However, all existing silkworm breeds and hybrids might not thrive on artificial diet on default; instead they should be made adapted to the diet through breeding process (Nair et al., 2013).

Each of the ingredients involved in the preparation of artificial diet can have a positive or negative effect on the main characters. According to some studies, ascorbic acid in food affects the yield of cocoon. Rearing of silkworms in their first and last instars on ascorbic acid-free diet has been shown to have a beneficial effect to cocoon production without affecting the survival rate or delaying the larval cycle (Cappellozza et al., 2005).

In Japan, where the sericulture industry is one of the most developed in the world, in about 50% of the silkworms farming an artificial diet is used during the young instars (Shimbo et al., 1994). Although the use of artificial diet is costly, since 6% of the costs are for agar-agar and mulberry leaves (Shimbo et al., 1994).

Some recipes have also been developed in order to feed silkworms on artificial diet using different percentages of mulberry leaves (5%, 15%, 25%) and applying the diet at different larval stages (Sbrenna et al., 2000).

According to (Cappellozza et al., 2011), hormone-based preparations can be added to the artificial diet, such as Methoprene (Manta) having a stimulating effect on the silkworms, with an increase in protein accumulation in the body that can be used later for biomedical purposes. Different diets could also have a negative effect on protein accumulation in the body, immune system, digestion, nutrient uptake, metabolism and silk synthesis (Zhou et al., 2008).

Sukirno et al. (2005) evaluate artificial diets that can be used to successfully culture the atlas silk moth, *Attacus atlas* L. The results of their studies show that in some of the characters there are no significant differences, while there are some in others. Based on the results that were obtained, they recommend adding extra protein to the artificial diet.

According to Saviane et al. (2014), the availability of quality mulberry leaves in the temperate climate zone is restricted to the spring-summer season, which is a limiting factor in the selection of silkworm strains. Therefore, they carry out coupled traditional rearing on mulberry leaves with rearing on an artificial diet, in order to obtain increased larval efficiency in converting food and high silk production.

Some authors (Tzenov et al., 2000) studied the pupation rate and fresh cocoon yield in different Bulgarian silkworm breeds and hybrids under optimal and adverse rearing conditions during the last two larval instars.

It was estimated that there existed clearly expressed genetically determined differences between the breeds and hybrids for pupation rate under adverse silkworm rearing conditions. However, no any correlation was detected between the pupation rate under the optimal and adverse rearing conditions in one the same strain.

MATERIALS AND METHODS

The experiments were conducted during the period 2017-2018 at the Agricultural University of Plovdiv. 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.

Two uni-bivoltine 4 molting pure lines, created in Bulgaria in 2005, namely Svila 1 and Svila 2, were tested.

From each strain, there were employed three replicates with 200 larvae each. Same strains but reared on mulberry leaves only were used as references to collate the main technological characters that have been studied.

The standard method for the summer season was applied for carrying out the incubation of silkworm eggs. The conditions for silkworm rearing on artificial diet compared to those applied for rearing with mulberry leaves only are shown in Table 1:

Table 1. Environmental conditions for silkworms rearing on artificial diet and on mulberry leaves

	Instar	Temperature, °C	Relative humidity, %
Larvae fed on fresh mulberry leaves	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
Larvae fed on artificial diet	I	29–30	90
	II	29–30	90
	III	27–28	80
	IV	26	70–75
	V	24	70
	Cocooning	25–27	55–60

It can be seen that the temperatures established for the rearing on artificial diet differs from environmental conditions required for rearing on fresh mulberry leaves, being slightly higher during the all instars and cocooning.
In regards to the different instars, the rearing temperature when artificial diet is used, is recommended to be kept higher (compared to rearing on mulberry leaves only): such as 29 to 30°C in the first and the second instars, 27 to 28°C in the third instar, and 26°C in the fourth instar. It is assumed that at a high temperature larva might gain more weight than at a low temperature. In the fifth instar however, there is also a difference (24°C for artificial diet-fed rearing) but it is still comparable with the temperature established for rearing on mulberry leaves only (20- 25°C).
In regards to the relative humidity, it can be seen from Table 1 that the required interval is about 50 - 85%. Lowering this parameter is not recommended as it can cause at least drying of the diet. Only daylight is applied during the incubation (no artificial light) and thus 12 hours light and 12-hours dark rhythm is established.
The "mass" hatching is observed on the 12th day after the incubation has started 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.
The following main technological characters were studied: fresh cocoon weight; cocoon shell weight; filament length and shell ratio.

For determining the fresh cocoon weight and shell weight, the following approaches were used: (1) all good quality cocoons/shells from the replicate were weighted and after that divided by their number; (2) a random sample consisting of 30 female and 30 male good quality cocoons/shells was taken and after weighting the weight is divided by the number. For shell percentage determination, the ratio between the weight of cocoon shell and the weight of fresh cocoon was used. The filament length was determined on a random sample of 30 good quality cocoons after single cocoon reeling test.

RESULTS AND DISCUSSIONS

The fresh cocoon weight has a direct effect on the yield of the cocoon and also on the filament length.
The results in Table 2 show that we have the highest values for the references of both strains in September 2017 and 2018.
The variation of the characters in silkworms fed on artificial diet ranges from 0.55 to 1.52. The lowest average values of the character were observed in July of 2017 in both the Svila 1 and Svila 2.
The highest values were recorded in September in both years 2017 and 2018 for the both strains that have been fed on artificial diet, probably due to the favourable climatic conditions.

Table 2. Fresh cocoon weight (mg)

Strains	Months	2017			2018		
		$\bar{x}$	*Sx*	*Vc*	$\bar{x}$	*Sx*	*Vc*
Svila 1	July	1743.33	28.57	2.32	1810	7.07	0.55
	July (reference)	1806.66	14.72	1.15	1870	7.07	0.53
	August	1763.33	10.81	0.86	1830	7.07	0.54
	August (reference)	1843.33	24.83	1.90	1836.66	22.73	1.75
	September	1810	7.07	0.55	1830	7.07	0.54
	September (reference)	1840	7.07	0.54	1870	7.07	0.53
Svila 2	July	1743.33	28.57	2.32	1790	7.07	0.55
	July (reference)	1800	7.07	0.55	1820	18.70	1.45
	August	1740	18.70	1.52	1843.33	14.72	1.12
	August (reference)	1803.33	10.81	0.84	1833.33	4.08	0.30
	September	1833.33	14.72	1.13	1850	7.07	0.54
	September (reference)	1870	7.07	0.53	1890	7.07	0.52

When observing the results of for Svila 1 strain in July, it can be seen that the values in both years had been decreased for larvae fed on artificial diet compared to the values of larvae fed entirely with mulberry leaf. The decrease compared to the reference is in the range of 63.33 mg in 2017 and 60 mg in 2018. The same trend is observed in the Svila 2 strain where we report a difference of 56.67 mg in 2017 compared to the reference and 30 mg in 2018 to reference.

Comparing the results from July and August of larvae fed on artificial food, we can see a decrease in the values of the cocoon weight at all. An increase in the values of the same character of the reference is observed in September. As a possible cause we consider the more favorable climatic conditions, since the summer months in our country exceed 30℃, which impedes the silkworms rearing and it leads to cocoons with lower weight. In September, the maximum temperature in reaches 25℃, which is the optimum temperature for silkworms rearing.

In regard to the cocoon shell weight (Table 3), it can be seen that the variation of the values is from 1.30 to 5.63%. We have a large variation within 5.63 % for Svila 1 reference values in September.

The highest average values of the character were observed in September and the lowest in August 2017 for the Svila 2 with a value of 410 mg. The values are slightly higher in July and August of 2018 again for Svila 2 - within 420 mg. In 2018, the highest reported mean values are observed for Svila 1 and the lowest - for Svila 2.

In regards to the results from July 2018, we see an increase in values compared to 2017, being higher with 28.34 mg. The results of the reference during same months retains relatively constant for both surveyed years. The lowest average values for the whole rearing period for larvae fed on artificial diet were observed in August (low values were noticed for both strains). In September there is already an increase in values, but at the same time we do not consider as significant the difference between individuals fed on artificial diet and those fed on mulberry leaves.

Table 3. Cocoon shell weight (mg)

Strains	Months	2017			2018		
		$\bar{x}$	*Sx*	*Vc*	$\bar{x}$	*Sx*	*Vc*
Svila 1	July	431.66	7.36	2.41	460	14.14	4.35
	July (reference)	460	7.07	2.17	466.66	14.71	4.46
	August	440	7.07	2.27	430	7.07	2.32
	August (reference)	453.33	4.08	1.27	460	7.07	2.17
	September	466.66	10.80	3.27	460	7.07	2.17
	September (reference)	470	18.70	5.63	460	7.07	2.17
Svila 2	July	443.33	10.80	3.44	420	14.14	4.76
	July (reference)	460	7.07	2.17	426.66	4.82	1.35
	August	410	7.07	2.44	420	7.07	2.38
	August (reference)	456.66	10.80	3.34	440	7.07	2.27
	September	476.66	10.80	3.20	446.66	4.08	1.30
	September (reference)	476.66	10.80	3.20	460	7.07	2.17

Table 4 shows the results of one of the most important technological characters - the filament length. The average filament length varies from 823.33 in Svila 1 in 2017 to 1016.66 m in the Svila 2 (reference values) in September 2018. The variation of this character is in the range of 1.05 to 2.90. The table clearly shows an increase in the reference values of the character in September 2018 for the Svila 2 and a decrease in the values in August for both tested strains in both years.

Table 4. Filament length (m)

Strains	Months	2017			2018		
		$\bar{x}$	*Sx*	*Vc*	$\bar{x}$	*Sx*	*Vc*
Svila 1	July	903.33	10.80	1.69	906.66	10.80	1.68
	July (reference)	930	18.70	2.84	940	7.07	1.06
	August	826.66	17.80	3.04	890	7.07	1.12
	August (reference)	910	7.07	1.10	923.33	10.80	1.65
	September	823.33	10.80	1.85	920	7.07	1.08
	September (reference)	886.66	8.16	1.30	976.66	10.81	1.56
Svila 2	July	883.33	10.80	1.73	900	7.07	1.11
	July (reference)	920	7.07	1.08	950	7.07	1.05
	August	910	18.70	2.90	980	7.07	1.02
	August (reference)	926.66	10.81	1.65	1010	7.07	10.80
	September	930	7.07	1.07	980	7.07	1.02
	September (reference)	976.66	10.80	15.6	1016.66	10.80	1.5

In regards to this technical character, as well as with the other technological characters that have been studied, the lowest values are observed during the summer months.

In September 2018 we registered the highest value for larvae fed on artificial diet, having in September 2018 filament length of 920 m (the same being 823.33 m in 2017). The difference comprises 96.67 m more in 2018, but despite the increase that has been observed, these values remain lower compared to the reference results for the same month and year. The difference between the reference and the test variant is 56.66 m in favor of the former.

The same trend is observed in Svila 2 where we have higher values in September for both years of rearing. In September 2018, the highest value of filament length is reported for Svila 2 - 1016.66 m for larvae fed on artificial diet. The difference between the lowest (833.3 m in July 2017) and highest (980 m in August and September 2018) values is within 146.67 m, which is slightly different than the observed values for the reference, where the difference is 96.66 m.

When considering the shell percentage (Table 5), we observed a gradual increase of the average values from July to August in 2017.

The variation in the same year ranges from 0.93 in August for Svila 1 (reference) to 3.75 for Svila 2 in August for the larvae fed on artificial diet. As with the other characters that have been analysed, the highest values were observed in September and the lowest values were registered in August.

Table 5. Shell percentage (%)

Strains	Months	2017			2018		
		$\bar{x}$	*Sx*	*Vc*	$\bar{x}$	*Sx*	*Vc*
Svila 1	July	24.77	0.56	3.21	25.41	0.68	3.79
	July (reference)	25.46	0.48	2.70	24.95	0.77	4.36
	August	24.96	0.51	2.9	23.49	0.29	1.77
	August (reference)	24.59	0.16	0.93	25.04	0.45	2.57
	September	25.78	0.64	3.50	25.13	0.29	1.62
	September (reference)	25.54	1.10	6.13	24.60	0.43	2.49
Svila 2	July	25.43	0.56	3.11	23.46	0.74	4.50
	July (reference)	25.55	0.29	1.61	23.44	0.46	2.77
	August	23.57	0.62	3.75	22.78	3.82	2.37
	August (reference)	25.32	0.46	2.58	23.36	0.41	2.54
	September	25.99	0.4	2.34	24.14	0.24	1.40
	September (reference)	25.49	0.64	3.56	24.33	0.28	1.64

CONCLUSIONS

Based on the results that have been obtained and the analysis that has been made therefore, the following conclusion can be drawn: rearing of silkworms on artificial diet in their young instars leads to a slight decrease in the values of the considered technological characters and provides a successful and economically viable cocoon crop.

Hence, as a summary, I recommend rearing of silkworms during their first three instars to be carried out on artificial diet in the summer months, in order to increase the competitive power of cocoon farmers and to beneficiate the silk entrepreneurs/ industries.

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VARIABILITY AND EVALUATION OF THE GROWTH PERFORMANCE OF THE KIDS GOATS IN THE ARGANERAIE OF AGADIR IN MOROCCO

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Abstract

One of the main sources of income for the rural argan grove population in the Agadir region is goat farming, and it is almost exclusively geared towards meat production. The goat herd is often led extensively where the rangelands are the main source of food. The objective of this study is to assess the variability in growth performance of kids of the main local breeds. Increasing the production of this herd requires improving the technical level of farms and the selection of individuals with good growth performance. The evaluation of the variability of growths was analyzed on 341 kids (167 males and 183 females). The data presented are adjusted for the factors of variation: litter size, sex of the kid, season of birth, age of the mother, birthing rank and breed of the mother. The birth weights, 10 days, 30 days and at 70 days are respectively 1.98 ± 0.32 kg, 2.61 ± 0.47 kg, 3.75 ± 0.74 kg and 5.45 ± 1.26 kg. The average daily gains (ADG 0-30 d) and (ADG 30-70 d) are 63.38 ± 6.68g and 43.99 ± 4.26g respectively. The local goat population studied is characterized by great variability for the measured growth parameters. The results of the Anova (GLM model) revealed differences between the different local breeds and revealed the influence of certain factors of variation analyzed on the growth of kids. Better control of the technical management of farms and selection of the best brood stock are necessary to have a better productivity.

Key words*: goats, local breeds, growth performance, variability, Arganeraie (argan forest).*

INTRODUCTION

Goat herds account for just over 1 billion goats worldwide, of which about 420 million head (40.9%) are raised in Africa. Morocco's goat herd currently accounts for 5.23 million head (FAO, 2017) is composed mainly of hardy local breeds which are characterized by a good adaptation to local climatic conditions and it is mostly concentrated in difficult and mountainous areas (Benlekhal and Tazi, 1996).
Goat farming is a key sector of agriculture and its versatile function, is of socio-economic importance and plays a dynamic role in the development of economic activity in rural areas. Knowing the growth dynamics of young animals may be used as one of the indicators to evaluate the level of adaptation under conditions of a given production system (Kume and Hajno, 2010).
The livestock system is extensive, traditional and oriented exclusively for the production of meat. The goat herd is heterogeneous and composed mainly of local breeds, with the dominance of the Atlas (Black) and Barcha breeds which represent approximately 80% of all goats (El Kheyyat and El Madidi, 2020). The main objective of this study is the analysis of the variability of the growth characteristics for the kids of the local goat breeds in a context of extensive system farming in the region of Agadir in Morocco.

MATERIALS AND METHODS

The study was conducted in Amskroud commune in the province of Agadir (Figure 1) with 35 goat farmers randomly selected in 9 villages.
For the monitoring of the animals of each farm, the goats were identified by ear tags with a number specific to each animal.
Live weight at birth (0), 30 and 70 days of kidding and parity of doe were recorded under the existing management conditions by recruited enumerators.
Birth date, birth weight, gemellarity and sex of kid were recorded within 24 h of the new birth.

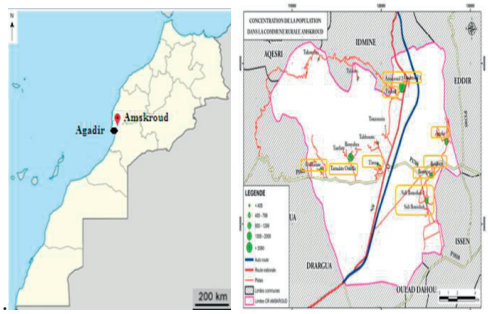

Figure 1. Map showing location of the study area (left) and location of villages sampled (right)

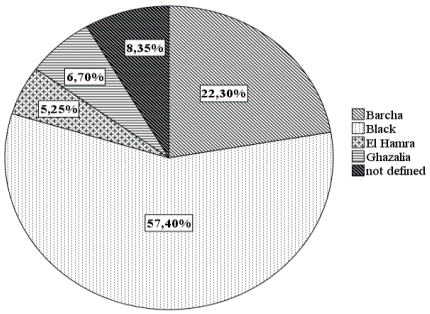

Figure 2. The percentages of the different goat breeds in the study area

Kids were weighed using Brecknell 235 10S Hanging Scale having 50 kg capacity and 200 g division within 24 h after birth. Growth traits of 341 kids were classified according to sexes and seasons. All the kiddings recorded during the period were classified into four seasons (winter, autumn, spring and summer). The births recorded were also classified according to their sexes.

The used GLM model of the following:

Yijk = m + Ai + Bj + Ck + eijk. Where the symbols of this model are: Yijk: the measured values of Breeds (i), season of kidding (j) and sexe (k) , m: Mean of total observed values, Ai: Effect of Breeds (i = 4), Bj: Effect of season of kidding (j = winter, spring, summer and autumn), Ck: Effect of sexe (males and females) and eijk: the residual error.

All statistical analyzes were performed using SAS version 9.3 software. The direct effects of the different factors on the weights at different ages were obtained by an ANOVA analysis using the PROC GLM.

RESULTS AND DISCUSSIONS

The goat population of the commune is composed mainly of 3 local breeds: Atlas (Black), Barcha, and Ghazalia with respectively 57.4, 22.3 and 6.70% (Figure 2).

The weight at birth, weights at 10 days, weights at 30 days and weights at 70 days varied between 1.43 to 2.76, 1.4 to 3.88, 2.05 to 5.41 and 2.87 to 9.55 kg, respectively. The average daily gains, ADG 1 (0-30) and ADG 2 (30-70) varied between 20.8 to 108.97 and 21.3 to 128.72g respectively (Table 1).

For weights at typical age, the coefficient of variation (CV) estimates ranged from 16.21% to 23.17% whereas for the average daily gain, we observed that the coefficient of variation are higher and are equal to 41.90 and 48.36 % for ADG 1 (0-30) and ADG 2 (30-70) respectively. Relatively low weights of kids are often obtained among local populations in extensive pastoral conditions, this is linked to the difficult breeding conditions and the availability of fodder (Alexandre and Mandonnet, 2005; Dereje et al., 2015; Tolera et al, 2000).

Under arid harsh conditions, environmental factors act on herd production by the bias of the resources variation and the climatic stresses intensity (Sghaier et al, 2007).

Table 1. Descriptive statistics for the measured characters (growth traits)

Parameters	N	Mean	Min	Max	SD	CV (%)
BW (Kg)	341	1.98	1.43	2.76	0.32	16.21
W10 (Kg)	341	2.61	1.4	3.88	0.47	17.95
W30(Kg)	331	3.75	2.05	5.41	0.74	19.81
W70(Kg)	318	5.45	2.87	9.55	1.26	23.17
ADG1 (g)	331	63.38	20.8	108.97	6.68	41.90
ADG2 (g)	318	43.99	21.3	128.72	4.26	48.36

ADG: Average Daily Gains, BW: birthweight, W30: weight at 30 days, Min: Minimum, Max :Maximum, SD: Standard deviation, CV: Coefficient of variation. ADG 1: (0-30 days) and ADG 2: (30-70 days).

Influence of breeds on the growth traits

Between breeds, the analysis of variance has showed a significant effect for W10 d, W30 d and highly significant effect for ADG (0-30) and ADG (30-70) (Table 2).

Table 2. Results of ANOVA (GLM)

Factors	ddl	WB	W30	W70	ADG1	ADG2
Breeds	3	0.78	3.16 *	1.16	4.58 **	3.98 **
KS	3	6.48 ***	6.51 ***	8.25 ***	5.39 **	7.46 ***
Sexe	1	3.51 *	4.69 *	2.95	2.70	0.65
Error	333					
Total	340					

WB: birth weight, W10: weights at 10 days, ADG:Average Daily Gains, KS:kidding Season. *, **, *** Significant at 0.05, 0.01 and 0.001 levels, respectively.

Table 3. Influence of breeds on the growth traits (Mean ± SD)

Breeds	N	WB (kg)	W70 (kg)	ADG(0-30)(g)
1 Black	74	1.92 ± 0.32 a	5.22 ± 1.27 a	61.47 ± 4.22 a
2 Barcha	30	1.93 ± 0.33 a	5.44 ± 1.38 a	59.48 ± 7.76 a
3 Ghazalia	38	1.97 ± 0.29 a	5.82 ± 1.35 a	69.02 ± 7.15 ab
4 Cross Pop	199	2.01 ± 0.30 a	5.47 ± 1.20 a	76.57 ± 7.82 b

Means followed by the same letter (s) are not significantly different at 5 % level of probability.

Figure 3 and Table 3 show that for birth weights the different breeds have almost similar mean values. For the weight at 70 days, we observe differences between the breeds but which remain statistically insignificant with average values which vary between 5.22 kg for Barcha and 5.82 kg for Ghazalia.

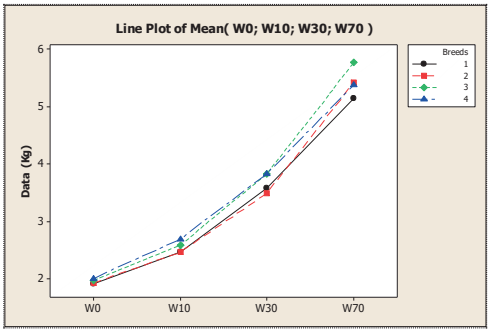

Figure 3. Growth trends of different goat breeds

For the average daily gains (ADG), we observed more pronounced and significant differences between breeds. For ADG1 (0-30), the lowest value is observed in the black breed (61.47 ± 4.22) while the highest value is recorded in the cross-population breed (Table 3 and Figure 4).

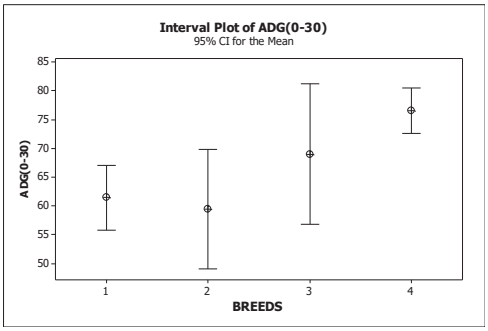

Figure 4. Effect of the breed on ADG (0-30)

Influence of the birth sex

Table 2, Table 4 and Figure 3 shows that the sexe of kids had significant influence on the BW, W10 and W30, However, kids's sexe had no significant effect on the W 70, ADG (0-30) and ADG (30-70).

The sex of the kids has a significant influence effect for WB, W10 and W30. The standard weights of males were significantly higher than those of females for all the growth traits measured (Figure 6). For birth weight, the average weights recorded are 2.03 kg in males and 1.94 kg in females (Figure 5).

For weight at 70 days, the average values recorded in males and females are 5.53 and 5.39 kg respectively.

These results are in agreement with those of several authors who have reported that the weights at typical ages of male kids goats are greater than the weights of female kids goats (Hagan et al., 2012; Meza-Herrera et al., 2014; Zahraddeen, 2008).

Table 4. Influence of the sex on the growth traits (Mean ± SD)

Sex	N	WB (kg)	W70 (kg)	ADG(30-70)(g)
1. Males	162	2.03 ± 0.90 a	5.53 ± 1.19 a	73.53 ±6.72 a
2. Females	179	2.04 ± 0.93 b	5.39 ± 1.30 b	68.61 ± 3.85 a

Means followed by the same letter (s) are not significantly different at 5 % level of probability.

The kidding season shows a highly significant effect ($P<0.01$) for all the growth parameters analysed (Table 2).

Table 5 presents the results of the influence of the season. This table shows that the birth season had significant influence on the WB, W70 and ADG (30-70). For the all growth parameters,

kids born in the rainy and mild season had higher growth than those born in the dry and hot season.

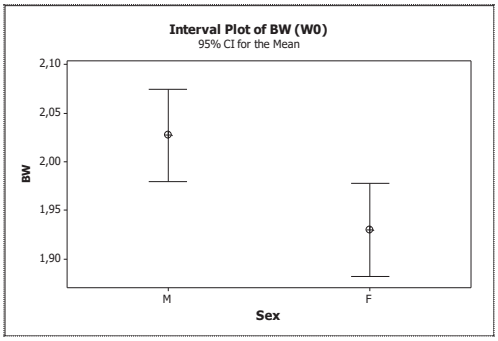

Figure 5. Effect of the birth season on birth weight

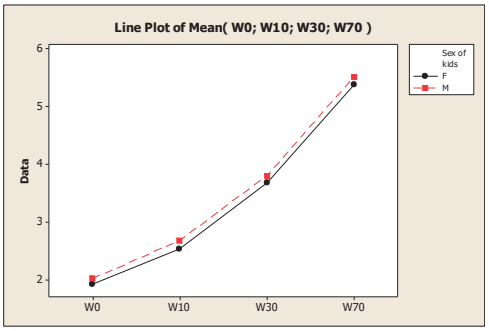

Figure 6. Growth trends by sex of kids

Influence of the birth season

For birth weight, the highest value is that recorded in winter (2.24 ± 0.33 kg) and the lowest is observed in summer (1.79 ± 0.30 kg) (Figure 7).
For weight at 70 days, the highest and lowest values are recorded in autumn (5.83 ± 1.24 kg) and summer respectively (4.87 ± 0.56 kg).

Table 5. Influence of the season on the growth traits (Mean ± SD)

Seasons	N	WB (kg)	W70 (kg)	ADG (30-70)(g)
1. Autumn	108	2.16 ± 0.30 a	5.83 ± 1.24 a	49.26 ± 5.14 a
2. Winter	157	2.24 ± 0.33 a	5.43 ± 1.33 a	44.16 ± 3.48 ab
3. Spring	45	1.98 ± 0.29 a	4.90 ± 1.08 b	36.62 ± 6.27 c
4. Summer	31	1.79 ± 0.30 b	4.87 ± 0.56 b	40.31 ± 3.89 bc

Means followed by the same letter (s) are not significantly different at 5 % level of probability.

The significant effect of season on growth trays of kids is reported in the literature (Ahuya et al., 2009; Elabid, 2008; Supakorn, and Pralomkarn, 2009). In a context where animal feed is based almost entirely on theextensive grazing, the strong seasonal variations in the availability of fodder act positively or negatively on growth parameters.

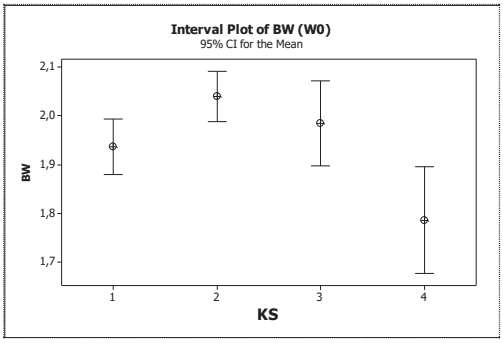

Figure 7. Effect of the birth season on birth weight

However the cold and rainy season, autumn and winter, are the favorable seasons for the WB. However the hot and dried seasons, spring and summer, have disadvantaged the production. This shows production is highly sensitive to the seasonal variations. Many researchers have stated that the season has a significant impact on growth traits (Al-Shorepy et al., 2002; Caro Petrović et al., 2012; Zhang et al., 2009).

CONCLUSIONS

The values recorded in this study are relatively low compared to those observed in other breeds and under more favorable conditions. Environmental effects estimated in this study are important and need to be taken into account for local goat management and breeding improvement. The improvement of technical farming conditions and the selection of more efficient individuals for growth traits are essential for improving productivity in this region.

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GROWTH PERFORMANCE OF SIMPLE- AND TRIRACIALI CROSSBRED LAMBS AND EWES PRODUCTIVITY OF R1 GERMAN BLACKFACE X TSIGAI CROSSED WITH FRENCH MEAT BREED

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Abstract

Crossbreeding of three French meat breeds rams and one R1 German Blackface (75%) x Tsigai (25%) ram with R1 crossbreed ewes German Blackface (75%) x Tsigai (25%) was carried out to determine the ram breed effects on productivity of ewes and growth performances of the lambs. A total of 151 crossbreed ewes R1 German Blackface x Tsigai were divided into four groups for mating with 4 rams from Berrichon du Cher (BC) - group 1, Blanche du Massif Central (BMC) - group 2, Mouton Vendèen (MV) - group 3, and R1 German Blackface (BF) - group 4, resulting in total 147 lambs. Ram breed had a significant influence on growth performances of the lambs. The lambs obtained from the R1 German Blackface ram were heavier than the lambs from groups 1 and 3 (P< 0.001) at birth, 28 day and 56 day. Significant differences were recorded between group 2 and 4 with regard at weight at 28 day (P< 0.01) and 56 day (P< 0.05). Body weight at 5 months in group 1 of crossbred lambs was significantly higher than to the crossbreed lambs obtained from the group 2 (P< 0.01), 3 (P< 0.05) and 4 (P< 0.01). The number of lambs born per ewe, number of lambs weaned per ewe, and lamb survival rate at weaning were similar among the four groups, while the lambs survival rate at 5 months at the lambs obtained from ewes mated to Blanche du Massif Central ram was higher compared to the others, the differences being significant (P< 0.05) compared to the lambs obtained from ewes mated to Mouton Vendèen ram.

Key words*: productivity, Berrichon du Cher, Blanche du Massif Central, Mouton Vendèen, German Blackface, Tsigai*

INTRODUCTION

The Tsigai sheep is the second most important sheep breed in Romania with 24.3% of the national sheep herds (the first is the Tsurcana sheep with 52.4%). Tsigai is a traditional triple-purpose sheep group, widely distributed across regions of Central, Eastern and Southern Europe (Cinkulov et al., 2008). Production levels vary greatly among countries and regions which rear Tsigai sheep (Kusza et al., 2008). Because the Tsigai race is a rustic breed, research conducted in Romania over time attempted to improve milk and meat production, most work was based on the use of industrial crossings with specialized imported breeds.

To improve meat production, the research was focused on increasing prolificacy, improving skills for meat production and carcass quality. Imported specialized breeds to improve meat production were: Suffolk, Ile de France, Merinofleisch, German Blackface. The obtained results were in all cases higher than those obtained from Tsigai breed (Ilișiu et al., 2010), but under the potential of improved breed (for lamb).

Recently, the Berrichon du Cher, Blanc du Massif Central and Mouton Vendeèn was introduced in Romania and crossed with Tsurcana and Tsigai breeds. The results have shown that the performances of lambs obtained from crossbreeding were higher than the lambs of local breeds (Borzan et al., 2017; Frujină et al., 2019). Blanc du Massif Central are kept in France in areas with harsh environmental conditions, and both robustness and good maternal abilities are their main characteristics. The Berrichon du Cher are kept in more favourable areas, are specialised in growth and

carcass traits and are widely used for terminal crossing (Hubby et al., 2003). The Mouton Vendeèn is well-adapted to temperature variations, as well as to alternating periods of drought and heavy rainfall. The meat production traits of the breed have gained a good reputation, resulting from good growth potential and excellent conformation. For these reasons, rams are often used for crossbreeding, which has extended the Vendéen's popularity towards the Limousin, central France, the Midi-Pyrénées, etc. as well as internationally (http://en.france-genetique-elevage.org). At the present there is little information to highlight the growth performance of the lambs obtained by crossing of the above mentioned three races with purebred or crossbreed ewes from other countries.

Within of some research projects funded by the Ministry of Agriculture and Rural Development of Romania, at the Research and Development Station for Sheep and Goats Reghin (the current Experimental Base Reghin of the Research Institute for Sheep and Goats of Palas Constanța), were achieving crossings between German Blackface x Tsigai breed – rusty variety in order to make a precursors for a meat sheep breed adapted to the submontane area. So far, has been obtained R1 crossbreed ewes (75% German blackface; 25% Tsigai – rusty variety). In order to choose the correct breed to continue the work to forming the sheep population above mentioned, it was decided that in 2018, the female R1 German Blakface x Tsigai to be crossed with males from french meat breed: Berrichon du Cher, Blanc du Massif Central and Mouton Vendeen.

The compatibility of breed crossing is essential for achieving high lambs performance. Knowledge of the occurrence rates of various health disorders is important for both veterinarians and researchers, in order to set-out, alarm thresholds', which will then help sheep breeders to monitor flock's health and in the decision making process (Gavojdian, 2015).

The objective of the current study was to evaluate the reproductive performance of R1 German Blackface (75%) x Tsigai (25%) ewes when mated with French meat breeds, and growth performances of crossbreed lambs reared intensively under Romanian conditions.

MATERIALS AND METHODS

The present research was conducted in Experimental Base Reghin of Research Institute for Sheep and Goat Palas Constanta, Mures 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).

Animals and management

A total 151 simple crossbreed ewes R1 German Blackface (75%BF) x Tsigai (25%TI) were divided into four groups and mated with three french meat breed rams and one R1 German Blackface x Tsigai (75% BF x 25% Tsigai) ram in 2018, as follow: groups 1 (41 ewes) Berrichon du Cher x German Blackface x Tsigai (BCxBFxTI), group 2 (31 ewes) Blanche du Massif Central x German Blackface x Tsigai (BMCxBFxTI), group 3 (40 ewes) Mouton Vendèen x German Blackface x Tsigai (MVxBFxTI). The control group (4) were formed of 39 R1 German Blackface x Tsigai (BFxTI) ewes. At the beginning of the experiment, all ewes and rams were kept in the shelter in the mating period (01.09.2018-20.10.2018) and feeded with hill heu (1 kg/head), maize (0.4 kg/head) and barley grain (0.2 kg/ewe). In the following period (October – November), the ewes grazed on natural pastures and were provided 0.6 kg barley grain per ewe. In winter (beginning of December until 13. May), the animals were held in the shelter and provided 0.4 kg maize, 0.1 kg sunflower groats and 2 kg hill heu per ewe.

A well recognized annual health program was carried out for all groups. Animals were treated with antiparasite drugs to control internal and external parasites. Lambs were born from January through March. 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 months age. Ewes and their lambs were kept together under the same management condition. Lambs were weaned at approximately 98 ± 9 days of age and kept under range conditions for two months. The diet was formulated for 300 g/head/day growth potential according to NRC (1985) requirements and digestible protein was 130 g. Flocks structure

and genotype used in the study are presented in Table 1.

Table 1. Number of lambs by gender and genotype

Gender	Genotype			
	BCxBFxTI[a]	BMCxBFxTI[b]	MVxBFxTI[c]	BFxTI[d]
Male	20	17	12	17
Female	18	14	27	22
Total	38	31	39	39

[a] Sire = Berrichon du Cher, Dam = German Blackface x Tsigai; [b] Sire = Blanche du Massif Central, Dam = German Blackface x Tsigai; [c] Sire = Mouton Vendèen, Dam = Blackface x Tsigai, [d] Sire = German Blackface x Tsigai, Dam= German Blackface x Tsigai.

The diet of lambs consisted of lucerne hay and concentrated fodder. The structure of concentrated fodder was as followed: 30% granulated fodder for lambs (16% CP), 30% maize, 15% barley, 12% wheat bran, 12% sunflower groats and 1% calcium. The fodder, water and salt were provided at discretion.

Traits definition

The traits investigated were classified as lamb and ewe traits. Early growth traits consisted of birth weight (BW); weight at 28 day (W28); weight at 56 day (W56); weaning weight (WW) (98.30 ± 8.66 days of age), post weaning weights at 4 months (W4M) and post weaning weights at 5 months (W5M).

Reproductive traits of R1German Blackface x Tsigai ewes included the prolificacy, fecundity number of weaned lambs per ewe mated (NWLEM); number of weaned lambs per ewe lambed (NWLEL); lamb survival rate at weaning*;* lamb survival rate at 5 months.

Statistical analysis

The mean comparisons between the four groups of the variables were carried out using independent samples t-test of the JASP procedure.

RESULTS AND DISCUSSIONS

Early growth traits

Least-squares means (±S.E.) for early growth traits of lambs by crossbreeds are presented in Table 2.

According to the traits considered, some variations were observed. The rams breed had significant influence on body weight at birth, 28 day, 56 day, at weaning, at 4 and 5 month. BW, W28 and W56 of group 1 and 3 were significantly higher (P<0.001) than of group 4. Also, significant differences were recorded in BW between the groups 1 (P< 0.05) and 2 (P< 0.001) when compared with group 3, as well as between group 1 and 2 (P<0.05).

Table 2. Least-squares mean (S.E.) for growth traits of crossbreed lambs (kg)

Trait	Genotype	N	Mean (kg)	Min. (kg)	Max. (kg)
BW	BFxTI	39	3.86 ± 0.10^{AC}	2.5	5.4
	BCxBFxTI	38	4.71 ± 0.18^{bcD}	2.3	7.6
	BMCxBFxTI	31	4.07 ± 0.16^{acD}	2.2	5.9
	MVxBFxTI	39	5.15 ± 0.13^{aBD}	4.0	7.2
W28	BFxTI	35	9.27 ± 0.56^{AbC}	4.5	15.1
	BCxBFxTI	38	13.09 ± 0.6^{BD}	4.4	20.4
	BMCxBFxTI	28	10.99 ± 0.38^{ad}	7.2	14.5
	MVxBFxTI	34	11.85 ± 0.27^{d}	8.8	15.6
W56	BFxTI	35	14.64 ± 0.75^{ABC}	6.0	25.0
	BCxBFxTI	37	19.97 ± 0.78^{AD}	7.1	30.0
	BMCxBFxTI	28	18.20 ± 0.60^{BD}	12.8	25.9
	MVxBFxTI	34	19.95 ± 0.51^{aD}	13.1	25.4
WW	BFxTI	35	27.10 ± 1.23^{abc}	15.1	42.6
	BCxBFxTI	35	28.95 ± 1.05^{b}	15.5	43.2
	BMCxBFxTI	28	25.88 ± 0.87^{a}	16.1	37.5
	MVxBFxTI	34	27.16 ± 0.98^{abc}	16.4	41.0
W4M	BFxTI	35	33.58 ± 1.43^{abc}	19.7	49.0
	BCxBFxTI	34	36.48 ± 1.13^{B}	26.1	47.7
	BMCxBFxTI	28	33.66 ± 1.20^{A}	21.6	46.3
	MVxBFxTI	33	33.56 ± 1.44^{abc}	17.8	48.5
W5M	BFxTI	34	39.69 ± 1.65^{AD}	22.0	59.0
	BCxBFxTI	32	45.56 ± 1.33^{BCD}	32.0	61.2
	BMCxBFxTI	28	39.52 ± 1.34^{AB}	21.6	54.5
	MVxBFxTI	31	$40.86 \pm 1.35^{a\,c}$	25.0	59.4

Means with different superscripts (^{a}, ^{b}, ^{c}, ^{d}) in each traits differ (P< 0.05).

Means with different superscripts (^{A}, ^{B}, ^{C}, ^{D}) in each traits differ (P< 0.01 and P< 0.001).

In W28, were significant differences recorded between group 1 and 2 (P<0.01), as well as between group 2 and 4 (P<0.05). Daily average gain is the primary and important indicator of selection (Frujină et al., 2019). At weaning (WW), only between groups 1 and 2 were the differences significant (P<0.05). In W4M the differences recorded were significant (P<0.01) between the groups 1 and 2. In W5M were recorded significant differences (P<0.01) between group 1 and the groups 2 and 4, as well as between the group 1 and 3 (P<0.05).

With regard at average daily gain, the BCxBFxTI crossbreed lambs had the highest ADG pre- and post-weaning (table 3), while the BFxTI lambs had the lowest ADG. It is observed the highest ADG to group 1 in the first month of birth, while in the second month of life the highest ADG were recorded to group 3, followed by group 2.

Significant differences (P<0.001) with regard at ADG in the first month of life were recorded

between the groups 1 and 3 and 1 and 4, as well as between the groups 1 and 2 (P<0.01).

Table 3. Least-squares mean (S.E) for average daily gain (ADG) of crossbreed lambs (g)

Trait	Genotype	N	Mean (kg)	Min. (kg)	Max. (kg)
ADG 0-28 days	BFxTI	35	195.00±20.28^{Abc}	50	409
	BCxBFxTI	37	307.54±18.07^{BCD}	79	554
	BMCxBFxTI	28	245.79±12.92 ^{ACD}	130	409
	MVxBFxTI	34	237.18± 7.25^{Ad}	154	329
ADG 28-56 days	BFxTI	35	191.87±15.46^{abc}	35	413
	BCxBFxTI	37	237.03±11.23^{Cd}	18	461
	BMCxBFxTI	27	258.04±11.21^{D}	139	406
	MVxBFxTI	34	289.29±14.03^{aD}	29	450
ADG birth – weaning	BFxTI	35	221.17± 9.32^{a}	104	350
	BCxBFxTI	35	253.66± 9.57^{d}	152	383
	BMCxBFxTI	28	226.00± 7.92^{ab}	151	322
	MVxBFxTI	34	225.74± 7.88^{ac}	133	339
ADG birth – 4 month	BFxTI	35	219.74± 8.75^{A}	112	321
	BCxBFxTI	34	251.88± 8.27^{bcD}	167	358
	BMCxBFxTI	28	223.32± 7.46^{ab}	159	320
	MVxBFxTI	33	224.09± 7.08^{ac}	94	319
ADG birth – 5 month	BFxTI	34	218.29± 8.65^{A}	107	328
	BCxBFxTI	32	263.72± 8.24^{BCD}	177	350
	BMCxBFxTI	28	227.75± 7.84^{AD}	128	310
	MVxBFxTI	31	228.94± 7.12^{AC}	148	325

Means with different superscripts (^{a}, ^{b}, ^{c}, ^{d}) in each traits differ (P< 0.05).
Means with different superscripts (^{A}, ^{B}, ^{C}, ^{D}) in each traits differ (P< 0.01 and P< 0.001).

Between the groups 2 and 4, as well as between 3 and 4, the differences founded were significant (P< 0.05). In the second month of life (28-56 day) were recorded significant differences (P<0.001) between group 4 and the groups 2 and 3, as well as between group 1 and 4 (P<0.05). Also, significant differences (P<0.05) were found between group 1 and 3. Pre-weaning, the differences recorded with regard at ADG were significant (P<0.05) between group 1 and another groups. Post-weaning was found significant differences (P<0.001) between group 1 and 4, as well as between the group 1 and the other two groups (P<0.01). From the data of the table 3, it can be observed the high variability of the daily average gain, the highest variability being recorded in the first 2 months of life, and inside of the group 1.

Reproductive Traits

The ram breed effect was not significant (P>0.05) for fecundity, prolificacy, NWLEM NWLEL and LSRW, expect LSR5M (P<0.05) (Table 4). The lambs obtained from ewes mated to Blanche du Massif Central ram had higher lamb survival rate at 5 months compared to the others, the differences being significant (P< 0.05) compared to the lambs obtained from ewes mated to Mouton Vendèen and not significant (P>0.05) compared to the others.

Table 4. Least-squares mean (S.E.) for reproductive traits of German Blackface x Tsigai ewes

Trait	Genotype				
	BCxBFxTI	BMCxBFxTI	MVxBFxTI	BFxTI	t-test
Fecundity	82.93 ± 5.95^{a}	83.87 ± 6.72^{a}	87.50 ± 5.30^{a}	84.62 ± 5.85^{a}	N.S
Prolificacy	114.71 ± 7.47^{a}	119.23 ± 7.88^{a}	108.57 ± 4.80^{a}	118.18 ± 6.82^{a}	N.S.
NWLEM ^{a}	0.90 ± 0.10^{a}	0.90 ± 0.10^{a}	0.83 ± 0.07^{a}	0.90 ± 0.10^{a}	N.S.
NWLEL^{b}	1.09 ± 0.09^{a}	1.08 ± 0.08^{a}	0.94 ± 0.06^{a}	1.06 ± 0.09^{a}	N.S.
LSRW ^{c}	108.82 ± 8.82^{a}	107.69 ± 7.69^{a}	94.29 ± 5.71^{a}	106.06 ± 8.64^{a}	N.S.
LSR5M ^{d}	97.06 ± 9.89^{a}	107.69 ± 7.69^{b}	85.71 ± 7.27^{b}	106.06 ± 8.64^{a}	*

^{a} Number of weaned lambs per ewe mated; ^{b}Number of weaned lambs per ewe lambed; ^{c}Lamb survival rate at weaning; ^{d}Lamb survival rate at 5 month; N. S. *P*> 0.05 and * *P*< 0.05, respectively. Means with different superscripts in each row differ (P< 0.05).

Discussions

As it can be seen from Table 2, the lambs obtained in the group 3 had an average weight at birth higher than the other three groups, the minimum weight recorded being 4.0 kg and the maximum weight 7.2 kg. However, the maximum birth weight of 7.6 kg was recorded in the group 1. The higher birth weight in the 1 groups has led to the occurrence of dystocia, so that in group 1, there were recorded 2 cases of dystocia, and in the case of group 3 was registered one case of dystocia.

BCxBFxTI lambs exceeded other crossbred lambs in early growth rate (up to 5 months) under intensive fattening system. We mention that the average daily gain was superior at BCxBFxTI during the 5 months, except for the second month of life, when was inferior to BMCxBFxTI and MVxBFxTI. In contrast with the groups 2 and 4, where the lambs had similar average daily gain in the first and second month of life, by groups 1 is recorded superior daily gain in the first month after birth, and by group 3, in the second month after birth. After weaning, except for the group

1, the evolution of the average daily gain was similar for the other 3 lots. The superiority of BCXBFxTI lambs can be attributed to growth characteristic of Berrichon du Cher breed. In the years 2016 and 2017, the same rams were used for mating and were crossed with the local Tsigai breed. The result reveals that the body weight at 90 days was superior to Blanch du Massif Central x Tsigai (26.82 kg) when compared with Mouton Vendeèn x Tsigai (21.74 kg), Berrichon du Cher x Tsigai (21.28 kg) and Tsigai pure breed (17.54 kg) (Borzan et al., 2017).

In one crossbreeding experiment who were used Mouton Vendeen and Blanc du Massif Central as paternal breeds and Tsurcana as maternal breed (Frunjină et al., 2019), at the same age as in our experiment, the crossbreed lambs had the following weights at the age of 98 days: 26.40 kg for female and 28.71 kg for male Mouton Vendeen x Tsurcana lambs, as well as 27.63 kg for female and 29.37 kg for male Blanc du Massif Central x Tsurcana lambs, compared to 19.87 kg for female and 21.40 kg for male Tsurcana pure breed.

During the 5 months, mortality was recorded in all 4 groups, the causes being different both between the groups, and during breeding periods, within the same group. Thus, from the data of Table 5 it can be observed that the highest mortality rate was registered during the birth-weaning period in the group of crossbreed MVxBFxTI. The highest mortality rate in the first months after lambing is due in particular to the insufficient milk supply of the mothers sheep, which could not support the accelerated growth rate of the lambs during the first 2 months of life, associated with the growth of the lambs from twins lambing. After weaning, with the exception of MVxBFxTI group, diarrhea was present in all the other three groups, which also led to the occurrence of deaths due to this cause.

On the other hand, within group 3 there were highlighted 2 cases of mortality due to respiratory diseases, pneumonia respectively, as well as one case due to indigestion, constipation respectively, followed by occurrence of rectal prolaps.

The same situation was encountered in group 1, where there were 2 cases of indigestion which led to the death of the lambs.

With regard at reproductive indices, it should be mentioned that the highest fecundity rate was recorded in group 3 and the smallest in group 1. In the same time, the highest prolificacy rate was found in the group 2 and the smallest in the group 3. In contrast with our finding, the smallest reproductive indices were found by natural mating of Tsigai ewes crossed with BMC ram (fecundity 90% and 102 prolificacy) (Borzan et. al, 2017).

Table 5. The mortality and disease occurrence rates

Genotyp	Total mortality, from which:								Proportion of occurrence of diarrhea after weaning	
	Befor weaning		After weaning							
	Insufficient milk to the sheeps mothers		Diarrhea		Indigestion		Pneumonia			
	No.	Proportion (%)	No.	Proportion (%)	No.	Proportion (%)	No.	Proportion (%)	No.	Proportion (%)
BCxBFxTI	2	5.30	3	7.90	2	5.30	0	0.00	8	22.20
BMCxBFxTI	3	9.70	0	0.00	0	0.00	0	0.00	7	25.00
MVxBFxTI	5	12.80	0	0.00	1	2.60	2	5.10	0	0.00
BFxTI	4	10.30	0	0.00	0	0.00	0	0.00	3	8.60
Total	14	38.10	3	7.90	3	7.90	2	5.10	18	55.80

At artificial insemination, the Tsigai breed ewes has showed superior value of reproductive indices (100% fecundity and 106% prolificacy) when was used seminal material coming from Tsigai ram, compared with them coming from Berrichon du Cher (96% fecundity and 104% prolificacy) and Mouton Vendeèn (94% fecundity and 106% prolificacy).

It is necessary to improve the reproductive indices (fecundity, prolificacy) and some technical parameters (e.g. the number of weaned lambs per ewe mated; the number of weaned lambs per ewe lambed; lamb survival

rate at weaning) due their ability to influence the economic indicators of sheep farms.

CONCLUSIONS

Improving of reproductive performances of the sheep through selection, as a measure to improve the economic efficiency of the farms. Due to growth performances of crossbreed lambs, the three French meat breeds could be used for crossbreeding to Tsigai pure breed and/or other simply crossed ewes to improve early growth, but due to dystocia, there is needed special attention for ewes at birth.
Based on our findings, we consider that further research on fattening technology of crossbreed lambs is necessary.

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EFFECTS OF PLUMAGE COLOUR ON MEASUREABLE ATTRIBUTES OF INDIGENOUS CHICKEN IN NORTH CENTRAL NIGERIA

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Abstract

The influence of plumage colour on measureable attributes of 6176 adult indigenous chickens of mixed sex from four states of the North Central Zone of Nigeria namely; Nasarawa, Niger, Benue, Kogi and the Federal Capital Territory (FCT) Abuja were assessed. The overall average body weight of the chickens was 1.95±0.03kg. The body weights of black, white, black/white, brown, black/brown, grey and mottled chicken however were 1.87±0.04, 1.94±0.04, 1.95±0.03, 1.93±0.03, 2.01±0.04, 1.96±0.04 and 1.94±0.14 kg respectively. Only body length did not vary by plumage colour. The others; body weight and width, shank, comb and breast length, breast height (P<0.001), beak and wing lengths (P<0.001) varied significantly. Generally, no colour was out rightly superior to others in all body measurements. However, body weight and breast height were both highest in black/brown chickens which also had the second highest breast length. Body width, shank, beak, comb and wing lengths were highest in grey chickens but lowest in those with white colour and combinations. Egg qualities were on the other hand mostly lowest in grey chickens. In selection for genetic improvement in body measurements, black/brown and grey chickens should be favoured. However, in view of the known negative relationship between body weight and egg attributes, selection in favour of grey plumage may result in chickens of poor egg attributes. Therefore, grey chickens should be selected against egg quality.

Key words*: body weight, characteristics, indigenous chicken, measurements, plumage colour.*

INTRODUCTION

Poultry production in Nigeria has undergone some transformation since the 1940s when exotic breeds and intensive production were introduced (Permin and Pedersen, 2000). The indigenous species utilized has been the un-improved domestic chicken (*Gallus gallus domestica*), guinea fowl (*Numida meleagris*), ducks (*Cairina spp*) among others.

The indigenous poultry species of an area are birds that have developed characteristics peculiar to a particular geographical location. Thus chicken which have adapted to the geography and environment of Nigeria are regarded as Indigenous Nigerian Chicken (Oluyemi et al., 1982). These birds have not attained their full potential, this is mostly due to several prevailing circumstances such as sub-optimal conditions, namely poor nutrition and other management practices, diseases, predators (Alemu and Tadelle, 1997) and lack of genetic improvement (Teketel, 1986).

Improvement in the sub-optimal conditions are key to better performance (Okoh et al., 2010). In addition, changes in genetic make-up of birds could produce strains that are ultimately superior to others. Selection to improve performance is a traditional procedure that can be applied even at farmers' level. For the procedure to be successfully carried out (at farmers' level) there should be available some easily observable phenotypic attributes (which the farmer can appreciate) that can indicate performance.

Plumage colour is a chicken's characteristics that can clearly be observed on the outside. Plumage colouration is due to melanin pigmentation and has genetic basis (Marl and Brusbargh, 1971; Mancha, 2004). The colour variations are also due to mixing as a result of crossing between colour types resulting in several other colour categories (Smith, 1965). If plumage colour is found to be related to performance of birds, it could be a useful indicator for selection.

This study apart from providing useful information on the variation in characteristics of chicken in the area according to plumage, could contribute to efforts at genetic changes and improvement.

MATERIALS AND METHODS

The study was carried out in the North Central Zone of Nigeria between January 2014 to March 2018. The area has an average elevation of 1,300 m above sea level. The longitudes and latitudes of the areas are; Benue (7º,12'N; 7º,29'N and 8º,45'E; 9º,24'E), Kogi (7º,12'N; 7º,56'N and 7º,11'E; 6º,58'E), Nasarawa (8º,35'N; 8º,37'N and 8º,09'E; 9º,02'E), Niger (9º,27'N; 9º,46'N and 6º,31'E; 7º,01'E) and the Federal Capital Territory (FCT) (9º,09'N; 9º,20'N and 7º,14'E; 6º,49'E) (Microsoft Encarta, 2008).

The North Central agro – ecological zone of Nigeria experiences a sub humid tropical climate with two distinct seasons, rainy and dry. The rainy season lasts from April to September and received from 1000- 2500mm of rain, while the dry begins in October and ends in March. The two seasons are due to the moisture laden south westerly-wind from the Atlantic Ocean and the dry dusty north-easterly from the Sahara Desert (BSN, 1982). Temperatures are high throughout the year averaging 30º C. Mean annual temperatures per state are Benue 30ºC, Nasarawa 31ºC, Kogi 29º C, Niger 30º C and the Federal Capital Territory 29ºC. The relative humidity ranged from 47 to 85 %. The study area experienced mean daily sunshine duration of 8 hours (TAC, 2002). The vegetation varies considerably. It is best described as savannah, a region of tall grasses and trees.

Farming is the main occupation in the area. Crops cultivated includes: Yam, Soya beans, Rice, Cowpea, Cassava, Sweet potatoes, Sorghum, Maize, Millet, Cocoyam etc. Livestock and poultry are mainly kept as part time farming activities; Cattle, sheep, goats, chicken, ducks, geese, turkeys, pigeons and guinea fowls are kept.

Birds used for the study were scavenging indigenous chickens found within the study area and data were collected as Out - and - On stations.

Traditional management was practiced in the study area. Marked areas were provided for birds at night but are allowed to scavenge freely in the day. Supplementary feeds in the form of house-hold refuse and grains were usually given in the morning before scavenging and later in the evening before gauge (calibrated in mm).

Internal egg characteristics were taken as follows:

Albumen width was determined by carefully separating the albumen and the width measured in rest. Water was supplied at various locations around the home. No vaccination was given and, diseases were controlled by using ethno-veterinary knowledge or slaughter of affected chicken.

Five local government areas (LGAs) were randomly selected from the four states and the Federal Capital Territory (Abuja) and 100 sets of questionnaires were distributed per LGA. Observable characteristics of plumage colours were identified. Body measurements were also carried on 6176 indigenous chickens as outlined by Adekoya et al. (2013) as follows:

- body weight was measured using a sensitive platform scale in kilogram to two decimal places. Linear measurements in centimetres were carried out using a flexible measuring tape;
- body length was measured between the first cervical vertebrate and the pygostyle;
- body width was measured as the distance between the right and the left flank of the body (hind breast);
- beak length was measured as the length of the upper beak rim;
- shank length was determined as the distance from the knee or knuckle (hock joint) to the region of the tarsus;
- wing length was determined as the distance from the caput humeral to the third carpal digit;
- bomb length was taken from the posterior of the comb as the longest distance;
- breast length was measured with a tape as the chicken was held on its back;
- breast height was determined as the distance from the base of the keel to the junction of the neck.

Egg characteristics were determined as follows:

- egg colour was carried out by visual observation and the use of colour chart;

- egg weight was measured using a sensitive platform scale in grams to two decimal places;
- egg length was determined as the distance between the two ends using a Vernier Calliper;
- egg width was measured as the diameter of the broadest part of the egg using a Vernier calliper;
- egg shell weight- The egg was broken and the shell, excluding membrane, immediately weighed in grams using a sensitive scale;
- shell thickness was measured, excluding the shell membrane, using a digital micrometre screw millimetres on a tripod using a micrometre screw gauge;
- albumen height was measured by pouring out the content of the egg into a plate and measured on a tripod micrometre screw gauge (calibrated in mm);
- yolk width was determined by carefully separating the yolk and measured in millimetre on a tripod using micrometre.

The Haugh Unit (HU) value was estimated from the relationship:

$HU = \log (H + 7.73 - 1.7W^{0.36}) 100$

where: H = albumen height; W= egg weight (Haugh, 1937)

Linear measurements, productivity parameters and egg characteristics were also subjected to analysis of variance using the SPSS version 17 (2008). The following model was utilized

$\mathbf{Y_{ijk} = \mu + P_i + S_j + e_{ijk}}$.

Where;

Y_{ijk} = an observation on variable;

μ = overall population mean;

P_l = effect of plumage colour;

S_i = effect of sex (for body measurements only);

e_{ijk} = residual error (Assumed to be randomly, independently and normally distributed with mean equal to zero).

Significantly different means in a subset were separated using the Ryan Einot Gabriel Welsch F- Tests in Statistical package for Social Sciences SPSS Version 17 (2008). Pearson's Correlation co-efficient was computed to test the relationship within and between body traits and egg parameters.

RESULTS AND DISCUSSIONS

The mean body measurements by plumage colour were as presented in Tables 1. Only body length did not vary significantly with plumage. The others (body weight, width, shank, comb, breast lengths and height were significantly ($P<0.01$) affected by plumage. Beak and wing lengths were also affected ($P<0.001$). In general, however, variation in body measurement with colour appears to be haphazard showing no particular trend, that is, no colour was out rightly superior to others in all measurement. However, body weight (2.011±0.040 kg) and breast height (11.77±0.13 cm) were both highest in black / brown chicken. Black/brown chicken also have the second highest breast length (18.24±0.18 cm).

Table 1. Mean body measurements of local chicken by plumage

	Mean ± Standard Error								
Parameter	Black	White	Black/White	Brown	Black/Brown	grey	Mottled	Overall	LS
Body weight(kg)	1.87±0.04^{b}	1.94±0.04^{b}	1.95±0.03^{b}	1.93±0.03^{b}	2.01±0.04^{a}	1.96±0.04^{b}	1.94±0.14^{b}	1.95±0.03	**
Body length(cm)	19.78±0.22	19.44±0.23	19.38±0.21	19.83±0.21	19.39±0.22	19.79±0.25	19.35±0.21	19.57±0.08	NS
Body width (cm)	18.45±0.23^{b}	17.58±0.24^{c}	18.25±0.22^{b}	18.45±0.22^{b}	18.33±0.22^{b}	18.61±0.26^{a}	18.55±0.22^{b}	18.32±0.08	**
Shank length(cm)	9.79±0.41^{b}	8.74±0.42^{b}	8.68±0.39^{b}	9.59±0.38^{b}	9.59±0.39^{b}	10.01±0.46^{a}	8.92±0.39^{b}	9.33±0.14	**
Beak length(cm)	3.03±0.05^{b}	2.93±0.05^{c}	3.13± 0.05^{b}	3.05±0.05^{b}	3.27±0.05^{a}	3.18±0.06^{b}	3.16±0.05^{b}	3.11±0.02	***
Comb length(cm)	2.65±0.04^{b}	2.51±0.41	2.51±0.03^{c}	2.71±0.03^{a}	2.51±0.03^{c}	2.75±0.04^{a}	2.71±0.03^{a}	2.65±0.01	**
Wing length(cm)	14.07±0.32^{b}	14.01±0.33^{b}	14.35±0.30^{b}	14.41±0.30	14.34±0.31^{b}	15.74±0.35^{a}	14.17±0.30^{b}	14.44±0.11	***
Breast length(cm)	18.37±0.19^{a}	17.77±0.19^{b}	17.77±0.18^{b}	17.95±0.18^{c}	18.24±0.18^{a}	18.22±0.21^{a}	18.23±0.18^{a}	18.084±0.06	**
Breast height(cm)	11.72±0.14^{a}	11.60±0.14^{a}	11.67±0.13^{a}	11.72±0.13^{a}	11.77±0.13^{a}	11.71±0.16^{a}	11.36±0.13^{b}	11.65±0.55^{a}	**

LS = Levels of Significant. NS = Not Significant (** = P<0.01, *** = P<0.001). Means in row with different superscripts are significantly different

Table 2. Mean body measurements by plumage and sex

Mean ± Standard Error										
Parameter	Sex	Black	White	Black/White	Brown	Black/Brown	grey	Mottled	Overall	LS
Body weight (kg)	M.	2.14±0.05^{a}	2.22±0.06^{a}	2.22±0.05^{a}	2.25±0.05^{a}	2.32±0.04^{a}	2.28±0.05^{a}	2.27±0.06^{a}	2.25±0.03	**
	F.	1.59±0.07^{b}	1.65±0.05^{b}	1.61±0.05^{b}	1.60±0.06^{b}	1.69±0.05^{b}	1.64±0.08^{b}	1.62±0.05	1.63±0.03	
Body length (cm)	M.	20.49±0.31^{a}	20.62±0.34^{a}	20.23±0.33^{a}	20.71±0.032^{a}	20.62±0.33^{a}	20.01±0.30^{a}	20.16±0.33^{a}	20.55±0.16	**
	F.	19.08±0.39^{b}	18.26±0.30^{b}	18.54±0.30^{b}	18.96±0.33^{b}	18.15±0.30^{b}	18.57±0.45^{b}	18.54±0.30^{b}	18.59±0.16	
Body width (cm)	M.	18.88±0.32^{a}	17.97±0.35^{a}	18.41±0.34^{a}	18.77±0.33^{a}	18.69±0.34^{a}	18.77±0.31^{a}	18.76±0.34^{a}	18.61±0.17	**
	F.	19.08±0.40^{b}	17.20±0.31^{b}	18.09±0.31^{b}	18.13±0.34^{b}	17.97±0.31^{b}	18.45±0.47^{b}	18.34±0.31^{b}	18.04±0.17	
Shank length (cm)	M.	9.25±0.56^{a}	8.38±0.61^{a}	8.38±0.59^{a}	8.93±0.58^{a}	9.47±0.60^{a}	9.64±0.58^{a}	8.52±0.60^{a}	8.94±0.29	**
	F.	10.33±0.71^{b}	9.09±0.54^{b}	8.99±0.55^{b}	10.25±0.60^{b}	9.71±0.54^{b}	10.37±0.82^{b}	9.32±0.55^{b}	9.72±0.29	
Beak length (cm)	M.	3.32±0.07^{a}	3.22±0.08^{a}	3.36±0.08^{a}	3.19±0.07^{a}	3.49±0.08^{a}	3.46±0.07^{a}	3.49±0.08^{a}	3.36±0.04	**
	F.	2.75±0.07^{b}	2.65±0.07^{b}	2.89±0.07^{b}	2.90±0.08^{b}	3.06±0.07^{b}	2.90±0.11^{b}	2.82±0.07^{b}	2.85±0.04	
Comb length (cm)	M.	3.10±0.05^{a}	3.11±0.06^{a}	2.90±0.05^{a}	3.16±0.05^{a}	3.08±0.05^{a}	3.28±0.05^{a}	3.27±0.05^{a}	3.13±0.02	**
	F.	2.20±0.07^{b}	2.25±0.05^{b}	2.12±0.05^{b}	2.25±0.05^{b}	1.94±0.05^{b}	2.27±0.08^{b}	2.16±0.05^{b}	2.16±0.02	
Breast length (cm)	M	18.58±0.26^{a}	18.13±0.28^{a}	18.13±0.27^{a}	18.23±0.27^{a}	18.35±0.28^{a}	18.48±0.26^{a}	18.65±0.28^{a}	18.39±0.13	
	F	18.16±0.33^{b}	17.23±0.25^{b}	17.40±0.25^{b}	17.66±0.28^{b}	18.13±0.25	17.97±0.38^{b}	17.81±0.25^{b}	18.39±0.13	**
Breast height (cm)	M	12.45±0.19^{a}	12.25±0.21^{a}	12.24±0.20^{a}	12.49±0.20^{a}	12.61±0.21	12.50±0.31^{a}	11.66±0.21^{a}	12.31±0.10	
	F	11.01±0.24^{b}	10.95±0.19^{b}	11.09±0.19^{b}	10.96±0.21^{b}	10.94±0.19^{b}	10.92±0.28^{b}	11.06±0.19^{b}	10.99±0.10	**

LS = Levels of Significant. NS = Not Significant (** = P<0.01). Means in column with different superscripts are significantly different.

Body weight, breast height together with breast length and girth are true measure of size in birds (Obioha, 1992). It would therefore appear that black/brown chickens were bigger than others. Body weight, shank, beak, comb and wing lengths were highest in grey plumage chicken but mostly lowest in chicken with white colour and combinations. They were clear and significant (P<0.01) sex dichotomy in body measurements with, as expected, the males being superior except in shank length (Table 2). Shank length were consistently longer in females for all colours and this did not translate into size (weight) advantage.

For egg quality measurements only egg weight, shell weight and thickness did not vary with plumage colour. The others varied significantly (P<0.01) (Table 3). Egg length, width, albumen height and width, yolk width and Haugh unit were highest in mottled (4.00±0.16 cm), white (4.00±0.07 cm), black/white (4.81±0.10 cm), white (19.11±0.28 cm), mottled (13.35±0.23 cm) and black/white (93.00±0.01 cm) chicken respectively. They were however, mostly lowest in grey chicken. It would therefore appear that the grey chicken has the poorest egg quality.

Correlation values were positive and significant at 1%. A few were correlated at 5% but most of them were not. Body weight showed a positive and significant (P<0.01) correlation with body length (0.493), breast height (0.476), and length (0.255), these characteristics are a measure of size in birds (Chineke, 2001). Most egg characteristic were negatively correlated with body weight.

The observation that grey chicken appears to have some of the highest body measurement but poorest egg quality attributes is in accordance with the negative relationship that is known to occur between body weight and egg production in birds (Moran, 1990). This is also supported by the generally negative relationship between them.

Table 3. Mean egg measurements by plumage

Mean ± Standard Error									
Parameter	Black	White	Black/White	Brown	Black/Brown	grey	Mottled	Overall	LS
Egg weight (g)	39.31±0.80	40.05±0.86	38.25±1.14	39.68±0.95	39.28±0.82	40.08±1.03	38.06±0.82	39.27±0.82	NS
Egg length (cm)	3.77±0.07^{b}	3.68±0.08^{b}	3.75±0.18	3.84±0.16	3.66±0.16	3.65±0.21	4.00±0.16	3.63±0.15	**
Egg width (cm)	3.83±0.07^{b}	4.00±0.07^{a}	3.94±0.10^{b}	3.96±0.08^{b}	3.80±0.09^{b}	3.71±0.11^{c}	3.74±0.09^{c}	3.87±0.06	**
Eggshell weight (g)	4.07±0.13	4.27±0.14	4.49±0.19	4.07±0.16	4.33±0.15	3.97± 0.19	4.47±0.15	4.08±0.09	NS
Eggshell thickness (mm)	1.03±1.12	1.93±1.20	0.81±1.60	2.35±1.33	0.42±1.00	0.72± 1.25	0.46±0.99	0.53±0.01	NS
Albumen height(cm)	4.48±0.10^{b}	4.57±0.10^{b}	4.81±0.10^{a}	4.67±0.08^{b}	4.47±0.12^{b}	4.33±0.16^{c}	4.55±0.1^{b}	4.48±0.06	**
Albumen width(cm)	18.87±0.1^{d}	19.11±0.28^{a}	19.01±0.10^{a}	18.58±0.23^{b}	18.75±0.20^{d}	18.46±0.26^{b}	19.05±0.12	18.89±0.17	**
Yolk width (cm)	13.32±0.20^{a}	13.28±0.21^{b}	12.60±0.28^{c}	12.57±0.24^{c}	12.07±0.24^{c}	12.41±0.30^{c}	13.35±0.23^{b}	13.09±0.16	**
Haugh unit	92.02±0.56^{b}	92.33±0.61^{b}	93.80±0.80^{a}	92.88±0.66^{b}	91.89±0.70^{c}	90.90±0.88	92.50±0.70^{b}	92.44±0.47	**

Note: - Ns = Not Significant, (**P<0.01). Means in row with different superscripts are significantly different

Table 4. Correlation of body weight and egg characteristics

	BW	BL	BWi	SL	WL	BeL	CL	BHT	BrL	EW	EL	EWi	ShT	ShW	AW	AHT	YWi	HU
BW		0.493**	0.210**	-0.051	0.293**	0.445**	0.670**	0.476**	0.255**	-0.019	-0.089	-0.005	-0.077	0.168*	-0.067	-0.061	-0.035	-0.057
BL			0.238**	- 0.021	0.284**	0.314**	0.570**	0.462**	0.301**	-0.036	-0.101	0.024	0.067	0.064	0,027	0.030	-0.002	0.037
BWi				-0.016	0.052	0.190**	0.203**	0.124*	0.095	0.024	0.030	0.009	-0.020	-0.039	0.036	-0.100	-0.154	-0.097
SL					-0.034	-0.001	-0.044	0.049	0.015	0.002	0.002	-0.081	-0.008	0.008	0.181*	0.117	0.100	0.113
WL						0.093	0.384**	0.311**	0.054	0.199	0.033	-0.113	-0.099	-0.019	-0.054	0.052	0.040	0.006
BeL							0.518**	0.290**	0.067	-0.037	0.183	-0.005	-0.029	-0.029	-0.100	-0.039	-0.079	-0.031
CL								0.508**	0.212**	0.012	-0.012	-0.100	0.070	-0.043	0.091	0.149*	0.155	0.142
BHT									0.215**	0.143*	0.068	-0.093	-0.018	0.264**	-0.074	0.108*	0.128*	0.075
BrL										-0.012	-0.063	-0.093	-0.029	-0.029	-0.100	0.039	-0.079	-0.031
EW											-0.076	-0.163	-0.018	0.040	-0.010	-0.122	0.003	-0.319
EL												0.099	-0.042	0.006	0.064	0.123	-0.153	0.129
EWi													-0.502	-0.005	-0.055	-0.145	0.126*	0.181*
ShT														0.007	0.003	0.129	-0.001	0.121*
ShW															0.016	-0.044	0.138*	-0.042
AW																0.065	0.008	0.069
AHT																	0.108*	0.97**
YWi																		0.114*
HU																		

* = Significant at P< 0.05 ** = Significant at P<0.01 .BW = Body weight, BL, = Body length, BWi= Body width, SL = Shank Length, WL, = Wing length BeL= Beak Length, CL= Comb Length, BHT= Breast Height, BrL= Breast Lenght. EW= Egg weight, EL= Egg Length, EWi= Egg width, ShT= Shell thickness ShW= Shell weight AW= Albumen weight, AHT = Albumen Heigth, YWi= Yolk width, = Haugh Unit

CONCLUSIONS

In this study, black/brown and grey plumage chicken were generally bigger than other categories but white were smallest. Grey chicken were however poorest in egg quality attributes.

For improved body weight, selection could be carried out in favour of black/brown and grey chicken. However, because of the lower egg quality attributes of grey chicken, care may probably have to be taken, this is because selection in favour of grey chicken may result in mostly poor quality eggs.

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EGG QUALITY ASSESSMENT OF INDIGENOUS CHICKENS IN THE NORTH CENTRAL AGRO ECOLOGICAL ZONE OF NIGERIA

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Abstract

Adult female indigenous chickens were assessed for egg characteristics in the North central agro ecological zone of Nigeria. Data were generated as Out/on station on all female chickens. Chickens were maintained as a single unimproved population and allowed to mate, lay and hatch naturally. External and internal qualities were assessed on the first generation eggs. Egg weight showed mean value of 39.59 ±0.06 g which did not vary significantly. There was however significant variation by state. Overall mean egg length was 3.80±0.01 cm, while on-station had 3.81± 0.01 cm. Mean egg width was 3.89 ± 0.01cm and on-station 3.88± 0.01 cm, but values varied significantly (P<0.01) by states. Mean shell thickness was 0.90±0.32mm while on-station value was 0.52±0.05 mm. There was significant difference in shell thickness (P<0.001) by states. Thickness varied from 0.35±0.03 to 0.790±0.08 mm in Nasarawa and Niger states. Shell weight was observed to be 4.18± 0.01 and showed non-significant difference by site and state. Internal quality showed that mean albumen width 18.78 ± 0.02 cm. Albumen height was observed to be 4.57± 0.03 cm and showed no significant difference by site but differed significantly (P<0.001) by state. The overall quality rating of the egg (Haugh unit) was 76.31± 0.16. Highest egg quality (Hu=78.61±0.22) was observed in Nasarawa and least (Hu = 73.44±0.16) in Niger state. Most correlation values were significant at 1%. Only egg weight is correlated with shell weight at 5 % (0.036; P<0.05). Most egg characteristic measured was lowly correlated. There was similarity of egg in the study area since most traits were non-significant. The high egg qualities are an indication of good reproductive performance in the chickens and the positive correlations showed that the traits could be used to predict each other and thus aid selection and hasten selection processes to develop the indigenous layer chickens.

Key words*: indigenous chickens, egg characteristics, egg quality, egg weight, Haugh unit.*

INTRODUCTION

Physical egg qualities are external and internal. The external quality refers mainly to outer observations on the intact egg by means of non-destructive examination but including shell quality while the internal quality refers to observations on the interior components which play important roles on integrity, processes of embryo development and successful hatching (Narushin and Romanov, 2007).

Nigerian indigenous chickens showed great variation in egg weight (Adedokun and Sonaiya, 2001; Peter et al. (2002). Msoffe et al. (2001) and Katule (1990). Research had shown that egg weights from indigenous chicken of various genetic group vary significantly. Egg weight measurement is important because of its direct relationship with the size of the day-old chick (Moran, 1990)

Egg length is the longest portion observed on the external surface or its long borders and the width which is the shorter portion of the egg (Gunlu et al., 2003) are a measure of surface area and can also be used to predict shell quality characteristics, hatchability and chick weight (Mebratu, 1997), and interior parameters (Narushin, 2005). Egg shape attracts consumers' attention, with preference being given to normal shapes (Narushin and Romanov, 2007). The soundness of egg shell is its fitness or strength or ability to allow the egg to go through handling processes without damage.

The assessment of the internal characteristics of the egg can be carried out through the destructive technique which involves breaking it (Narushin and Romanov, 2007) or the nuclear-magnetic resonance, computer vision and acoustics. These methods are non-destructive assessment; the internal characteristics of the

egg have to do with the consistency of contents (Kuchida et al., 1999; Coucke et al., 1999). Albumen is formed from the layers of secretion of the anterior section of the hen's oviduct (Scott and Silversides, 2000). The primary natural purpose of the albumen is to protect the yolk and provide additional nutrition for the growth of the embryo (when fertilized) (Stevens, 1996). The albumen has a major influence on the overall interior egg quality (Jacob et al., 2000). Albumen quality is influenced by genetic and environmental factors such as temperature, humidity, presence of carbon dioxide, pH and storage time (Fayeye et al., 2005). Others include nutrition and the hen's age (Roberts and Ball, 2004). Loss of water from the egg through evaporation during storage is influenced by temperature and humidity and is detrimental to internal egg quality (Scott and Silversides, 2000). There are several measures of egg yolk quality, as the yolk ages it absorbs water from the albumen and increases in size; this weakens the perivitelline membrane (Kirunda and Mckee, 2000). The yolk is also flattened and often displaced to one side as opposed to the fresh egg whose round yolk stays in a central position surrounded by the thick albumen (Jacob et al., 2000).

The research is aimed at assessing the physical egg qualities of the Nigerian Indigenous chicken within the North Central Agro- ecological Zone to provide further data which may be useful for future genetic / reproductive studies.

MATERIALS AND METHODS

The study was carried out in the North Central Zone of Nigeria between January 2014 to March 2018. The area has an average elevation of 1,300 m above sea level. The longitudes and latitudes of the areas are; Benue (7º,12'N; 7º,29'N and 8º,45'E; 9º,24'E), Kogi (7º,12'N; 7º,56'N and 7º,11'E; 6º,58'E), Nasarawa (8º,35'N; 8º,37'N and 8º,09'E; 9º,02'E), Niger (9º,27'N; 9º,46'N and 6º,31'E; 7º,01'E) and the Federal Capital Territory (FCT) (9º,09'N; 9º,20'N and 7º,14'E; 6º,49'E) (Microsoft Encarta, 2008)

The North Central Agro – ecological Zone of Nigeria experiences a sub humid tropical climate with two distinct seasons, rainy and dry. The rainy season lasts from April to September and received from 1000- 2500 mm of rain, while the dry begins in October and ends in March. The two seasons are due to the moisture laden south westerly-wind from the Atlantic Ocean and the dry dusty north-easterly from the Sahara Desert (BSN, 1982). Temperatures are high throughout the year averaging 30º C. Mean annual temperatures per state are Benue 30ºC, Nasarawa 31ºC, Kogi 29º C, Niger 30º C and the Federal Capital Territory 29ºC. The relative humidity ranged from 47 to 85 %. The study area experienced mean daily sunshine duration of 8 hours (TAC, 2002). The vegetation varies considerably. It is best described as savannah, a region of tall grasses and trees.

Farming is the main occupation in the area. Crops cultivated includes: Yam, Soya beans, Rice, Cowpea, Cassava, Sweet potatoes, Sorghum, Maize, Millet, Cocoyam etc. Livestock and poultry are mainly kept as part time farming activities; Cattle, sheep, goats, chickens, ducks, geese, turkeys, pigeons and guinea fowls are kept.

Birds used for the study were scavenging indigenous chickens found within the study area and data were collected as Out - and - On stations

Traditional management was practiced in the study area. Marked areas were provided for birds at night but are allowed to scavenge freely in the day. Supplementary feeds in the form of house- hold refuse and grains were usually given in the morning before scavenging and later in the evening before rest. Water was supplied at various locations around the home. No vaccination was given and, diseases were controlled by using ethno veterinary knowledge or slaughter of affected chickens.

Data were generated for external and internal egg qualities on outdoor (Out station) from birds kept by local farmers. Sixty-two indigenous chicken of both sexes were randomly selected from four states and the Federal Capital Territory (Abuja) and kept indoors (On station). These birds were kept as un-improved and maintained as a single mating population in an open sided poultry house screened with wire mesh for protection. They were allowed to mate, lay and hatch naturally. Data were collected from their first generation progenies for egg quality assessment. Both indoor and outdoor (On station /Out station) egg qualities were determined as follows:

Egg weight was measured using a sensitive platform scale in grams to two decimal places.
Egg length was determined as the distance between the two ends using a Vernier calliper.
Egg width was measured as the diameter at the broadest part of the egg using a Vernier Calliper.
Egg shell weight was determined by breaking the egg and the shell, excluding membrane, immediately weighed in grams using a sensitive scale.
Shell thickness was measured, excluding the shell membrane, using a digital micrometre screw gauge (calibrated in mm).
Internal egg characteristics were taken as follows:
Albumen width was measured by carefully separating the albumen and the width measured in millimetres on a tripod using a micrometre screw gauge.
Albumen height was measured by pouring content of the egg into a plate and measured on a tripod micrometre screw gauge (calibrated in mm)
Yolk width - The yolk was carefully separated and measured in millimetre on a tripod using micrometre.
The Haugh Unit (HU) value was estimated from the relationship: HU= log (H + 7.73- $1.7W^{0.36}$) 100, Where H = albumen height W= egg weight (Haugh, 1937).
Egg characteristics were subjected to analysis of variance using the SPSS version 17 (2008).
Significantly different means in a subset were separated using the Ryan Einot Gabriel Welsch F- Tests in Statistical package for Social Sciences SPSS Version 17 (2008). Pearson's Correlation co- efficient was computed to test the relationship in egg parameters measured.

RESULTS AND DISCUSSIONS

Egg weights of indigenous chickens for out-station and on station are presented in Tables 1 and 2. There was significant variation by state with highest (39.86 ± 0.15 g) and lowest ($39.32 \pm 0.16\ g$) values reported in Nasarawa and Kogi State respectively. The mean egg weight obtained in this study is higher than the 28 g and 29.37 g mentioned by Williamson and Payne (1978) and Mbap and Zakar (2000) but similar to 36–41 g (Sonaiya, 2003) for indigenous chickens in Nigeria.

Table 1. Overall mean egg characteristics by site

Parameter	Out-Station	On-Station	LS	Combined Mean
Egg weight (g)	39.59± 0.06	39.69± 0.05	NS	39.64± 0.04
Egg length (cm)	3.80± 0.01	3.81± 0.01	NS	3.80± 0.01
Egg width (cm)	3.89±0.01	3.88 ±0.01	NS	3.89±0.01
Shell thickness(mm)	0.90±0.32	0.77± 0.26	NS	0.84±0.20
Shell weight (g)	4.18±0.01	4.17±0.01	NS	4.18±0.01
Albumen width (cm)	18.78±0.02	18.78± 0.01	NS	18.78±0.01
Albumen height (cm)	4.57±0.03	4.61±0.02	NS	4.59±0.02
Yolk width (cm)	12.76±0.02	12.75±0.02	NS	12.75±0.01
Haugh Unit	76.31 ± 0.16^{b}	67.45 ± 0.11^{a}	***	71.88±0.13

Note:- LS = Level of significant. NS = Not Significant.
Significant at *** = (P<0.001)

For improved breeds Obioha (1992) and Narushin and Romanov (2007) gave 50- 60g as standard weights. This is an indication that the local chicken egg weights are inferior to their exotic counterpart. Overall mean egg length for out- station was 3.80±0.01, while on-station had 3.81 ± 0.01cm. The egg length values were not significantly different by site and state. Mean egg length in the present study is lower than 5.77 – 6.12 cm reported by Mbap and Zakar (2000). Overall mean egg width for out-station of 3.89 ± 0.01 cm and on-station 3.88±0.01cm, were similar but values varied significantly (P<0.01) by states. The highest width ($3.95 \pm 0.02\ cm$) was observed in Benue State and the least ($3.84 \pm 0.02\ cm$) in Nasarawa State.

Table 2. Egg characteristics by state

Parameter	Benue	Kogi	Nasarawa	Niger	Abuja	Overal Mean Out- Station	On- Station	LS
Egg weight(g)	39.41± 0.15^{b}	39.32±0.16^{b}	39.86± 0.15^{b}	39.73± 0.15^{b}	39.50±0.15^{b}	39.56±0.18	39.86± 0.05^{a}	***
Egg length(cm)	3.78± 0.01	3.78±0.01	3.79± 0.01	3.81± 0.01	3.82±0.01	3.80±0.01	3.81± 0.01	NS
Egg width(cm)	3.95± 0.02a	3.89±0.02^{ab}	3.84± 0.02^{a}	3.90± 0.02^{ab}	3.89±0.02^{ab}	3.89±0.02	3.88± 0.01^{a}	**
Shell thickness	0.52± 0.02^{b}	0.52±0.01^{b}	0.35± 0.03^{a}	0.79± 0.08^{b}	0.52±0.01^{b}	3.80±0.01	0.52± 0.05^{b}	***
Shell weight(g)	4.23± 0.03	4.18±0.03	4.18± 0.03	4.23± 0.03	4.18±0.03	4.20±0.03	4.16± 0.01	NS
Albumen wi(cm)	18.78± 0.04	18.69±0.04	18.75± 0.04	18.85± 0.04	18.82±0.04	18.78±0.04	18.77± 0.01	NS
Albumen ht(cm)	4.57± 0.07^{a}	3.66±0.07^{b}	4.53± 0.07^{a}	4.59± 0.07^{a}	4.62±0.07^{a}	4.39±0.07	4.71± 0.02^{a}	***
Yolk width(cm)	12.75± 0.05	12.71±0.06	12.79± 0.06	12.78± 0.06^{a}	12.76±0.05	12.76±0.05	12.75± 0.02	NS
Haugh Unit	76.22±0.02^{a}	74.34±0.52^{a}	78.61±0.22^{a}	73.44±0.162^{a}	78.14±0.52^{a}	76.31±0.162	67.45±0.11^{b}	***

Note:- Ns, not Significant, (***P<0.001, (**P<0.01) Means in a row with different superscripts are significantly different.
Where: wi = width, ht = height

Overall mean shell thickness was 0.90±0.32 mm for out-station while on-station value was 0.52±0.05 mm. There was no significant difference in shell thickness by site but it was significant (P<0.001) by state. Thickness varied from 0.35±0.03 to 0.790±0.08 mm in Nasarawa and Niger State respectively. Shell weight was observed to be 4.18± 0.01 and 4.16± 0.01 g for out/ on stations respectively; and showed no significant difference by site and state.

Mean albumen width for out-station was 18.78 ± 0.02 cm while that of on-station was 18.79± 0.01 cm. There was no significant difference in albumen width by site and state. Overrall mean albumen height was observed to be 4.57±0.03 cm at out- station while that of on-station was 4.71±0.02 cm and showed no significant difference by site but differed significantly (P<0.001) by state. It varied from 3.66± 0.07 to 4.62± 0.07 cm in Kogi State and Abuja respectively. The mean yolk width recorded for out-station was 12.76 ± 0.02 cm and 12.75 ± 0.02 cm for on- station, which showed a non- significant difference by site and by state. The non-significant difference in egg length, shell weight, albumen weight and yolk width between states is a reflection of the similarity of the indigenous chickens in the study area. The egg weight, length and width values of indigenous chickens also showed that they were smaller compared with exotic breed. The overall quality rating of the egg (Haugh unit) at out-station was 76.31± 0.16 while on-station value was 67.45± 0.11. There was significant difference (P<0.001) by site and by state. Highest egg quality (Hu=78.61±0.22) was observed in Nasarawa and least (Hu = 73.44±0.16) in Niger state. The egg quality rating (Haugh unit) of 84.36 – 91.16 reported by Awosanya et al. (1998) is lower than the value obtained in this study. The egg shell thickness obtained in this study is higher than 0.34 – 0.35mm and 0.36±0.01 reported by Awosanya et al. (1998) and Mancha (2004) respectively. Mebratu (1997) and Chineke (2001) also gave lower values of 0.31–0.38 mm and 0.31±2.37 mm respectively for egg thickness. The importance of good shell thickness is that it enables the best use of nutrients contained in the egg by the embryo (Sergeyeva, 1976). There are lesser chances of bacteria penetration (Fisinin et al., 1990), dehydration (Roque and Soares, 1994) and also offers the best protection from mechanical damage (Sergeyeva, 1976).

Correlation coefficients between egg measurements are presented in Table 3. Most correlation values were significant at 1%. Only egg weight is correlated with shell weight at 5 % (0.036; P<0.05).

Most egg characteristic measured was lowly correlated. Albumen height is correlated with yolk width (0.389; P<0.01). There was no significant correlation between egg weight and shell thickness (-0.024) and albumen weight (-0.014). Egg length was not significantly affected by shell thickness (0.004) and shell weight (0.006).

Table 3. Correlation among egg parameters

Egg weight	Egg Length	Egg Width	Shell Thickness	Shell Weight	Albumen Wt	Albumen ht	Yolk Width
EW	0.036**	0.073**	-0.024ns	0.036*	-0.014ns	0.048**	0.041**
EL		0.180**	0.004ns	0.006ns	0.018**	0.018**	0.018**
EWi			-0.014ns	0.070**	-0.053**	0.108**	0.096**
STH				0.054**	0.008ns	0.013ns -	0.012ns
SW					0.017**	0.047**	0.009ns
A.W						0.036**	0.122**
A.H							0.389**
Ywi							

* = Significant at *P<0.05
** = Significant at **P<0.01

CONCLUSIONS

The positive correlations between egg quality characteristics showed that the traits could be used to predict each other and could hasten selection processes to develop the indigenous chicken for egg.

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INVESTIGATING THE RELATIONSHIP BETWEEN THE PARITY AND SOME PLACENTAL TRAITS IN GOATS

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Abstract

The relationship between the parity, the litter weight (LW) and some placental traits with goats was investigated in this study. 94 goat were investigated in total, 28 of which from first parity, 27 from second parity, 15 from third parity, 12 from forth parity and 12 from fifth parity. One-way ANOVA was used for statistical comparison, and Pearson correlation was used to determine the relationships between the variables. The goats' placenta from the first parity weighs the least and the forth parity's placenta is significantly ($P<0.05$) the heaviest. Significant differences in placental efficiency between the investigated animals were not discovered. Positive correlation was discovered between the weight of the placenta and the birth weight of the kid in the first, second and fifth parity. The results of this study show negative correlation between the weight of the placenta and the density of the cotyledons in goats of the first, second, third and fifth parity. Further studies are required to investigate the relationship between parity and placental traits in goats.

Key words*: goats, parity, placental efficiency, placental weight.*

INTRODUCTION

An important indicator of vitality in small ruminants is the placental traits. When the embryo grows in size, the processes of diffusion through which it nourishes become insufficient to sustain its life (Sen and Önder, 2016; Ocak et al., 2014), and then the placenta starts play a major role.

According to Bell and Ehrhardt, (2002), in late pregnancy, placental size is closely related to transport capacity and can be changed by maternal nutrition.

Goats have polycotyledonary placenta and placentomes which carry out exchanges between the maternal and foetal circulatory system (Sen and Önder, 2016; Ocak et al., 2014).

The low placental and cotyledon traits of kids who died during the early postnatal period showed that placental and cotyledon traits have an effect on kids' vitality (Ozyurek, 2019).

Sen et al. (2013) and Sen and Önder (2016) establish that the number of placentomes and the placental size of goats determines the exchange capacity of substances between the mother and the fetus. According to them, the size, which is related to the nutrient transfer capacity of the placenta, plays an important role in determining the prenatal growth of the fetus, and then the birth weight and postnatal viability.

The mother's womb directly influences the fetuses' growth and restricting the nutrition of the mother during the pregnancy leads to a lowering in the fetuses' weight and the weight of the placenta but, it also leads to the increase of placental efficiency (Konyalı et al., 2007; Osgerby et al., 2002).

The birth weight is of significant importance for the vitality of the newborn. It influences not only the neonatal vitality, but the morbidity and the fatality in adult animals too (Fowden and Forhead, 2009).

It is important to mention that according to Konyalı et al., (2007), the lower placental efficiency and density of the cotyledons leads to a longer period of time until the kid stands up after birth. They conclude that the increase of placental efficiency leads to an earlier standing up of the kid and less time until the first sucking. Furthermore, this is also one of the factors for the establishing of a proper connection between the goat mother and its kid and it also leads to the increase of probability of the kid surviving.

There are a number of determining factors for the development of the placenta – the goat mother's nutrition, the litter size, sex, breed, age and parity of the goat etc.

In their research, Dwyer et al. (2005) conclude that the placental weight and the placental efficiency are influenced by the age of the mother and the parity of the sheep. With the increasing number of births, the placentas become heavier which is likely due to the reproductive maturity of the animals. The weight of the placenta increases with the age of the goat mother, but the placental efficiency and the density of the cotyledons is not influenced by the age of the mother.

The placentas of the goats with three or more pregnancies weigh more and have a higher number of cotyledons in comparison with these from the first or second pregnancy (Ocak et al., 2013). According to Özyürek (2019), the cotyledon number is formed on the 30th day of the pregnancy and there was no change in cotyledon number in the later periods although there was a change in size.

Ozyurek (2019) investigates the relationship between kids vitality and placental characteristics in hair goats. He concludes that the placental efficiency was affected by the vitality of kids ($p<0.05$) and observed that there was no x large cotyledon (>51 mm diameter) in the dead kids. This means that the size of the cotyledons is of great importance for the prenatal and postnatal survival of the kid.

The purpose of our research was to investigate the relationship between the parity of goats, litter weight and some placental traits like placental weigh, cotyledon number, placental efficiency and cotyledon density.

MATERIALS AND METHODS

The study was conducted in the goat farm of the Research Institute on Mountain Stockbreeding and Agriculture in the town of Troyan, Bulgaria. The facility is located at an altitude of 380 m, (42° 53' 39" N / 24° 42' 57" E).

The study involved 94 goats of Bulgarian White Dairy breed (BWD) and its crossbreeds with Anglo-Nubian (AN) and Togenburg (TG).

The parity of goats were classified as 1st, 2nd, 3rd, 4th and 5th . 94 goat were investigated in total, 28 of which from the first parity, 27 from the second parity, 15 from the third parity, 12 from the forth parity and 12 from the fifth parity.

All goats were housed and cared for under the same conditions. During the winter period animals were kept in a barn and fed with a ration containing of 2 kg hay, and 0.8 kg concentrated fodder per head. There was free access to water and salt.

In the spring months (May-November) goats were grazing.

Goats were vaccinated against enterotoxemia, treated for parasites, and given vitamins A, D, and E (Vialiton, Biovet).

Kidding of goats took place in February and March. Before kidding goats were separated in individual pins and were under surveillance (Figure 1).

Figure 1. Goat separated in individual pins and newborns kids

Placentas (chorioalanantois and related fetal cotyledons) were collected immediately after natural delivery and weighed fresh in digital scales.

The kids' were weighed right after birth. When twins were born the weight of each placenta was summed up.

The Cotyledon number (CN) from each delivered placenta was counted and recorded. Cotyledons were classified by size according to Konyali et al. (2007). Cotyledon number (<10 mm diameter); Cotyledon number (between10 and 30 mm diameter); Cotyledon number (≥3 mm diameter).

Placental efficiency was defined as the ratio of total kid birth weight (g) to Placental weight, (g) (Molteni et al., 1978).

Cotyledon density was defined as the number of cotyledons per gram of Placental weight (Ocak et al., 2013).

One-way ANOVA was used for statistical comparison. Relationships between the placental traits of kids and goats were determined with Pearson correlation analysis.

RESULTS AND DISCUSSIONS

The placental traits (PW, CN, PE and CD) and the litter weight based on the parity are shown in Table 1.

Placental weight increased with the goat's parity. The placenta of the goats that gave birth for the first time (First parity) were the lightest, while the goats that gave birth for the fourth time (Fourth parity) had significantly ($P<0.05$) the heaviest placenta.

The placenta of the second parity was 140 g heavier than the placenta of the first parity, while the difference between the first parity and the fourth parity was 188 g.

Table 1. Placental traits and the total weight of the kids based on the parity

Goat's age	Litter weight, g LW $x\pm S_x$	Placental weight, g PW $x\pm S_x$	Cotyledon number CN				Placental efficiency PE $x\pm S_x$	Cotyledon density CD $x\pm S_x$
			total $x\pm S_x$	≤1 $x\pm S_x$	1-2 $x\pm S_x$	≥3 $x\pm S_x$		
I – Parity	*4601.79± 276.56	541.07± 31.30	89.82± 4.44	12.89± 1.57	72.19± 4.57	4.00± 0.87	8.74± 0.40	0.17± 0.01
II – Parity	5685.19± 397.87	641.11± 40.81	85.04± 4.63	8.26± 1.27	71.22± 4.56	5.44± 1.16	9.12± 0.49	0.14± 0.01
III – Parity	5440.00± 483.91	682.67± 56.82	72.47± 4.34	4.79± 1.17	57.53± 4.20	10.27± 2.22	8.55± 0.94	0.12± 0.01
IV - Parity	*6500.00± 564.48	729.17± 55.90	94.42± 8.62	6.17± 2.11	75.92± 8.24	12.33± 1.86	9.04± 0.53	0.14± 0.01
V - Parity	4815.00± 435.89	665.00± 85.04	74.20± 8.69	7.40± 3.23	55.10± 8.55	11.80± 1.81	7.69± 0.57	0.12± 0.01

*($p<0.05$)

With goats from the fifth parity there was a lowering of the weight in comparison with the forth parity.

The fetuses' weight is linked to the weight of the placenta. It is a functional unit which forms the fetus and the mother's tissue or in other words the placenta is a determining factor which limits the growth of the fetus (Konyalı et al., 2007; Ocak and Onder, 2011).

Using the placenta, the fetus receives nutrients and oxygen from the mother and exudes the unnecessary substances from the exchange. Glucose is major metabolic substrate for the exchange of the placenta and the fetus (Bell and Ehrhardt, 2002).

According to Konyalı et al. (2007), it is likely that smaller placentas are more efficient than the bigger ones which need more nutrients.

With the increase of a goat's births we could see an increase in litter weight. The lightest kids came from goats that were giving birth for the first time, while the heaviest come from goats giving birth for the fourth time.

In past experiments (Hristova et al., 2012; Stoycheva, 2014) we have found that with the increase of a goat's age (Parity), the kid's birth weight increases.

Martinez et al. (2009) remarks that goats from the breed Murciano – Granadina give birth to lighter kids in their first births than births from the second or higher parity.

Elabid (2008) finds that kids from the second parity are heavier in comparison with the first parity, but the difference is insignificant.

According to Elabid (2008) and Islam et al. (2009), a major factor in the increase of birth weight in kids is the change in live weight of the mother during the pregnancy.

The increase of the live weight of the kid found by us in the birth of older mothers is likely due to the decrease of expenses of nutrients for growth with aging of the goats and their redirection to the pregnant uterus. The birth weight of the kids corresponded with the nutrition of the goats, their breed and the homeoretical capacity of the mothers too.

The results of our study showed that cotyledon number and cotyledon density decreased with parity which is in agreement with the findings of Sen and Önder (2016) in Saanen goats and Özyürek and Türkyilmaz (2020) in Morkaraman sheep.

An exception makes the placentas of the fourth parity in which the number of cotyledons was the highest.

The placental efficiency determines how many grams of the fetus fall per 1 g of placenta (Wilson and Ford, 2001) which means that the placental efficiency determines the capacity of the mother (Wilson and Ford, 2001), or in other words the total weight of the placenta which the mother can carry up to the birth (Bennett and Leymaster, 1989; Wilson et al., 1999).

Previous studies reported that placental efficiency increased with parity in beef, sheep and cattle (Dwyer et al., 2005; Echternkamp, 1993).

In this study we did not find an essential connection between the weight of the placenta and its effectiveness which is in agreement with Konyali et al. (2007). According to them it does not change with the parity of goats and sheep respectively. Placental efficacy is assumed to be genetically based, but on the other hand, it is also significantly influenced by environmental factors during pregnancy. The age and the reproductive maturity of the goat mothers may cause a significant impact on the placental efficiency, but it is interesting what the limit at which parity begins to have a negative effect is (Ocak et al., 2013).

Pearson's Correlation Ratio was used to characterize the relationships between the studied placental parameters.

Table 2 presents the correlations between the individual indicators according to the parity of the goats.

Table 2. Pearson correlation coefficient of placental parameters according to goat parity

	LW	CN	PE	CD
I – Parity				
PW	0.69	0.54	-0.37	-0.60
LW		0.24	0.37	-0.60
CN			-0.45	-0.28
PE				-0.05
CD				
II – Parity				
PW	0.66	0.65	-0.31	-0.53
LW		0.59	0.48	-0.23
CN			-0.07	0.25
PE				0.28
CD				
III – Parity				
PW	0.49	0.34	-0.53	-0.86
LW		0.74	0.43	-0.08
CN			0.17	0.17
PE				-0.73
CD				
IV - Parity				
PW	-0.75	0.69	-0.27	-0.08
LW		0.44	0.42	-0.37
CN			-0.30	0.57
PE				-0.42
CD				
V – Parity				
PW	0.66	0.56	-0.68	-0.83
LW		0.75	0.06	0.10
CN			0.00	0.52
PE				0,66
CD				

A moderate to significant positive correlation was found between the placental weight in the goats from the first parity, the second parity and the fifth parity and this is logical because with the increase of the placental weight the litter weight increases too. Our findings are in agreement with Sen and Önder's (2016) findings, who also find a positive correlation in

Saanen goats of different parities. Ocak et al. (2014) find the opposite to be true in Damascus goats though.
In goats from the fourth parity the relation was negative
We saw a positive correlation between the placental weight and the number of cotyledons in the first parity, the second parity, the fourth parity and the fifth parity.
Negative correlation between the placental weight and the placental efficiency was discovered in the third parity and the fifth parity.
A negative correlation was discovered between the placental weight and the cotyledon density of the first parity, the second parity, the third parity and the fifth parity. The findings reported by Sen and Onder, (2016), Özyürek and Türkyilmaz, (2020) in Morkaraman sheep supported our results.
A negative correlation was discovered between the litter weight and the density of the cotyledons in goats and first parity kids.
There was a positive correlation between litter weight and the total number of cotyledons in goats that had given birth more than one time.

CONCLUSIONS

The results of this study showed that the parity of the mother has a significant impact on the weight of the placenta and the litter weight in the goats we examined.
Young mothers gave birth to kids with lower live weight when the placental weight was lower.
A negative correlation was discovered between the placental weight and the density of the cotyledons in the first parity, the second parity, the third parity and the fifth parity.
The acknowledgement of these facts could determine a different approach in the raising and feeding of young pregnant goats which could compensate in some level their reproductive immaturity.
Further studies are required to investigate the relationship between parity and placental traits in goats.

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CARCASS TRAITS FOR THE KIDS OF TWO INDIGENOUS GOAT BREEDS IN BULGARIA

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Abstract

The Kalofer longhaired goat (KLG) and the Bulgarian screw-horned longhaired goat (BSLG) are indigenous goat breeds in Bulgaria, reared in some mountainous regions of the country. 12 male kids (6 of KLG and 6 of BSLG), born as singles, were slaughtered immediately at weaning at 90 days of age, without a period of intensive fattening after weaning. Based on the results of slaughter analysis of suckling kids from the two local goat breeds, it can be concluded that the average weight of the cold carcass was 9.88 kg for male suckling kids of KLG and 9.79 kg for kids of BSLG. Dressing percentage of chilled carcass, without the offal was 48.87 % and 50.5% for KLG and BSLG, respectively. The linear measurements of the carcass of male suckling Kalofer longhaired kids at 90 days of age were as follows - big carcass length – 49.50 cm, length of ham – 32.16 cm, circumference of ham – 32.83 cm. The area of m. longissimuss at 11th ribs was 10.39 cm². The ratio meat:bones in the carcass of suckling Kalofer longhaired kids at 90 days of age was relatively high - 2.99:1. For males Screw-horned kids, the linear measurements of a carcass were as follows – big carcass length – 56.66 cm, length of ham – 30.16 cm, circumference of ham – 30.18 cm. Area of m. longissimuss at 11th ribs was 10.0 cm². The ratio meat:bones was 2.87:1.

Key words*: indigenous goat breeds, local goat breeds, slaughter characteristics, carcass traits*

INTRODUCTION

The meat from suckling kids, slaughtered at early age, is a highly valuable product, with marked dietary and taste qualities (Rubino et al., 1999; Arias and Alonso, 2002; De Gea et al., 2005; Zimerman et al., 2008; Bonvillani et al., 2010). The studies of a bulgarian authors on this problem are few, especially the researches on the meat characteristics of local goat breeds in Bulgaria. At the beginning of the 19th century, the different local populations of the native goat breeds in Bulgaria, was united by the researchers in a common group called "local goats". They were defined as a low-productive, without a pronounced breed type, and were recommended to be improved by cross-breeding with imported, high-yielding goat breeds (Kadiyski, 1952; Kadiyski, 1958; Balevska and Tyankov, 1971; Solomonov et al., 1984). The integration of all local populations of native goats into a common group, was detrimental to indigenous goat in the country. Undoubtedly, the breed has a high impact on productivity, and especially on some meat characteristics in goats (Dhanda et al., 2003; Monte et al., 2007; Ekiz et al., 2010; Sanudo et al., 2012).

The Kalofer longhaired goat (KLG), and the Bulgarian screw-horned longhaired goat (BSLG) are indigenous goats breeds in Bulgaria, endangered by extinction (Vuchkov et al., 2011), (Figure 1 and Figure 2). In the contemporary aspect of the problem related to the preservation of genetic resources in livestock breeding, the establishment of the status "threatened" by the local Bulgarian goat breeds - Kalofer longhaired and the Bulgarian screw-horned longhaired goats, it is imperative to carry out more in-depth studies on their productive qualities (milk and meat production), and the possibility of increasing the economic effect of keeping the local breeds of goats in our country.

The farmers traditionaly growing these breeds mainly in mountainous and semi-mountainous regions of the country. The possibilities for its sustainable preservation and development over time, is inevitably linked to the diversification of products and raw materials derived from these native breeds. KLG and BSLG are typical breeds with combined productivity - grown for meat, milk and fur. In traditional technology of rearing in Bulgaria, the kids are slaughtered immediately after weaning at three months of

age, without a period of intense fattening after weaning (so-called “milk kids”). In all farms with KLG and BSLG, the meat from young kids, slaughtered at early age is the main income for the farmers. This requires a detailed study of the meat characteristics of kids at an early age, and the possibility for obtaining meat with high taste and dietary qualities.

MATERIALS AND METHODS

The purpose of this study was to investigate some meat features and qualities of carcass from kids of two indigenous goat breeds in Bulgaria, slaughtered immediately at weaning in 90-days of age.

For the purpose of the study, was carried out a complete slaughter analysis on 6 Kalofer longhaired and 6 Bulgarian screw-horned longhaired kids, born in farms in the Kresna region. The experimental groups were aligned by sex and type of birth for the two breeds - male, single.

During the suckling period the kids were traditionally grown together with their mothers, the mother's milk was the main food, and after the 20th day of birth, the kids had free access to lucerne hay and concentrated fodder (corn, barley and sunflower meal - 40:40:20).

The age at slaughter of the kids - 90 days, immediately after weaning. The goats, mothers of the kids, were selected according to their age, 2-4 years of age. The daily ration of goats consisted mainly of grazing. The individual slaughtering judgment was carried out of the carcasses, after 24 - hour’s refrigeration. Linear dimensions of the carcass and its separated parts were made. The slaughter analysis was done according to the method of Zahariev and Pinkas (1979). The data were processed by a variation-statistical method.

RESULTS AND DISCUSSIONS

The results of the slaughter analysis of males kids from KLG and BSLG, at 90 days of age, and the linear dimensions of some parts of carcass, and the bones-meat ratio are presented in tables 1-5.

Within the sample, the live weight before slaughter (after 24 hours of starvation) was 20.16 kg and 19.40 kg, for kids from KLG and BSLG, respectively (Table 1). The formation of a relatively high live weight after a 90-days suckling period in both local goat breeds were representative. To a large extent, during this early period of its development, the growth of the kids was directly depends on the quantity and quality of the milk produced by their mothers. The high dry matter content of the milk of the local long-haired goats, significantly determines a high growth rate of their kids at an early age, and relatively higher live weight at weaning in 90 days of age.

Compared to the others local kids, in studies by Bulgarian authors, the Kalofer kids shows the highest growth rate at an early age, and significantly higher live weight for a 90-days suckling period (Tsonchev, 1974; Zunev and Uzunov, 1994).

Table 1. Carcass traits of Calofer longhaired and Bulgarian screw-horned longhaired kids at weaning in 90 days of age (n = 6 kids/breed)

Signs/breeds	Kalofer longhaired kids				Bulgarian screw-horned kids			
	SX	SD	max	min	SX	SD	max	min
Live weight before slaughter, kg	20.160	0.683	21.0	19.5	19.400	1.799	23.0	18.2
Weight of cold carcass, kg	9.883	0.762	10.8	9.1	9.799	0.960	11.7	9.1
Dressing percentage of cold carcass, %	48.970	2.671	52.5	45.5	50.503	1.988	52.1	49.5
Skin, kg	1.775	0.147	1.9	1.5	1.316	0.147	1.7	1.1
Legs (no skinned), kg	0.633	0.121	0.7	0.4	0.650	0.063	0.75	0.6
Head (skinned), kg	0.883	0.116	1.0	0.7	0.850	0.070	0.95	0.75

The higher live weight of kids from KLG at 90-days of age, is indirectly a prerequisite for better meat production, compared to the others goat breeds in Bulgaria.

Carcass weight is an important sign of the meat production in the small ruminants, as well as a key indicator for the classification of the carcasses by the SEUROP system. The average weight of chilled carcasses (excluding internal fat) was 9.883 kg and 9.799 kg for kids from KLG and BSLG, respectively. Lower scores cite Markovic et al. (2011) in 90 days old male kids of Balkan goat breed in the Republic of Montenegro. The kids of KLG and BSLG outperform by weight of the chilled carcass, kids of some Turkish local goat breeds - Gokceada, Maltese, Hair Goats (Yalcintan et al., 2010).

The dressing percentage of cold carcass was relatively high - 48.9% in the kids from KLG, and 50.5% in the kids from BSLG. Account the early age at slaughter (90 days of age), and that the kids were still in a suckling period, the high values of dressing percentage could be attributed to the lower losses of tissue fluid at slaughter, and underdeveloped digestive system in young animals.

The abomasum had a priority in development of the various parts of complex stomach in the young ruminants.

With age, and increasing the share of plant foods in the ration, as well as the early weaning and habituation to plant foods, stimulating the earlier development of the digestive tract, in particular the stomach and intestines.

This fact influences the calculation of the dressing percentage in cold carcass. In kids of KLG, slaughtered at 90 days of age, we found that the weight of the empty stomach and intestines were 0.550 kg and 0.941 kg, respectively (Table 2).

Expressed in % to the live weight before slaughter, empty stomach and intestines constitutes 2.727% and 4.651%, respectively. Similar values were also found in kids from BSLG. The values which we have established for empty stomach and intestines are lower than those cited by Markovic et al., 2011) at the Balkan goat from Montenegro.

Undoubtedly, with increasing age, as well as with the early weaning of the kids, their transition to whole plant ration (concentrated and coarse feed, without fresh milk) increases development of the different parts of digestive system. For comparison, in early weaned local kids and subsequently fattened up to 110 days of age, weighing 23.40 kg, Stankov et al. (1999) found a higher percentage of stomach and intestine to the live weight before slaughter.

Table 2. Weight of offal and fat tissue in chilled carcass of Kalofer longhaired and Bulgarian screw-horned longhaired kids at weaning in 90 days of age (n= 6 kids/breed)

Signs/breeds	Kalofer longhaired kids				Bulgarian screw-horned kids			
	SX	SD	max	min	SX	SD	max	min
Lunges, kg	0.408	0.020	0.45	0.4	0.391	0.049	0.45	0.3
Liver, kg	0.433	0.025	0.45	0.4	0.508	0.066	0.6	0.45
Heart, kg	0.106	0.010	0.12	0.1	0.103	0.08	0.12	0.1
Spleen, kg	0.048	0.004	0.05	0.04	0.048	0.004	0.05	0.04
Kidneys, kg	0.113	0.020	0.14	0.08	0.111	0.018	0.14	0.09
Internal fats (omentum, sweetbread), kg	0.701	0.055	0.8	0.63	0.658	0.153	0.85	0.45
Intestines (empty), kg	0.941	0.107	1.3	0.93	0.733	0.075	0.8	0.6
Stomach (empty), kg	0.550	0.077	0.65	0.45	0.541	0.058	0.6	0.45

The thinner skin was also important for the relatively high dressing percentage in 90 days old kids from the two indigenous goat breeds in Bulgaria. The raw skin of the Kalofer kids was 1.775 kg. For Bulgarian screw-horned longhaired kids, the skin was significantly lighter – 1.316 kg. Expressed as % of the live weight before slaughter, raw skin was 8.80% for the kids of KLG, and only 6, 399% for the kids of BSLG. The lighter skin gave a logical explanation for the higher dressing percentage on the Screw-horned, compared to the dressing percentage on the Kalofer kids.

During the suckling period in local long-haired kids, the subcutaneous and internal fat depositions in the carcass were very poorly

expressed. In the kids of KLG, the internal fats (included mesenterium and mesenteric lymph nodes) weigh 0.701 kg, and in the kids of BSLG - 0.658 kg. Related to the weight of the chilled carcass, they made up a relatively low percentage – 3.478% and 3.267% respectively. These results can be explained with the early slaughtering age and the lack a period of intensive fattening of the kids.

In intensively fattened local kids, for example, the percentage of internal fat increases (Zunev and Uzunov, 1994). Some authors cited that in kids from specialized dairy goat breeds, with increasing age, delamination of internal fat is more pronounced (Dhanda et al. 2003; Ekiz et al., 2010; Yalcintan et al., 2010).

The local longhaired kids weaned at 90 days of age have a relatively higher dressing percentage (48.97% at Kalofer and 50.50 at Screw-horned) compared to intensively fattened local Strandzha kids up to 23.40 kg and 110 days old at which the dressing percentage was 46.58% (Stankov et al., 1999).

The our data for the dressing percentage of 90-days old kids of KLG and BSLG were higher than those cited by Zunev and Uzunov (1994) for local kids, intensively fattened up to 160 days. Markovic et al. (2011) cited lower values for this sign in the 90 days old kids from local goat breed in Montenegro - Balkan goat.

A much higher percentage of dressing percentage is cited by Dhanda et al. (1999) in cross-bred kids with specialized meat goat breed - Boer (50-55%).

Table 3. Ratio between some offal and live weight before slaughter of Kalofer longhaired and Bulgarian screw-horned longhaired kids at weaning in 90 days of age (n = 6 kids/breed)

Signs/breeds	Kalofer longhaired kids				Bulgarian screw-horned kids			
	SX	SD	max	min	SX	SD	max	min
Skin: live weight before slaughter, %	8.806	0.774	9.774	7.500	6.399	0.366	7.573	5.288
Legs: live weight before slaughter, %	3.132	0.552	3.500	2.051	3.255	0.116	3.365	3.030
Head: live weight before slaughter, %	4.373	0.501	5.000	3.590	4.271	0.355	4.670	3.788
Internal fats: live weight before slaughter, %	3.478	0.220	3.810	3.231	3.267	0.536	3.788	2.473
Intestines: live weight before slaughter, %	4.651	1.074	6.190	3.077	3.680	0.322	4.040	3.261
Stomach: live weight before slaughter, %	2.727	0.371	3.250	2.308	2.725	0.333	3.297	2.273

In the analysis of some linear dimensions of the carcass of the Kalofer longhaired and the Bulgarian screw-horned longhaired kids (Table 4), there is a tendency for pronounced carcass compactness. Shorter, more compact, carcasses give the impression of better muscularity, and more prominent muscle profiles (Figure 3). This is very important for their commercial appearance. The length of the carcass was 49.50 cm in the Kalofer kids. The carcasses of the Screw-horned kids were not much longer - 56.6 cm.

The length and the circumference of the ham are criteria for the muscles development of the carcass. It was noteworthy that the length and the circumference of the ham in the Kalofer kids were with almost same values - 32.16 cm and 32.83 cm respectively (Table 4). This fact gave the impression of excellent muscularity and good filling of the ham. Similar values were observed in the Screw-horned kids - 30.16 cm and 30.18 cm, respectively - length and circumference of the ham.

Based on the fact that local longhaired goats are breeds with combined productivity (meat, milk and skins), the linear carcass dimensions obtained were logical. Specialized milk breeds have a longer carcasses, and longer and narrower hams, compared to these in local breeds from the combined productivity (Sanudo et al., 2012). Ekiz et al. (2010) also highlight differences on productive type and body size.

In intensively fattened local Strandzha kids at 110 days of age Stankov et al. (1999), indicated higher values of carcass length, and at the same time, lower values for the circumference of

ham. The area of m. Longissimus in Kalofer longhaired kids was 10.396 cm². A similar results for this sign we found in Bulgarian screw-horned longhaired kids - 10.00 cm². In intensively fattened to older age (110 days), and to higher live weight (23.40 kg) local Strandzha kids, Stankov et al. (1999) established similar values - 10.32 cm².

The both signs - area of m. Longissimus and circumference of the ham are representative for the meat content of the carcase.

The obtained values of these indicators determine the relatively good qualities of the carcass of the kids from KLG and BSLG, slaughtered at weaning at 90 days of age. The local kids from the Balkan goat breed in Montenegro, at the same age, had longer carcasses than Kalofer kids. The length of the carcass was 53.13 cm, with a slaughter live weight 13.50 kg (Memisi et al. 2009).

Table 4. Linear measurements of carcass of Kalofer longhaired and Bulgarian screw-horned longhaired kids at weaning in 90 days of age (n = 6 kids/breed)

Signs/breeds	Kalofer longhaired kids				Bulgarian screw-horned kids			
	SX	SD	max	min	SX	SD	max	min
Big length of cold carcass, cm	49.500	1.760	52.0	47.0	56.666	2.732	61.0	53.0
Length of ham, cm	32.166	1.940	34.0	29.0	30.166	0.983	31.0	29.0
Circumference of ham, cm	32.833	1.169	34.0	31.0	30.185	1.169	32.0	29.0
Area of m. Longissimus (at 11 ribs), cm²	10.396	1.599	12.25	7.76	10.006	0.326	10.3	9.6

In order to provide a more complete assessment of the meat productivity of the 90 days old suckling kids from KLG and BSLG, the "meat: bone" ratio in different parts of the carcass was determined.

The results presented in Table 5, shows that the meat: bone ratio in the left half of the carcass of the Kalofer kids was 2.997: 1. In the Screw-horned kids meat: bone ratio was 2.871: 1. This defined a relatively high meatiness class for the carcasses of suckling kids from KLG and BSLG.

This ratio was maintained at approximately constant limits in the various parts of the carcass - neck, shoulder, ribs, loin and ham.

The lack of detachable subcutaneous fat were a criterion for the high dietary properties of meat from weaned at 90 days of age, local longhaired kids.

Table 5. Ratio meat: bones in left half of the carcass of Kalofer longhaired and Bulgarian screw-horned longhaired kids at weaning at 90 days of age (n = 6 kids/breed)

Signs/breeds	Kalofer longhaired kids				Bulgarian screw-horned kids			
	SX	SD	max	min	SX	SD	max	min
Meat: bones (left half)	2.997	0.167	3.174	2.835	2.871	0.257	3.271	2.581
Meat: bones (neck)	2.961	0.262	3.222	2.500	2.610	0.368	3.090	2.125
Meat: bones (shoulder)	3.368	0.275	3.640	2.959	3.559	0.518	4.240	2.888
Meat: bones (ribs)	2.263	0.275	2.781	2.022	2.097	0.215	2.484	1.840
Meat: bones (first quarter)	2.728	0.195	3.029	2.503	2.644	0.260	3.045	2.267
Meat: bones (loin)	2.954	0.497	3.538	2.291	2.447	0.366	2.875	2.000
Meat: bones (ham)	2.977	0.214	3.379	2.760	2.733	0.349	3.225	2.250
Meat: bones (second quarter)	3.447	0.242	3.861	3.193	3.253	0.350	3.634	2.720

Despite the lower slaughter age (90 days), the meat: bone ratio in the carcass of the investigated animals allows the slaughter to be made at the designated weaning age in order to obtain light carcasses. Slaughtering at this early age does not adversely affect of the economic strategy of the farmers and the nutritional and dietary values of the carcasses of kids. This free niche, namely the production of light carcasses (up to 13 kg), could be a good alternative for sustainable development of the Bulgarian local goat breeds.

Figure 1. KLG - Kalofer longhaired goat breed (doe). © Sedefchev.

Figure 2. BSLG - Bulgarian Screw-horned longhaired goat breed (doe). © Sedefchev.

Figure 3. Carcasses of a suckling kids, slaughtered at weaning 90 days of age (KLG – left, BSLG – right). © Vuchkov.

CONCLUSIONS

On the basis of the results obtained of slaughter traits of suckling kids of the Kalofer longhaired goat (KLG) and Bulgarian screw-horned longhaired goat (BSLG) breeds, several conclusions are drawn.

The average weight of cold carcass was 9.88 kg for males suckling Kalofer longhaired kids and 9.79 kg for males Bulgarian screw-horned longhaired kids, at 90 days of age. Dressing percentage of chilled carcass, without offal was 48.87%, and 50.5% for kids from KLG and BSLG, respectively.

The linear measurements of the carcass of males suckling Kalofer longhaired kids at 90 days of age was respectively - big length of carcass - 49.50 cm, length of the ham - 32.16, circumference of ham - 32.83 cm. The area of m. Longissimuss at 11 ribs was 10.39 cm².

Determination the linear measurements of the carcass of males suckling Bulgarian screw-horned longhaired kids at 90 days of age was respectively - big carcass length - 56.66 cm, length of the ham - 30.16 and circumference of ham - 30.18 cm. The area of m. Longissimus at 11 ribs was 10.0 cm².

The ratio meat:bones in the carcass of suckling Kalofer longhaired kids at 90 days of age was significantly higher - 2.99:1. The value of this sign in the Bulgarian screw-horned longhaired kids was 2.87:1.

These results allowed that the slaughter of the kids from Bulgarian local breeds, can be done in the specified age of weaning (90 days of age), with the aim to producing light carcasses (type Capretto), and without a negative impact on economic strategy of the farmers. This free niche, namely the production of light carcasses (up to 13 kg), could be a good alternative for sustainable development of the Bulgarian local goat breeds.

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GENOTYPICAL PARTICULARITIES OF THE TYPE OF SHEEP MOLDAVIAN KARAKUL

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Abstract

The purpose of the present research was to reveal and evidence the genotypic particularities of the type(race) of sheep Moldavian Karakul. The experiments were performed on sheep populations from the Experimental Household of the National Institute of Zootechnics and Veterinary Medicine (INZMV), and from the Agricultural Cooperative of Production "Agrosargal", Republic of Moldova. Research they demonstrated that the hereditary capabilities of the Moldavian Karakul sheep are well genetically consolidated and are constantly transmitted through descent. In particular, the morpho-productive characters, which determine the quality of the furskin, have significant genotypic (mother-descendant) correlations, such as: loop length ($r=0.47\pm0.08$; $t_r=5.9$; $P<0.001$); extension of the loop ($r=0.45\pm0.09$; $t_r=5.6$; $P<0.001$); silk fiber ($r=0.40\pm0.09$; $t_r=4.4$; $P<0.001$); fiber luster ($r=0.30\pm0.10$; $t_r=3,0$; $P<0,01$); fiber length ($r=0.30\pm0.10$; $t_r=3.0$; $P<0.01$); body length of lamb at birth ($r=0.36\pm0.02$; $t_r=18.0$; $P<0.001$); body mass of lamb at birth ($r=0.30\pm0.03$; $t_r=10.0$; $P<0.001$) and evaluation class ($r=0.48\pm0.07$; $t_r=6.8$; $P<0.001$). The large body mass of the sheep (meat production aptitude) is constantly transmitted through heredity. There is an obvious genotypic correlation between this character of the father-rams and the young-descendants ($r=0.55\pm0.09$). The heritability coefficient of body mass is not high ($h^2=0.3$), but quite significant ($t_r=2.6$; $P<0.01$). The value of the repeatability coefficient (r_w) of body mass at different ages of young sheep and adult sheep is 0.23–0.47. The consolidated genotypic capacities of the Moldavian Karakul sheep are also manifested in the transmission through heredity milk production. The value of the heritability coefficient (h^2) of this character is not high, but quite significant ($h^2=0.316$; $P<0.001$). Most breeding rams have valuable hereditary capabilities. Out of the total 112 rams tested for descendants qualities, 30 rams were recognized as improvers, which is 26.8%. The genotypic qualities of improvers have been repeatedly confirmed. The type of sheep Moldavian Karakul possesses a karyotype with specific genetic characteristics, which is identified by the classical Asian Karakul race and the local race Tusca, after the erythrocyte antigenic markers of the 7 blood groups (A, B, C, D, M , R and I), which include 15 erythrocyte antigens (Aa, Ab, Bb, Bd, Bg, Be, Bi, Ca, Cb, Da, Ma, R, O, I and i). Moldavian Karakul sheep are characterized by a high frequency (0.6005-0.9504) of antigens Bb, Bd, Bg, Be, Ca, Cb, Da, Ma, O and I, a medium frequency (0.3447-0.5796) of antigens Aa and R, as well as a reduced frequency (0.0496-0.2260) of antigens Ab, Bi and i. In the diagram of the genetic clusters, the sheep population Moldavian Karakul occupies an intermediate position in cluster B with the genetic distance of 0.2586, located between the two related populations, as is the local breed Tusca of the cluster A with the genetic distance of 0.1280 and, the race Asian Karakul from cluster C with genetic distance of 0.3154. In the population studied were found 136 genotypes and 48 genetic allele with specific frequencies. The degree of homozygosity of the investigated population constitutes 0.58, which shows sufficient genetic consolidation for the growth "in itself".

Key words*: race, sheep, Moldavian Karakul, heritability, furskin, milk, antigens.*

INTRODUCTION

According to the communication of Bertone B.F. (official representative of FAO at the first International Symposium for the Karakul race in Vena, 1967), quoted by Ştefănescu et al. (1973), Karakul sheep are raised on all continents of the world, except Oceania.

Analyzing the dynamics and distribution of sheep flocks in the growing countries of Karakul we concluded that, they are concentrated, raised and exploited efficiently in countries and regions that have huge territories of natural pastures, and also poor (Central Asia, Africa), but extended, with a climate that allows them to be maintained throughout the entire calendar year without expensive shelters (Buzu, 2018). In addition, Karakul sheep are also found in some European countries, where the local breeds for milk-furskins are

traditionally grown, such as: Socoliskaia and Resetilovca - in Ukraine, Turcana White and Greyish - in Romania, and Tusca - in the Republic of Moldova.

In the countries of the regions and parts of the above-mentioned world, multiple intraracial types of Karakul sheep have been created, as well as regional types of elite within this world megarace, which are distinguished by a whole range of phenotypic morpho-productive and genotypic particularities.

Thus, in **South West Africa (Namibia**), using his own specific selection procedures, Tompson (quoted by Филлингер, 1975), he created at the Neydam Experimental Station a new intraracial type of Karakul sheep with flat buckling. According to the communication of Мостерт (1975), at the same Station, through the reciprocal crossings of the Karakul race with the Somali and White African race, a new intraracial type of white Karakul sheep was created. The German researcher Шефер (1975a, 1975b), described the particularities of the heritability of the furskin characters in these types. Нел (1975a, 1975b), he studied the phenotypic and genotypic correlations, as well as the heritability of a furskin character string in the Karakul sheep of South African types, proposed some specific methods of testing the rams according to the descent qualities.

In Uzbekistan, under the influence of Namibian fashion, the karakulists reoriented the selection of Karakul sheep in the direction of creating new types of sheep with flat and costal curls, of different colors and coloration (Buzu, 2016). Thus, Букаев et al. (2016) announces the creation of a new type of elite "Саржальский" of black Karakul sheep with the type of coastal loop. Юсупов et al. (2016) created the new type of elite "Бухароишарифский" of sheep Karakul on silver with the type of flat loop. Рахимов et al. (2016) communicate about the creation of a new type of elite "Сарибельский" of sheep Karakul gray silvery with the type of flat loop. Юсупов et al. (2016) obtained a new type of elite "Авазчульский" of black Karakul sheep with the type of coastal loop. Солиев et al. (2016) created the new type of elite "Узбекистанский - Uzbekistanski" of sheep Karakul gray silvery with the type of looping jacket. Очилов et al. (2016) created the new intra-racial type "Турткульский" of Karakul sheep from the Karakalpak type. About the creation of new types of sheep Karakul in this country of Central Asia informs us Гигинейшвили (1976) and Жиряков (2008).

In Kazakhstan, Шамекенова (2011) communicate on the creation of a new intra-racial type of Karakul gray sheep through the absorption crossings of local Kurdiuk and Edilbaev sheep, with Karakul gray sheep. Юсупбаев (2011) report on the creation of a new intra-racial type of white Karakul sheep "Отырарский", by crossing white sheep for fine wool and black Karakul, with white rams from the Kazakh race of Kurdiuk and white sheep of the Karakul type from Nurata.

Алибаев et al. (2014) created, a new type of elite sheep Karakul greyish of blue colorated. Алимбаев (2011) and Ескара et al. (2014), the communicates on the creation of a new type of elite black sheep Karakul of «Жоматский» with the tubular type loop.

In Romania, SCPCO Popăuţi researchers created a new type of intra-racial (race) Karakul from Botosani, by crossing the black and greyish Turcana sheep and mating with the purebred Karakul sheep (Ştefănescu et al., 1961; Taftă, 1997b; Ursu et al., 1995). Subsequently, genetic improvement of sheep populations was carried out under the pedo-climatic conditions specific to the area, creating within the race lines and new populations with varieties of greyish (Bosânciuc et al., 1994; Filote et al. , 1997), gray, brown and white (Ursu et al., 1994, 1995, 1997; Ursu, 1997), estimating the morpho-productive and genetic parameters of these newly created populations (Bosânciuc et al., 1997; Groza et al., 2014; Nechifor et al., 2015).

In Ukraine, at the Institute of Scientific Research in Animal Husbandry for the Steppe Districts "Askania-Nova", Herson region, according to the communication of Перегон И.Л. (1972), through cross-breeding activities of the Karakul and Romanov races, selection and breeding of artisans of different generations, the researchers managed during the years 1935-1970 to create a new intra-racial type of prolific Karakul sheep with superior performances, compared to the Asian one. The prolific Karakul sheep from Askania are also widespread in the Cernăuţi region, based on

which, by crossing with the local sheep, measures are being taken to create a new type of sheep from Karakul of Bucovina (Дронык et al., 2010; Lesyk et al., 2016).

In all the new intra-racial and elite types created by the Karakul sheep, in all the countries and regions where they were created, the creator-authors described, quite broadly and in detail, the morpho-productive phenotypic particularities of the sheep. At the same time, the genotypic particularities of the newly created sheep populations, in most countries (with the exception of South-West Africa, Romania and, in part, Uzbekistan), have not been sufficiently elucidated, which makes it impossible to genotypically compare the biodiversity of genetic resources of the Karakul sheep from the spreading areas of this world megarase.

In South West Africa, the genotypic particularities of the local types of the Karakul race have been largely described by the scientific researchers of the Neydam Experimental Station (Филлингер, 1975; Мостерт, 1975; Шефер, 1975a, 1975b; Нел, 1975a, 1975b), which highlighted the hereditary capacities of the sheep from the populations subjected to selection, determined the genotypic correlations and the heritability coefficients of the main selected characters, described the genetic characteristics of the sheep genealogical lines within the race.

In Uzbekistan, for the first time in the world, at the Union Institute of Scientific Research for Karakultura (Samarkand city), research was carried out on the antigenic factors of blood groups in sheep from the Karakul race, identifying the possibilities of their use as genetic markers in the selection sheep (Ата-Курбанов, 1986).

In Romania, the genotypic characteristics of the type of sheep Karakul de Botosani have been elucidated by describing the hereditary particularities of the color varieties and the genetic parameters for the main properties of the buckling (Ştefănescu et al., 1961; Ursu et al., 1994; Bosânciuc et al., 1994; Bosânciuc, Taftă, 1997; Nechifor et al., 2015), as well as by the inheritance characteristic of the antigenic factors of the blood groups (Hrincă et al., 1989, 1994, 2001; Hrincă, 2012).

In the Republic of Moldova, the genotypic particularities of the Moldavian Karakul sheep have been elucidated by us in separate works describing the hereditary capabilities of the sheep and the breeding value of the reproducing rams (Buzu, 2012, 2015, 2014c), determining the phenotypic and genotypic correlations, as well as the heritability coefficients of the main selected characters (Buzu, 2000, 2001, 2014a, 2014b). Research on the frequency and inheritance of blood group antigenic factors in sheep from local populations was performed for the first time by Люцканов (1990, 2009) and Марзанов (1991). At the same time, when presenting the materials to the State Commission for the approval of the type of sheep Moldavian Karakul, it was necessary to systematically highlight the genotypic particularities, the comparative characteristic with the original races and the passport of the sheep population after the antigenic markings of the blood groups.

The revealing of these particularities, being the purpose of the present work, is a very current scientific problem.

MATERIALS AND METHODS

The genotypic particularities of the type of sheep Moldavian Karakul have been investigated on the sheep populations from the Experimental Household of the National Institute of Zootechnics and Veterinary Medicine (INZMV) in the village Maximovca, the district Anenii Noi and from the Agricultural Cooperative of Production "Agrosargal", village Sarata Galgenă, Hânceşti district.

These particularities were highlighted based on the research of the hereditary capacities of the sheep for the descending transmission of the main morpho-productive characters: the quality of the furskin, the body mass and the milk production.

Based on establishing the heritability coefficients (h^2) of the characters, the calculations of the squares of the genotypic correlation coefficients, existing between the characters of the parents and their descent, were applied, applying the electronic programs ("STATISTICA-12"), known in the computer

in the biometric processing statistics of population genetics.

The capacities of hereditary transmission of the furskin qualities of the Moldavian Karakul lambs were established following the analysis of the results of the inheritance by the lambs, of the main selected characters, both quantitative and qualitative. The lambs' value was made 1-2 days after birth, according to the Karakul sheep guidelines Instructions with amelioration principles in the Republic of Moldova (Buzu et al., 1996). In order to determine the breeding capacities of the rams breeders, during the creation of the type of sheep, the Moldavian Karakul was carried out their permanent testing according to the qualities of the descent, according to the methods elaborated by us (Buzu, 2014c). At the beginning of the type creation period, the rams were tested only once, regardless of the age at which they were tested. Subsequently, during the period of approval of the new type, the multiple testing (several times) of the rams was applied according to the qualities of the descent. According to the mentioned methods, the rams were divided into different categories and degrees, according to their value of improvement (1st degree improver, 2nd grade improver, ordinary grade improver, or reducer).

During the period of approval of the new type of sheep Moldavian Karakul, using monospecific sera from the INZMV immunodiagnostic bank, the genetic markers (erythrocyte antigens) of 7 blood groups A, B, C, D, M, R and I were investigated, include 15 antigens: Aa, Ab, Bb, Bd, Bg, Be, Bi, Ca, Cb, Da, Ma, R, O, I and i (Люцканов, 1990, 2009). As a result, the characteristics of the Moldavian Karakul sheep population were given according to the antigen, genotype and allele profile in the blood groups, determined the indices of the genetic distances between the related initial races (Asian Karakul and Tusca), constructed the genetic cluster diagram, calculated the degree of homozygosity and the number of efficient alleles. The frequency of antigenic factors, genotypes and alleles in the sheep populations investigated was determined by the methods of Животовский and Машуров (1974).

The indices of the genetic distances between the sheep populations investigated were calculated based on the squared deviations of the summary differences of the antigen frequencies of the blood groups according to the methods of Серебровский (1970).

The analysis of the genetic cluster and the construction of the diagram was performed according to the methods of Машуров and Черкащенко (1987).

The index of the degree of homozygosity and the number of efficient alleles in the sheep populations investigated were determined, according to the methods of Robertson A., 1956.

The data obtained as a result of the researches were processed, systematized and generalized, their certainty being appreciated, according to the variational biometric statistics, according to the methods of Плохинский (1989).

RESULTS AND DISCUSSIONS

The results of the researches showed that, the genotypic particularities of the type of sheep Moldavian Karakul are manifested by the ability to transmit by heredity the main morpho-productive characters selected, through the capacities of genetic improvement of the rams-breeders, as well as by the specific structure of the immunogenetic markers of the groups blood in the systems of the sheep population.

The inheritance of the main productive morph characters.

The degree of heritability of the characters selected in the sheep population can be appreciated both by calculating the genotypic correlations of these characters between parents and descent, as well as by determining the heritability coefficient (h^2).

In the Karakul race, the furskin characteristics, taken separately, influence the quality of the furskin as a whole, which constitutes a primordial morpho-productive character.

Knowing the degree of their inheritance in the specific sheep population, subject to genetic selection and breeding, is of significant scientific interest, both for the efficiency of the selection process and for the genotypic characteristic of the population (race).

The results of the research of a series of genotypic correlations of the characters, which condition the quality of the furskin as a whole,

showed that, in most of the selected characters, there are correlation coefficients (mother-descendant) of medium and sub-level, with quite significant criteria of certainty (Table 1).

Table 1. Genotypic correlation (mother - descendant) of the main specific furskin characters sheep population Moldavian Karakul

Characters	N	$r \pm m_r$	t_r
Length of the loop	51	0.47 ± 0.08	5.9***
Extension of the loop	49	0.45 ± 0.08	5.6***
Silkiness	43	0.40 ± 0.09	4.4***
Luster	47	0.30 ± 0.10	3.0**
Degree of closure of loops	70	0.09 ± 0.11	0.8
Loop modeling	70	0.09 ± 0.11	0.8
Modeling type	70	0.12 ± 0.11	1.1
Fiber sowing	70	0.10 ± 0.10	1.0
Reserve the skin	70	0.08 ± 0.11	0.7
Constitution	47	0.06 ± 0.14	0.4
Body length lamb at birth	65	0.36 ± 0.02	18.0***
Lean body mass lamb at birth	41	0.30 ± 0.03	10.0***
Class at evaluation	49	0.48 ± 0.07	6.8***

Remark: **t_r< 0.01; ***t_r< 0.001.

The results obtained allow us to find that, in the type of sheep Moldavian Karakul, hereditary capacities are sufficiently consolidated, a fact confirmed by the existence of a series of highly significant genotypic correlations. This refers, first of all, to the most important morpho-productive characters that determine the quality of the furskin and its surface, such as: the length of the loop, the extension of the loops, the silkiness and the luster of the hair fibers, the body mass of the lambs at birth, the length of the body and the class of evaluation.

Of the basic characters, which allow the appreciation of hereditary capacities for the production of furskins, they are constantly inherited and have significant genetic correlation the length of the loop (r = 0.47 ± 0.08), the extension of the loop (r = 0.45 ± 0.09), silkiness (r = 0.40 ± 0.09) and fiber luster (r = 0.30 ± 0.10). This fact is extremely important for increasing the efficiency of the selection of furskin qualities in the new type of sheep. Highly significant genotypic correlations also exist at the body mass of lambs at birth (r = 0.30 ± 0.03; t_r = 10.0; P <0.001) as well as at body length (r = 0.36±0.02; t_r = 18.0; P <0.001). These indices are very important for the creation of the new type of corpulent Moldavian Karakul sheep. Individuals with body length and large body mass were continuously selected for reproduction throughout the entire breeding period.

Genetic correlation with significant mean values (r = 0.48 ± 0.07) was also recorded at the inheritance of the most important polygenic character, such as the lamb evaluation class, given that it is a synthetic character, which accumulate a range of qualities of the furskin, appreciated in lamb during the evaluation.

It is remarkable that, the new type of sheep Moldavian Karakul constantly transmits by heredity not only the qualities of furskin, but also other production skills, such as the large body mass (meat production ability) of sheep at all ages. In our previous research (Buzu, 2012, 2014a) it has been demonstrated that, between the body mass of the rams and the body mass of the descent at birth there is an obvious genotypic correlation (r = 0.55 ± 0.09). The heritability coefficient of body mass is not high (h^2 = 0.3), but quite significant (t_r = 2.6; P <0.01). This is also confirmed by the fact that the body mass of the young Moldavian Karakul sheep has a fairly obvious repeatability at different ages and periods of ontogenetic development, including until adulthood.

The value of the coefficient of repeatability (r_w) of the body mass at different ages of the young sheep, as well as in the adult sheep, varies between 0.23 and 0.47 (Table 2).

Table 2. Genetic repeatability (r_w) of body mass in young sheep Moldavian Karakul

The age of the young sheep	The value coefficient of repeatability, $r_w \pm m_r$	The criterion of certainty, t_{rw}	The certainty threshold after the Student
At birth - 20 days	0.47 ± 0.07	6.7	P < 0.001
At birth –90 days	0.39 ± 0.09	4.3	P < 0.001
At birth - 6 months	0.26 ± 0.08	3.2	P < 0.01
At birth-18 months	0.23 ± 0.09	2.6	P < 0.01
In adulthood	0.25 ± 0.09	2.8	P < 0.01

This means that the more young the sheep develops at an early age, the greater the adult sheep mass will be.

These parameters confirm the fact that the type of Moldavian Karakul sheep has a high inheritance of high body mass, and the

selection of sheep according to this important morpho-productive character of the youth at different ages, as well as in the adult age of the sheep, is quite effective.

The genotypic capabilities of the type of Moldavian Karakul sheep are also manifested in the transmission by hereditary of such a polygenic character, as is the milk production.

Our previous research (Buzu, 2014b) has shown that, from parents with increased milk production, in principle, offspring with increased milk production skills are obtained (Table 3).

Table 3. Milk production of Moldavian Karakul daughter sheep according to the productivity of mothers

Milk production a mother-sheep, kg	Daughter's milk production, kg			
	N	M ± m	σ	C_v, %
> 110	7	87.10 ± 8.24***	21.77	25.0
90 - 109	13	77.69 ± 4.51***	16.40	16.5
70 - 89	21	68.10 ± 5.37*	24.60	36.1
50 - 69	28	65.36 ± 3.23**	17.10	26.2
< 50	8	48.75 ± 5.18	14.57	29.9

Remark: * - P < 0.05; ** - P < 0.01; *** - P < 0.001; Compared to daughters of the mother batch„<50 kg

Thus, from mother sheep with low milk production, up to 50kg, were obtained daughter offspring with low milk production (48.75 kg). With the increase of the milk productivity level of the mother sheep from 50 kg to 109 kg, the milk production of the daughters increased on average by 28.94 kg or 59.4% (P<0.001). The highest milk production (87.10 kg) was obtained from the daughters of the mother-sheep who had the highest milk quantity, over 110 kg.

The analysis of these data confirms that the milk production skills in sheep are constantly transmitted through heredity.

The hereditary power and the degree of influence of the parents on the structure of the genetic variability of the offspring after milk production, are determined by the value of the heritability coefficient (h^2), which in our research was not high, but quite significant (h^2 = 0.316; P <0.001), being in accordance with the level 0.3-0.4 of the information of Taftă et al. (1997a).

The research data confirm that the third part of the general phenotypic variability of the character of milk production is conditioned by the inheritance of the parents, and the other two thirds of variability of this character are according to other factors, mainly external ones, which generates a variability quite broad phenotypic. The coefficient of variation (C_V) of milk production is quite high, falling in values from 16.5 to 48.6%.

At the same time, this variability of milk production indicates the fact that there are still wide possibilities for the selection of sheep population by this important productive character.

From the data presented above we can conclude that, one of the genotypic particularities of the type of Moldavian Karakul sheep is the constant inheritance of the main selected characters, which demonstrates the sufficient genetic consolidation of the new type of sheep created and the possibility of maintaining at a high level, as well as of further enhancing the productive potential of this type.

Improver capabilities of ram-reproductive.

One of the genotypic particularities of the type of Moldavian Karakul sheep presents the improver capacities of the ram-reproductive, which are selected and used for reproduction with a high intensity. The most valuable reproductive are considered those rams, who have confirmed their improver status in multiple tests carried out in several years in a row, on different categories of females with different types of curl and evaluation classes. These rams possess a valuable genotype with well-established heredity.

During the period of approval of the type of Moldavian Karakul sheep, during the five years, a total of 112 breeding rams were tested, including 46 heads in the experimental household of the National Institute of Zootechnics and Veterinary Medicine (INZMV) and 66 heads in the Agricultural Cooperative of Production «Agrosargal» (Table 4).

Of the group of rams tested according to the descent qualities of INZMV, 34 rams were tested once, 2 rams - twice (doublutest), 5 rams - tripletest and 5 other rams were tested four and more times.

Table 4. Test results of reproducing rams by descent qualities over five years

Specification	INZMV	«Agro-sargal»	Total
Total rams tested, heads	46	66	112
including: unitest	34	47	81
doubletest	2	12	14
tripletest	5	5	10
cvadrutest and above	5	2	7
Total improver were found, heads	11	19	30
% of total	23.9	28.8	26.8
inclusive:			
1st grade improver	2	7	9
II grade improver	2	1	3
ordinary grade improver	7	11	18
They confirmed the value of the improver, heads:			
once	6	12	18
twice	2	4	6
three times	3	3	6

At ACP «Agrosargal» 47 rams were unitested, doubletested -12 heads, tripletested - 5 heads and quadrutested - 2 rams. In total, 81 reproductive ramsthey were only tested once, twice -14 rams, three times -10 rams and four times more -7 rams were tested at both sheep farms once.

According to the results of the descent testing in the two households, more than ¼ of the tested rams were recognized improver. In both farms, 30 improver rams were found out of the total 112 rams, which is 26.8%.

At INZMV were revealed 11 of the 46 test ramsimprovers, which is 23.9%. At the CAP «Agrosargal» were revealed 19 improver rams - which makes up 28.8% of the rams tested. In the group of rams improver of superior value rate (I degree improver) were divided into a total of 9 rams, including 2 - in farm INZMV 7 - in CAP "Agrosagal". They were assigned the highest category of improver.Three rams were assigned to the category - 2nd grade improver, of which 2 heads at the INZMV farm and 1 head at the CAP «Agrosargal». Ordinary improvers totaled 18 heads, including 7 heads at INZMV farm and 11 heads at CAP «Agrosargal».

According to the test results, they have repeatedly confirmed the value of 12 improver ramsreproducing. At the INZMV farm, 2 rams confirmed their improver value twice, and 3 rams - three times.

At the CAP farm "Agrosargal", 4 rams were confirmed twice the value of improver for the flock in which they were used, and 3 rams - three times.

Based on the data presented, we can conclude that reproducing rams, which have been used for breeding new intraracial sheep, are quite valuable and preponderant. The genetic qualities of improvers have been repeatedly confirmed.

From these rams in the herd, numerous valuable descendants were obtained, which inherited the superior morpho-productive qualities of the improver parents and contributed to the genetic improvement of the population in subsequent generations. The most valuable individuals of these generations were selected as founders and continuators of the elite lines (Buzu I., 2015).

Antigen frequency of blood groups and genetic distances.

The research results of the erythrocyte antigenic factors of the 7 blood groups (A, B, C, D, M, R, I), which include 15 erythrocyte antigens (Aa, Ab, Bb, Bd, Bg, Be, Bi, Ca, Cb, Da, Ma, R, O, I, i) they demonstrated the type (race) of Moldavian Karakul sheep possesses a karyotype with specific genetic characteristics, which identifies it highlighted by the classical sheep race of Asian Karakul, as well as by the local race Tusca.

Research on the immunogenic characteristics of sheep has shown that, in Moldavian Karakul sheep, they have a high frequency of antigens Bb, Bd, Bg, Be, Ca, Cb, Da, Ma, O and I, an average frequency of antigens Aa and R, and , a reduced (low) frequency of Ab, Bi and i. antigens (Table 5).

In the Tusca race, it was found that animals of both colours (greyish and black) were characterized by a certain similarity of blood factors, namely, by the high frequency of antigens Aa, Ma and I (0.6226 - 0.9275), low antigen of Be and R (0.1698 - 0.2754) and average of antigens Bb, Ca, O and i (0.3188 - 0.4928).

Between the greyish and the black sheep, essential differences in antigen frequency were not detected.

In order to improve the furskin qualities of the Tusca race, at the first stage of creation the

type, have been used the pure-race Asian Karakul rams were imported from Uzbekistan.

Table 5. Frequency of antigens in sheep races Karakul and Tusca

System	Antigen	Moldavian Karakul (N=383)	Tusca		Asian Karakul (N=113)
			greyish (N=53)	black (N=69)	
A	Aa	0.5796	0.6226	0.6956	0.2920
	Ab	0.1514	0.3019	0.1159	0.3197
B	Bb	0.6527	0.3774	0.4928	0.8407
	Bd	0.6867	-	-	-
	Bg	0.7232	-	-	-
	Be	0.6266	0.2642	0.2464	0.6637
	Bi	0.2260	-	-	-
C	Ca	0.9060	0.3396	0.4783	0.1239
	Cb	0.7467	0.6226	0.3768	0.5398
D	Da	0.7415	0.6226	0.5508	0.8673
M	Ma	0.8172	0.8868	0.9275	0.9735
R	R	0.3447	0.1698	0.2754	0.7522
	O	0.6005	0.4528	0.4203	0.2478
I	i	0.0496	0.3584	0.3188	0.0000
	I	0.9504	0.6416	0.6812	1.0000

As a result, the immunogenetics of new type sheep has undergone changes in the structure of antigens, genotypes and alleles.

According to the analyzes carried out, the newly created type inherited from the imported sheep a high frequency of Bb, Be and I antigens, and from the local breed Tusca of black colour - Cb antigen.

As a result of the marked genetic restructuring within the Aa antigen, the frequency of which was high in the local population of Tusca and reduced to that imported from Uzbekistan, the new type currently has an average frequency according to this factor. The same change also occurred within the R antigen, in particular that its frequency, in the initial race Tusca, was reduced, and in the Asian - Karakul breed - high, as a result, in the Moldavian Karakul type, the frequency of the R antigen is average (0, 3447).

It has also been found that the reduced frequency of the Ab type antigen in the new type was inherited from the black-colored Tusca sheep. The frequency of Ma and I antigens in all three populations, studied, is quite high.

Based on the immunogenetic analysis and the frequency calculations of the antigenic factors, the genetic distances were determined and the analysis of the genetic clusters was performed in their diagram (Fig. 1).

The obtained data demonstrates that the smallest genetic distance (0.1280) is between the sheep Tusca race at the black and greyish colour, which make up the cluster A.

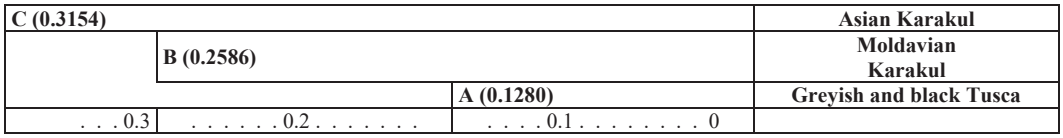

Figure 1. Diagram of genetic clusters of Karakul and Tusca sheep populations

Within cluster B, the new type of sheep Moldavian Karakul has a tangency, both with the local race Tusca and with the Asian Karakul race.

Thus, according to the genetic distance (0.2586) the new type sheep population occupies an intermediate position between the two related populations.

The pure Karakul race, imported from Uzbekistan, is at the greatest genetic distance, namely at point C (0.3154), which reflects its interrelation with the Tusca race and the type of Moldavian Karakul sheep.

Thus, the diagram analyzed confirms that the new type of Moldavian Karakul sheep corpulent, for the milk - furskins, has a specific genome of antigenic frequencies and was created by the method of crossing two different races, with the subsequent selection of the requested genotypes, according to the standard-purpose.

Genotypes and genetic references.

By analyzing the phenotypes of the families, the frequencies of the genotypes were detected and calculated in the Moldavian Karakul sheep population (Table 6).

Overall, 136 genotypes were detected by type. The highest frequency of genotypes is found in system A, D, M and I.

Table 6. Frequency of genotypes in the Moldavian Karakul sheep population

System	Its genotype and frequency			
A	a/a - 0.2115 a/- - 0.2402	a/b - 0.0679 ab/- - 0.0366	b/b - 0.0131 b/- - 0.0339	-/- - 0.3968
B	b/b - 0.0131 b/bg - 0.0026 b/bge - 0.0026 b/bdg - 0.0052 b/bdgi - 0.0026 b/bde - 0.0026 b/g - 0.0052 b/gde - 0.0026 b/ge - 0.0026 b/gei - 0.0026 b/d - 0.0104 b/dg - 0.0287 b/dge - 0.0131 b/dgei - 0.0026 b/de - 0.0157 b/e - 0.0104 b/- - 0.0183 bg/bg - 0.0052 bg/bge - 0.0026 bg/bd - 0.0026 bg/bde - 0.0026 bg/g - 0.0209 bg/ge - 0.0078 bg/d - 0.0862 bg/dg - 0.0052 bg/de - 0.0444 bg/dei - 0.0131 bg/di - 0.0052	bg/e - 0.0104 bg/ei - 0.0052 bg/- - 0.0026 bgde/- - 0.0026 bge/bge - 0.0052 bge/g - 0.0026 bge/ge - 0.0026 bge/d - 0.0235 bge/e - 0.0078 bge/i - 0.0026 bge/- - 0.0104 bgei/bde - 0.0026 bd/b - 0.0026 bd/g - 0.0052 bd/ge - 0.0052 bd/gei - 0.0026 bd/d - 0.0026 bd/e - 0.0052 bd/- - 0.0104 bdg/b - 0.0026 bdg/g - 0.0026 bdg/d - 0.0104 bdg/e - 0.0209 bdg/ei - 0.0052 bdg/- - 0.0157 bdge/b - 0.0026 bdge/bg - 0.0052 bdge/bdg - 0.0026	bgde/d - 0.0078 bgde/i - 0.0131 bgde/- - 0.0235 bdgei/bge - 0.0026 bdgei/- - 0.0026 bdgi/bge - 0.0026 bdgi/- - 0.0052 bde/b - 0.0052 bde/g - 0.0078 bde/gi - 0.0026 bde/d - 0.0052 bde/i - 0.0026 bde/- - 0.0026 be/g - 0.0052 be/d - 0.0052 be/dg - 0.0052 be/- - 0.0078 bei/g - 0.0026 bei/dg - 0.0052 bi/dge - 0.0052 bi/e - 0.0026 g/g - 0.0131 g/gei - 0.0026 g/d - 0.0078 g/e - 0.0261 g/- - 0.0209 g/ge - 0.0052 ge/- - 0.0052	gi/e - 0.0026 d/g - 0.0235 d/ge - 0.0104 d/gei - 0.0026 d/d - 0.0157 d/de - 0.0052 d/e - 0.0078 d/- - 0.0209 dg/d - 0.0104 dg/dg - 0.0026 dg/e - 0.0104 dg/ei - 0.0052 dg/- - 0.0104 dge/e - 0.0026 dge/- - 0.0131 dgi/g - 0.0026 dgi/d - 0.0026 de/g - 0.0104 de/gi - 0.0052 de/d - 0.0026 de/de - 0.0131 de/e - 0.0052 e/e - 0.0235 e/- - 0.0418 ei/d - 0.0026 -/- - 0.0131
C	a/a - 0.1880 a/- - 0.0287	a/b - 0.6868 ab/- - 0.0026	b/b - 0.0496 b/- - 0.0104	-/- - 0.0339
D	a/a - 0.5196	a/- - 0.2089	-/- - 0.2715	
M	a/a - 0.7180	a/- - 0.1070	-/- - 0.1750	
R	RR - 0.1621	Rr - 0.2033	rr - 0.6346	
I	II - 0.9373	Ii - 0.0131	ii - 0.0496	

In system A, the highest frequency had the genotypes -/- (hyphen/hyphen) and a/-, constituting 0.3968 and 0.2402 respectively.

In system B the frequency of genotypes is generally quite low. In this system, the highest frequency is at genotypes $B^{bg/d}$ - 0.0862, $B^{bg/de}$ - 0.0444, $B^{e/-}$ -0.0418, $B^{b/dg}$ - 0.0287 and $B^{g/e}$ - 0.0261.

Basically, it was found that, compared to other systems, the frequency of genotypes in system B is the lowest, obviously, out of the total number of genotypes, 67-74% are genotypes with frequency below 0.01.

This phenomenon indicates that, in the created type, high heterozygosity can be maintained, without returning to infusion with other types of sheep Karakul.

In system C, as well as in system A, 7 genotypes were detected, including 3 homozygotes and 4 heterozygotes. The highest frequency of homozygotes is in the $C^{a/a}$ genotype - 0.1880, and in the heterozygotes, in the $C^{a/b}$ genotype - 0.6868. Of all the genotypes in this system, the lowest frequency is at $C^{ab/-}$ - 0.0026 genotype.

Regarding the other systems (D, M, R and I), 3 genotypes were detected, of which 2-homozygous and 1-heterozygous. The most common genotypes in the nominated system sare: $D^{a/a}$ - 0.5196, $M^{a/a}$ - 0.7180, $R^{r/r}$ - 0.6346 and II - 0.9373.

Along with determining the frequency of genotypes, the frequency of alleles, which is closely related to each other, was calculated. A total of 48 alleles were detected in the sheep population analyzed (Table 7).

Table 7. Frequency of alleles in the type Moldavian Karakul sheep

System	Alleles and their frequency			
A	A^a - 0.3655	A^b - 0.0640	A^{ab} - 0.0183	A^- - 0.5522
B	B^b - 0.0836	B^{bi} - 0.0039	B^{bgde} - 0.0235	B^{bde} - 0.0170
	B^g - 0.0862	B^{ge} - 0.0196	B^{bgei} - 0.0013	B^{gde} - 0.0013
	B^d - 0.1397	B^{gi} - 0.0052	B^{bdg} - 0.0313	B^{dge} - 0.0170
	B^e - 0.1031	B^{dg} - 0.0431	B^{bdge} - 0.0052	B^{dgi} - 0.0039
	B^i - 0.0091	B^{de} - 0.0509	B^{bdgi} - 0.0052	B^{dgei} - 0.0013
	B^{bg} - 0.1084	B^{di} - 0.0026	B^{bdgei} - 0.0026	B^{dei} - 0.0065
	B^{bd} - 0.0183	B^{ei} - 0.0091	B^{bde} - 0.0170	B^{gei} - 0.0052
	B^{be} - 0.0117	B^{bge} - 0.0379	B^{bei} - 0.0039	B^- - 0.1110
C	C^a - 0.5457	C^b - 0.3981	C^{ab} - 0.0013	C^- - 0.0548
D	D^a - 0.6240	D^- - 0.3760	-	-
M	M^a - 0.7715	M^- - 0.2285	-	-
R	R - 0.2637	r - 0.7363	-	-
I	I - 0.9439	i - 0.0561	-	-

In system A, a high frequency of the A^- - (0.5522) and low - of the A^{ab} - (0.0183) allele was detected.

System B contains a multitude of alleles, similar to those of the aforementioned genotypes.

Thus, by a comparatively higher frequency, the alleles B^d - 0.1397, B^- - 0.1110, B^{bg} - 0.1084 and B^e - 0.1031 are distinguished. A slightly lower frequency is at allele B^g - 0.0862 and allele B^b - 0.0836. With regard to the other 26 alleles of this system, a reduced frequency was established in most cases.

Within system C, the highest frequency has the C^a allele - 0.5457, and the lowest one - the C^{ab} allele - 0.0013.

Regarding systems D, M, R, and I it was found that the synthesis of red cell antigens is controlled by two alleles. High frequency alleles in these systems include: D^a (0.6240), M^a (0.7715), R^r (0.7363), and I (0.9439). The lowest frequency in these four systems was found in alleles M^- (0.2285), R (0.2637) and I^i (0.0561).

These are the characteristics of the frequency characteristics of the immunogenetic alleles of the new type of Moldavian Karakul sheep.

Degree of homozygosity.

Particular importance for the genetic and zootechnical characteristics of the races and types of sheep is determined by the degree of hetero- and homozygosity, which can confirm the effectiveness of the selection process and the genetic improvement works.

Following the analyzes carried out, the level of homozygosity and allele efficiency in the population of Moldavian Karakul was calculated (Table 8).

Table 8. Degree of homozygosity and allele efficiency in the Moldavian Karakul sheep population

System	Degree of homozygosity	Number of efficient alleles
A	0.62	1.6129
B	0.10	10.000
C	0.27	3.7037
D	0.89	1.1236
M	0.40	2.5000
R	0.79	1.2658
I	0.99	1.0101
General	0.58	3.0309

The data show that the degree of homozygosity in the blood group systems in the new type of sheep is different, depending on the antigenic system.

The highest degree of homozygosity was recorded in systems I (0.99), D (0.89), R (0.79) and A (0.62). A lower degree of homozygosity was found in systems B (0.10), C (0.27) and M (0.40). The general homozygosity in the sheep population Moldavian Karakul constituted 0.58. The degree of homozygosity greater than 0.50 demonstrates sufficient genetic consolidation of the newly created races and types.

The highest allele efficiency was recorded in system B (10.0) and C (3.704). The overall efficiency per allele is 3.0309 and falls within the medium limits of sufficiently efficient characteristic of the selection process.

Therefore, based on the immunogenic characteristics we can conclude that, the new type of sheep Moldavian Karakul is genetically

well consolidated, having a karyotype with a specific genetic structure, which distinguishes it from other types within the Karakul race.

CONCLUSIONS

The main genotypic particularity of the type (race) of Moldavian Karakul sheep is that, the hereditary capacities of the sheep are well consolidated genetically and are constantly transmitted through descent.

In particular, the morpho-productive characters, which determine the quality of the furskin, have significant genotypic (mother-descendant) correlations, such as: loop length (r=0.47±0.08; t_r = 5.9; P <0.001); extension of the loop (r = 0.45±0.09; t_r = 5.6; P <0.001); silk fiber (r = 0.40±0.09; t_r = 4.4; P <0.001); fiber luster (r = 0.30±0.10; t_r = 3.0; P <0.01); body length of lamb at birth (r = 0.36±0.02; t_r = 18.0; P <0.001); body mass of lamb at birth (r = 0.30 ±0.03; t_r = 10.0; P <0.001) and evaluation class (r = 0.48±0.07; t_r = 6.8; P < 0.001).

The large body mass of the sheep (meat production aptitude) is constantly transmitted through heredity. Between the body mass of the father-rams and the body mass of the descent at birth there is an obvious genotypic correlation (r = 0.55±0.09). The heritability coefficient of body mass is not high (h^2 = 0.3), but quite significant (t_r = 2.6; P <0.01).

The value of the coefficient of repeatability (r_w) of body mass at different ages of young sheep and adult sheep is 0.23–0.47.

The consolidated genotypic capacities of the type of Moldavian Karakul sheep are also manifested when transmitting milk production through hereditary. The value of the heritability coefficient (h^2) is not high, but quite significant (h^2 = 0.316; P <0.001).

Most breeding rams have valuable hereditary capabilities. Out of the total 112 rams tested for descent qualities, 30 rams were recognized as improvers, which is 26.8%.

The genotypic qualities of improver have been repeatedly confirmed. Breeding rams were still used to for reproduction new type sheep.

The type (sheep) of the Moldavian Karakul sheep has a karyotype with specific genetic characteristics, which is identified by the classical Asian Karakul race and the local race Tusca, after the erythrocyte antigenic markers of the 7 blood groups (A, B, C, D, M , R and I), which include 15 red cell antigens (Aa, Ab, Bb, Bd, Bg, Be, Bi, Ca, Cb, Da, Ma, R, O, I and i).

Moldavian Karakul sheep are characterized by a high frequency (0,6005-0,9504) of antigens Bb, Bd, Bg, Be, Ca, Cb, Da, Ma, O and I, a medium frequency (0.3447-0.5796) of antigens Aa, R and, a reduced frequency (0.0496-0.2260) of antigens Ab, Bi and i.

In the diagram of the genetic clusters, the sheep population Moldavian Karakul occupies an intermediate position in cluster B, between the two related populations with a genetic distance of 0.2586, compared to the local raceTusca of the cluster A with the genetic distance of 0.1280 and in front by the Asian Karakul of the C cluster with the genetic distance of 0.3154.

Overall, by type, 136 genotypes and 48 alleles with specific frequencies were detected. The degree of homozygosity as a whole by type constitutes 0.58, which shows sufficient genetic consolidation for the growth "in itself".

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COMPARATIVE STUDY ON THE PERFORMANCE OF BEES POPULATIONS OF BUCKFAST HYBRID AND THE NATIVE RACE *Apis melifera carpatica*, EXISTING IN THE AREA OF IAȘI

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Abstract

Given the performances of the bees of the native breed Apis melifera and those of the Buckfast hybrid, it was desired to find out what are the performances of some products obtained from the crossing of the two populations. The study was conducted between 15.04.2017 and 10.06.2018 at the acacia harvest, where four families of different breeds were selected, respectively 2 families from the native breed Apis melifera carpatica (A1 and A2) and 2 families from the Buckfast hybrid. (B1 and B2). The families taken in the study were chosen from the hive depending on the degree of development. The characters pursued for the obtained products were: the quantity of honey harvested, the gentleness, the instinct of roasting, the building of artificial honeycombs as well as the amount of pollen harvested in the ascending and descending population during 2017 and 2018. Regarding the amount of pollen harvested in 10 days, the B2 family recorded 2.6 kg, for honey production, the harvest is significant in favor of the daughters of the Buckfast hybrid.

Key words: *bees, honey, pollen.*

INTRODUCTION

The improvement of bee populations implies the conservation and improvement in purebred, based on the artificial selection of productive and adaptability to the environment that the population has acquired during its evolution (Drăgănescu et al., 2006; Bienefeld et al., 2007) In a balanced population (Hardy-Weinberg law), in natural conditions (geographical breed) in bees, the performance of the offspring is intermediate between the performance of the parents. The situation occurs when a character is controlled by additive genes (honey production).

Interracial hybridization (crossbreeding between individuals belonging to different races) causes the effect of heterosis or hybrid vigor, a phenomenon found in all plant and animal species (Boeking et al., 2000). In bees, the effect is controlled only in order to obtain the first generation (F1), the results leading to the improvement of certain performances only in the first generation. Performance improvement occurs only at the level of the individual and not to improve the performance of the population that is subject to selection (Meixner et al., 2013).

The phenomenon of heterosis that occurs in a hybrid can be explained as follows: the products resulting from the crossing of individuals belonging to different genetic populations (hybrids) are more vigorous (heterozygous) than purebred individuals (parents), these being homozygous (Căuia et al., 2009).

The superiority of hybrids is given by the manifestation of the phenomenon of more intense heterozygosity (different alleles of a gene appear on the same locus) as well as of non-additive genetic interactions (dominance, superdominance, epistaxis). The resulting individuals may be more vigorous or productive compared to their parents (Horia and Pascal, 2005; Bura and Pătruică, 2011).

The honey bee is the only species of economic interest that is totally dependent on the natural environment. It has evolved for millions of years,

being largely under the control of natural selection (Căuia and Siceanu, 2009).
Due to the species' biology, breeding and developed technologies, human intervention has focused on maintaining and supporting the development of bee families in order to ensure optimal production conditions. But the most important role is played by the environment, which is the space for obtaining food naturally (nectar and pollen) and reproduction (Drăgănescu et al., 2008; Ruttner, 1980).
The Romanian bee has been studied since 1937-1939 (Fişteag and Farcaş), when it was proposed as an independent breed - *Apis melifera carpatica*. In 1965, at an international level, Foti conducted extensive studies that are currently being continued by the Research and Development Institute for Beekeeping. In 1958, the Romanian Beekeepers Association, and in 1974 the Research-Development Institute for Beekeeping conducted research on the conservation and improvement of the local genetic fund (Foti et al., 1965).
Considering the performances of the bees of the native breed *Apis melifera* and those of the hybrid Buckfast, it was interesting to see the performances of some products obtained from the crossing of the two populations. Thus, the aim of the paper is to study the performance of the bee population obtained from the hybrid Buckfast and the native breed *Apis melifera*, bred in the Oţeleni area, Iaşi county. For this, the characters were followed: the amount of honey harvested, gentleness, family development, swarming instinct, artificial honeycomb building and the amount of pollen harvested from the ascending and descending population, during 2017 and 2018.

MATERIALS AND METHODS

The biological material was represented by four families of bees, exploited in a private PFA apiary, located near the forest of the Forest District Iloaei Forest and has a herd of 150 bee families, of which 120 families produce honey and other bee products. The difference of 30 families are intended for reproduction. The study was conducted between 15.04.2017 and 10.06.2018.
At the acacia harvesting, 4 production families from different breeds were chosen, respectively: 2 families from the native breed *Apis melifera carpatica*; 2 families from the Buckfast hybrid.
The families studied were chosen from the apiary, depending on the degree of development. During the study, the characteristics of gentleness and instinctive swarming were followed, continuing with a series of production characteristics: the amount of pollen harvested, the amount of honey extracted, the degree of development of families.
1. Two families with native bee *Apis melifera carpatica* (A1 and A2) (Figure 1) from breeding families. The selected families have achieved in the past years the following results: the amount of honey: rapeseed honey 8 kg/family; acacia honey 19.5 kg/family; lime honey 18 kg/ family; sunflower honey 17 kg/family; pollen harvested 2 kg/family.

Family A1

Family A2

Figure 1. *Selected families of the native breed Apis melifera carpatica*

2. Two families with the Buckfast hybrid (B1 and B2) (Fig. 2) from instrumentally seeded breeding families. The selected families have been tested for several years, their results in previous years were: the amount of honey: rapeseed honey 14 kg/family; acacia honey 42 kg/family; lime honey 40 kg/family; sunflower honey 41.5 kg/family; pollen harvested 5 kg/family.

Family B1

Family B2

Figure 2. Families selected from the Buckfast hybrid

The characteristics of the breed were also followed in terms of meekness and the manifestation of the swarming instinct.

RESULTS AND DISCUSSIONS

Pollen harvest results

Pollen harvesting from Oţeleni commune was carried out between 15.04.2017 and 15.10.2017. For 2017, the mother family *Apis melifera* recorded a production of 3.4 kg pollen/year, compared to the amount of 5.0 kg pollen/year harvested from the hybrid mother family Buckfast (+1.6 kg pollen/year) (Table 1).

Table 1. The amount of pollen harvested in 2017

Specification	The amount of pollen collected (kg/an)	
	Apis melifera	*Hybrid Buckfast*
The mother family	3.4	5.0
Daughter 1	3.6	5.3
Daughter 2	3.2	5.0

Thus, daughter 1 of the *Apis melifera* breed collected a quantity of 3.6 kg pollen/year, and daughter 2 - 3.2 kg pollen/year. Comparing the amount of pollen with that harvested in Buckfast hybrid daughters, the results show a harvest of 5.3 kg pollen/year in daughter 1 (+1.7 kg pollen /year) and 5.0 kg pollen/year in daughter 2 (+ 1.8 kg pollen/year).

The study on pollen harvesting of fruit trees in the commune of Oteleni, was conducted between 15.04.2017 and 10.06.2017, corresponding to the harvest of acacia, the results being shown in Table 2.

The research period was 10 days, after which 1.2 kg of pollen were recorded in family 1 *Apis melifera* and 1.45 in family 2 *Apis melifera*. In the Buckfast families, the harvest recorded a value of 2.1 kg/family in B1 and 2.6 kg in B2.

The analysis of Table 2 shows the performance of Buckfast hybrid families, the differences being significant (+0.9 kg pollen in family B1 and 1.15 kg pollen in B2).

Table 2. The amount of pollen harvested in 2018

Specification	***Apis melifera***		***Hybrid Buckfast***	
Pollen (kg)	*Family A1*	*Family A2*	*Family B1*	*Family B2*
	1.2	1.45	2.1	2.6

Results on the amount of honey extracted

The amount of honey extracted was recorded for all types of harvesting: rapeseed, acacia, raspberry, linden, sunflower and hay (polyfloral honey). For 2017, the mother family *Apis melifera* recorded a honey production of 81.5 kg of honey/year, compared to the amount of 166.5 kg of honey/year harvested from the Buckfast hybrid mother family (+85.0 kg of honey/year) (Table 3). The largest quantities of honey were extracted at the harvest of acacia (19.5 kg of honey/year), at the harvest of lime

(18 kg of honey/year), followed by the harvest of sunflower (17 kg of honey/year), and for polyfloral honey (11 kg of honey/year).
From the daughters *Apis melifera*, coming from the mother family, in 2017, 87.5 kg of honey / year were extracted from daughter 1 *Apis melifera* and 77.0 kg of honey/year from daughter 2 *Apis melifera* (Table 3).
Analyzed by types of harvest, the daughters of *Apis melifera* recorded the following quantities of honey: from daughter 1 *Apis melifera*, the best yields were obtained by picking lime (22 kg of honey/year), by picking acacia (21 kg honey/year), followed by sunflower harvesting (16 kg honey/year); from daughter 2 *Apis melifera,* the best yields were obtained by picking acacia (19 kg of honey/year), by picking linden (18 kg of honey/year), followed by picking sunflower (15 kg of honey/year). year (Table 3).

Table 3. The amount of honey harvested for 2017

Assortments of honey	***Apis melifera***			***Hybrid Buckfast***		
	Mother family	Daughter 1	Daughter 2	Mother family	Daughter 1	Daughter 2
Rape	8.0	10.0	9.0	14.0	16.0	11.0
Acacia	19.5	21.0	19.0	42.0	43.0	38.0
Raspberry	8.0	9.0	7.0	16.0	12.0	13.0
Linden	18.0	22.0	18.0	40.0	39.0	41.0
Sunflower	17.0	16.0	15.0	41.5	48.0	18.0
Polyfloral	11.0	9.5	9.0	13.0	15.0	16.0
Total kg/year	**81.50**	**87.50**	**77.00**	**166.5**	**173.0**	**137.0**

Also for 2017, the parent family of the Buckfast hybrid recorded a honey production of 166.5 kg of honey/year.
The largest quantities of honey were extracted at the harvest of acacia (42 kg of honey/year), at the harvest of sunflower (41.5 kg of honey/ year), followed by the harvest of linden (40 kg of honey/year).
From the daughters of the Buckfast hybrid, coming from the mother family, in 2017, 173 kg of honey/year were extracted from daughter 1 Buckfast and 137 kg of honey/year from daughter 2 Buckfast. The number of dairy cows has continuously decreased during the period 1990-2010, with a negative impact upon milk production.
Milk yield is the only positive aspect, because it has increased reaching 3,980 kg per cow in the year 2010.
As a consequence of the reduced number of cows but an increased milk yield, milk production has continuously increased, except the year 1995 when it recorded the lowest level.
The North Eastern region is traditionally suitable for cow rearing, due to its pastures and meadows, the important number of cow livestock and possibilities to produce ecological milk.
The differences in harvested honey production are significant in favor of the daughters of the Buckfast hybrid, with a difference of +85.5 kg honey/year for daughter 1 Buckfast and +60 kg honey/year for daughter 2 Buckfast.
For 2018, the differences in harvested honey production are significant in favor of the daughters of the Buckfast hybrid, with a difference of +85.5 kg honey/year for daughter 1 Buckfast and +60 kg honey/year for daughter 2 Buckfast (Table 4).
For the native breed *Apis melifera*, the amount of honey harvested was 18.6 kg from the two warehouses and 4.3 kg of honey harvested from the nest on the marginal frames of the A1 family.

Table 4. Quantities of honey extracted from the studied families

SPECIFICATION	***Apis melifera***		***Hybrid Buckfast***	
	Family A1	*Family A2*	*Family B1*	*Family B2*
Store	18.6	17.4	31.5	28.8
Nest with framed frames	4.3	2.60	10.5	7.2
TOTAL	22.9	20.0	42.0	36.0

In family A2, the amount of honey harvested was 17.4 kg (+ 1.2 kg compared to A1) and 2.6 kg of honey from the nest (-2 kg compared to family A1). The total amount of 20 kg of honey was distributed as follows: 87% of the warehouses and 13% harvested from the nest on the marginal frames. For the families from the Buckfast hybrid, the quantities of honey varied from 42 kg in the B1 family to 36 kg harvested in the B2 family. The B1 family produced 4 warehouses ½ reduced of merchandise honey, and due to the selection and improvement of this hybrid 75% of the merchandise honey was found in the 4 warehouses (31.5 kg) and 25% in the nest, placed above the brood located on frames (7.2 kg). The B2 family made 3 warehouses ½ reduced merchandise honey, of which 80% of the honey was found in warehouses (28.8 kg) and 20% in the nest, located above the 10 frames with brood (7.2 kg).

In order to analyze the development of bee families in 2017, observations were made in several periods, taking into account the following elements: - between 15.04.2018 and 15.07.2017 bee families developed reaching a varied number of frames, between 7 and 12 frames in *Apis melifera* bees and between 10 and 15 frames in Buckfast hybrid families (Table 5); - after 20.07.2017 and artificial swarms were formed, as a result of which extracts of framed frames were made, 3 for each family, so that the number of frames varied from this period until 15.09.17 between 9 and 6 frames in the *Apis melifera* families and in a number of 9 frames in the Buckfast families

Table 5. The development of the families studied in 2017

Assortments of honey	***Apis melifera***			***Hybrid Buckfast***		
	Mother family	Daughter 1	Daughter 2	Mother family	Daughter 1	Daughter 2
15.04.2017	9	7	8	10	10	9
15.05.2017	10	8	9	12	11	10
15.06.2017	12	11	11	14	12	11
15.07.2017	12	11	11	15	12.5	12
ARTIFICIAL SWARM FORMATION - FRAMEWORK HARVESTING						
20.07.2017	-3	-3	-3	-3	-3	-3
15.08.2017	9	8	8	9	9	9
15.09.2017	7	6	6	9	9	9
FORMATION OF WINTER NESTS ON BEES						
15.10.2017	8	8	8	10	10	9

Starting with 15.10.17 the bee families regardless of breed or hybrid went into hibernation, the family being composed of 8 frames for *Apis melifera* and between 9 and 10 frames for the Buckfast hybrid

Family development in 2018

To the acacia harvest, when the families reached their apogee and registered a uniform development on the nest side, the warehouses for honey merchandise were added.

Therefore, for *Apis melifera*, family A1, which had 7 frames with brood, 2 warehouses of honey goods were added, and for family A2, which had 8 frames with brood, 2 warehouses were added (Table 6).

Table 6. The development of the families studied in 2018

SPECIFICATION	***Apis melifera***		***Hybrid Buckfast***	
	Family A1	*Family A2*	*Family B1*	*Family B2*
Frames puiet	7	8	9	10
Honey merchandise stores	2	2	4	3
TOTAL	9	10	13	13

For the Buckfast hybrid, the B1 family, which had 9 frames with brood, added 4 warehouses of honey, and for the B2 family, which had 10 frames with brood, 3 warehouses were added.

Manifestation of bee gentleness in 2017 During 2017, stings were recorded only from the *Apis melifera* families. Thus, from the mother family received 9 stings, 7 stings in daughter 1 *Apis melifera* and 13 stings in daughter 2 *Apis melifera* (Table 7).

Table 7. The result on gentleness in bee families studied in 2017

Specification	*Apis melifera*			*Hybrid Buckfast*		
	Mother family	Daughter 1	Daughter 2	Mother family	Daughter 1	Daughter 2
Number of stings	9	7	13	0	0	0

The manifestation of the bees' gentleness in 2018, in the Buckfast hybrid families, the bees showed gentleness, not being cases of attack on the beekeeper (Table 8).

Table 8. Assessment of the character of gentleness in the studied families

SPECIFICATION	*Apis melifera*		*Hybrid Buckfast*	
	Family A1	*Family A2*	*Family B1*	*Family B2*
Number of stings	7	13	-	-

The manifestation of the swarming phenomenon in 2017

In 2017, in the studied families, the swarming phenomenon was manifested only in the native breed, *Apis melifera* (Table 9).

The manifestation of the swarming phenomenon in 2018

After the honey extract, the two autochthonous families underwent directed multiplication, obtaining two artificial swarms, reducing one store for each family. In the harvest season for fruit trees, the A1 family built 3 barrels, and the A2 family a number of 8 barrels. At the acacia harvest, the A1 family built a number of 5 hulls, compared to the A2 family, with 12 hulls built (Table 10).

Table 9. Manifestation of swarm instinct and number of barrels

Specification	*Apis melifera*			*Hibrid Buckfast*		
	Mother family	Daughter 1	Daughter 2	Familia mama	Daughter 1	Daughter 2
Picking acacia	3	7	11	0	0	0
Pick lime	3	23	7	0	0	0
Total boots	6	30	18	0	0	0

Table 10. Manifestation of swarm instinct and number of barrels in 2018

COMB	*Apis melifera*		*Hybrid Buckfast*	
	Family A1	*Family A2*	*Family B1*	*Family B2*
Number of barrels of fruit trees	3	8	0	0
Number of acacia barrels	5	12	0	0
Total combs	8	20	0	0

Number of artificial honeycombs in 2017 For 2017, the number of artificial honeycombs built by the studied bee families ranged from 1 honeycomb in polyflora harvesting to 6 honeycombs in acacia harvesting in the mother family *Apis melifera* (Table 11), compared to 2 honeycombs when picking lime from the Buckfast mother family, up to 7 honeycombs when picking acacia. Throughout 2017, the studied mother families recorded a total of 16 honeycombs in *Apis melifera* and 22 honeycombs in the Buckfast hybrid (+6 honeycombs) (Table 11).

Table 11. Number of artificial honeycombs in 2017

The type of picking	*Apis melifera*			*Hybrid Buckfast*		
	Mother family	Daughter 1	Daughter 2	Mother family	Daughter 1	Daughter 2
Rape	2	2	2	3	3	3
Acacia	6	5	5	7	6	6
Linden	4	4	4	6	6	6
Sunflower	3	3	3	4	4	4
Polyfloral	1	1	1	2	2	2
Total combs	16	15	15	22	21	21

Analyzing table 11, it is observed that among the daughters, the number of artificial honeycombs per year was maintained at 15 for the daughters *Apis melifera* and at 21 for the hybrid daughters Buckfast.

The number of artificial honeycombs in 2018

The families from the Buckfast hybrid did not show swarming instinct and therefore no seedlings were harvested, being maintained to test the predisposition to swarm. As of June 10, 2017, the hybrid families did not show signs of swarming, but a greater development of the brood area comprising 5 reduced frames.

The F1 family managed to build a number of 4 artificial honeycombs when picking fruit trees and 6 honeycombs when picking acacia. The F2 family managed to build a number of 3 artificial honeycombs when picking fruit trees and 5 honeycombs when picking acacia.

Table 12. Number of artificial honeycombs formed in 2018

COMB	*Apis melifera*		*Hybrid Buckfast*	
	Family A1	*Family A2*	*Family B1*	*Family B2*
Harvesting from fruit trees	2	3	4	6
Acacia picking	3	4	3	
Father honeycombs	5	7	7	

CONCLUSIONS

Following the study performed on the productive performances and the characters of the native breeds *Apis melifera* carpatica and of the hybrid Buckfast, the following conclusions can be drawn:

For 2017, the mother family *Apis melifera* recorded a production of 3.4 kg pollen/year, compared to the amount of 5.0 kg pollen/year harvested from the hybrid mother family Buckfast (+1.6 kg pollen/year). For 2018, the research period was 10 days, following which 1.2 kg of pollen were registered in the A1 family and 1.45 in the A2 family. In the Buckfast families, the harvest recorded a value of 2.1 kg/family in B1 and 2.6 kg in B2.

In 2017, the differences in harvested honey production are significant in favor of the daughters of the Buckfast hybrid, with a difference of +85.5 kg honey/year for daughter 1 Buckfast and +60 kg honey/year for daughter 2 Buckfast.

In 2018 the differences registered between the 4 families were in favor of the Buckfast hybrid, which compared to the same family of *Apis melifera* registered an increase of: Family B1 - 12.9 kg for honey harvested from warehouses and +5.9 kg for honey harvested from the nest and for Family B2 - +11.4 kg for honey harvested from warehouses and +4.6 kg for honey harvested from the nest.

For 2017, between 15.04.2018 and 15.07.2017, the bee families developed reaching a varied number of frames, between 7 and 12 frames for bees from the *Apis melifera* family and between 10 and 15 frames for Buckfast hybrid families, and after 20.07.2017 and artificial swarms were formed, as a result of which extracts were made with brood frames, 3 for each family, so that the number of frames varied from this period until 15.09.17 between 9 and 6 frames in the *Apis melifera* families and in a number of 9 frames in the Buckfast families. For the year 2018, the A1 family that presented 7 frames with broods introduced 2 warehouses of honey goods, and for the A2 family that presented 8 frames with broods, 2 warehouses were also introduced. For the Buckfast hybrid, the B1 family which had 9 frames with brood was

introduced 4 warehouses of honey, and for the B2 family which had 10 frames with brood, 3 warehouses were also introduced.

In both years, in the Buckfast hybrid families, the bees showed gentleness, with no cases of attack on the beekeeper. Reverse situation in the autochthonous race, where both families showed a high aggressiveness towards the administrator.

In 2017, 6 barrels were registered for the mother family, while for the daughters the highest number was 23 barrels, made by the daughter 1 *Apis melifera*, when the lime was harvested. Daughter 2 *Apis melifera* made 11 barrels, but at the acacia harvest

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GENETIC DIVERSITY OF PINZGAU CATTLE BREED: A SYSTEMATIC REVIEW

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Abstract

The present work aims to review the most important aspects regard to genomic characterization of Pinzgau cattle breed. In Romania, this cattle breed is part of the country´s genetic and cultural heritage and faces the impact of bottleneck and the lack of diversity due to a significant decline in population. Natural populations´ genetic structure is influenced by a limited gene flow that occurs when the geographic distances between them increase. In our country, the Food and Agriculture Organization of the United Nations (FAO) maintained the status of ' endangered-maintained ' cattle breed in 2000 considered to be threatened with extinction. This paper wants to highlight the fact that the conservation of this breed is a national priority and also, reviews the most relevant information in the literature on the genetic diversity of this cattle breed.

Key words: *Pinzgau cattle, genetic diversity, cattle breeds.*

INTRODUCTION

The Pinzgau breed is named after its origin area, near Salzburg, Austria, and is a mountain cattle breed. The breed originated from local mountain breeds in the 19th century and evolved in three directions: traction, milk and meat. In Romania, the Red Pinzgau breed has been established since the second half of the nineteenth century, after the absorption of crosses made between the local cattle breeds Grey Steppe, Mocanita and Pizgau of Austria. Also, "Cow of Dorna" or "Black Pinzgau" was created by the crossing of local cattle with specific mountain breeds: Pinzgauer, Mölltal, Zillertal etc. (Popa et. al., 2012).

Over the last few decades, the biodiversity of cattle has decreased dramatically. The genetic degradation observed was mainly due to the specialization of livestock production in favor of cosmopolitan and high quality breeds, so, the control of the genetic diversity of cattle has therefore become an important concern in the management of livestock breeding programmes (Kukučková et. al., 2017).

The Pinzgau breed, currently, meets in more than 25 countries around the world. Special color mottled red-brown spots on the side of the body and white line became the character of the breed.

In Romania, the Pinzgau breed is meets in three areas: the NW of Moldova, the SW of Transylvania and the W of Transylvania-Apuseni. Transylvanian Pinzgau breed has a strong constitution, lively temperament, docile disposition, average precocity, high endurance, good adaptation ability, resistance to disease and environment (Kadlecik et. al., 2004).

Dorna cow is less than 1-2 cm tall than Red Pinzgauer, the rectangular body is more pronounced, the bones and muscles are better developed and the background is black (Fisteag, 1958).

According to FAO reports, the Pinzgau breed is threatened with extinction, entering the category of endangered cattle. FAO studies often draw attention to the numerical decline of different species, the classification of different breeds, taking into account the number of individuals for each breed, the ratio of females to males, and their inclusion in active conservation or maintenance programs by companies or research institutions, as one of the following categories: extinct, critical, endagered, critical-

maintained, endangered-maintained, not at risk (Scherf, 2000).
Due to its rusticity, resistance to a particular hilly and mountainous climate, with a remarkable successful longevity and survival, the expression of its genetic characteristics distinct from other breeds, Pinzgau must be considered a component of national genetic resources.
The main objective of this study was to provide a thorough insight into the genomic characterisation of the Pinzgau cattle breed (especially the Romanian Pinzgau breed) through the use of high-performance molecular information.

MATERIALS AND METHODS

In order to reach the objectives of this study, 13 bibliographic sources from the specialized literature were consulted. The main issues addressed refer to the morphological and productive evaluation of the Pinzgau breed, especially in our country, as well as information regarding the genetic diversity of this breed, which is currently in danger of extinction.
The research methods used in this study were the observation, analysis and graphical interpretation of data from the specialized literature regarding numerical evolution, morpho-productive characteristics and genetic analysis of the Pinzgau cattle breed.

RESULTS AND DISCUSSIONS

1. The morphological and productive characteristics of the Pinzgau cattle breed

The Pinzgau breed was first developed between 1690 and 1740 by the crossing of local red bulls with the Bern type of Switzerland, after 1740, the resulting animals were used for breeding in the true breed and is located in the mountain areas of Romania at an altitude of over 1000 m. This breed is originates in Austria, Salzburg, Tyrol, alpine and subalpine areas (Maciuc, 2006).
In 1820, Pinzgau breed were exported to countries such as Romania, Yugoslavia, the Czech Republic and Slovakia. Throughout South Africa, Canada, the USA and Australia, even under the harshest weather conditions, Pinzgau thrived (Kadlecik, 2004).
An extensive study on the morphological and production characteristics of Pinzgau cattle, from the Apuseni Mountains area and from the Hațeg and Petroșani Depression was carried out between 1956-1962 by Dincă et al., whose synthesis is presented below. The average body weight of the cows was 382.3 kg, the cows in the area of the valleys had 392 kg body weight, and those in the mountain area and in the premonton area 371 kg.
Figure 1 shows the weight of the Pinzgau (females) breed, for 2014, in comparison with the cows from the breeds: Brown, Romanian Black Spotted, Romanian Spotted and other beef cattle breeds, undefined.

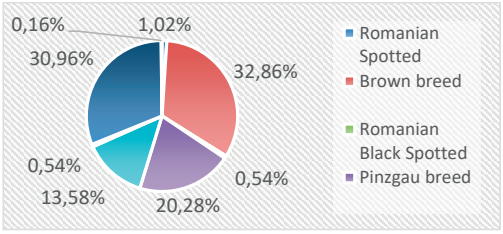

Figure 1. The share of Pinzgau cattle breed from the total of cows in Romania, registered in the herd book, 2014

In Romania, as mentioned by FAO-1993, the Pinzgauer cattle included 1092 females registered in the national herd book. However, the recorded population trend was considered to be decreasing. According to figure 1, in 2014, the Pinzgau breed represented 20.28% of the total number of cattle from the bovine species, in the territory of our country.
For 2015, the situation regarding the weight of the Pinzgau breed from the total number of cattle in Romania is presented in figure 2.

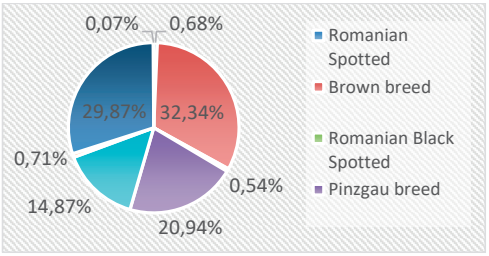

Figure 2. The share of Pinzgau cattle breed from the total of cows in Romania, registered in the herd book, 2015

According to figure 2, in 2015, the Pinzgau breed represented 20.94% of the total number of cattle from the bovine species, in the territory of our country, a higher percentage by 0.66 compared to the previous year.
Regarding the exterior, the conformation and the constitution, in general, the cattle of this breed present a pleasant, attractive and harmonious exterior, having a characteristic conformation to the breeds with intermediate production skills (Georgescu et. al., 1998).
The body development is relatively variable, depending on the area of spread and the local geoclimatic conditions (table 1).
Within the breed there is a variety known as Dorna Cow or Black Pinzgau. It is a special type of breed, widespread in the area of the same name, especially around the localities of Vatra Dornei, Câmpulung Moldovenesc and Gura Humorului.
The researchers conducted by Acatincăi in 2004, show that there are morphological differences between the cattle of the Pinzgau breed, found in the northwest of Moldova, the Apuseni Mountains or the Dorna Depression.
The phenotypic performances in the direction of milk production are different, with limits between 1900-3500 kg and 3.62- 4% fat. As for the herds from the official production control, they have a very sinuous evolution (table 2).

Table 1. The main morphological characteristics of the Pinzgau breed

Characteristic (cm)	*NW Moldovei (cattle)*	*Apuseni Mountains (cattle)*	*Cows*	*Bulls*	*Dorna Cow*
Waist	126.3	129.4	128.6	134	125
Length of the trunk	151.2	155.0	153.1	163.1	151
Thoracic depth	67.4	67.9	67.5	74.3	64
Thoracic perimeter	178.8	179.3	179.1	199.7	176
Body weight	469.2	482.8	471.1	662.9	444

**Acatincăi, 2004*

Table 2. Number of lactation, average milk production and fat content

Pinzgau cattle breed			
Lactation	I	IV	VII
Average milk production (kg)	2849.79	3313.89	3300
Fat content of milk (%)	3.77	3.75	3.73

*Official Control of *Production*, during 2014-2015

Regarding the evolution of milk production according to the lactation rank, it has a linear character.
Imported animals of the Pinzgau breed have produced a lower yield of milk under the conditions of processing in Romania than in Austria. The protein, fat and lactose content of milk was also lower (Gîlcă & Gîlcă, 2012).

2. Genomic uniqueness of the Pinzgau cattle breed

The genome of many breeds of cattle was studied by researchers and the research results have been published over time in numerous articles. The research was centered on the analysis of genetic markers correlated with the characteristics of the productions, in the case of cattle breeds threatened with extinction (as is the case with the Pinzgau breed), this was useful for the understanding of the importance of the survival of genetic capital relevant to animal origin, the degree of uniformity of the breed and, corroborated by several other important molecular markers, their place of development and domestication. The genetic structure of natural populations is determined by the minimal gene flow that happens as the spatial differences between them increase. Genetic segregation of the breeds decreased the variation at the molecular stage, which can be controlled by growing homozygosity (Felius et al., 2014).
Investigating Pinzgauer populations in Austria, Bavaria, Germany, Erhardt (1996) discovered a new K-casein variant (K-CN G) with a frequency of 0.003 by isoelectric focusing in polyacrylamide gels and by alkaline polyacrylamide gel electrophoresis. K-CN G

was not present in milk samples of Limpurger, another endangered breed.

A. Caroli et al. analized in the original Pinzgauer cattle, milk protein genetic variation and casein haplotype structure. A total of 485 dairy specimens from Original Pinzgauer from Austria (n=275) and Germany (n=210) were isoelectrofocused to evaluate the genetic variation influencing the protein amino acid charge in dairy proteins αS1-casein, β-casein, π-casein, α-lactalbumin and β-lactoglobulin. A rather elevated genetic variation influencing the amino acid charge of dairy proteins characterizes the Original Pinzgauer breed, with a total of 15 alleles, 12 of which were discovered at a frequency > 0.05. With 4 alleles identified, the most polymorphic protein was β-casein. CSN1S1*B, CSN2*A2, CSN1S2*A, CSN3*A, LGB*A, and LAA*B were the predominant allles. A comparatively high frequency of CSN1S2*B (0.202 in the entire information set) was discovered, primarily occurring within the haplotype C-A2-B-A (in the order CSN1S1-CSN2-CSN1S2-CSN3), which appears to be unique to the original Pinzgauer, potentially due to the survival of an ancient haplotype or Bos indicus introgression.

A specific white spotting phenotype, termed finching or line-backed spotting, is known for all Pinzgauer cattle and occurs occasionally in Tux-Zillertaler cattle, two Austrian breeds. The so-called Pinzgauer spotting is inherited as an autosomal incompletely dominant trait. Based on 777k SNP data, a genome-wide association study using 27 white spotted and 16 solid-colored Tux-Zillertaler cattle revealed a strong signal at the Kit locus on chromosome 6. Haplotype analyzes described the Kit coding region's critical interval of 122 kb downstream. Whole-genome sequencing of a Pinzgauer cattle and comparison with 338 control genomes disclosed a complicated structural version composed of a deletion of 9.4-kb and a reversed duplication of 1.5 kb fused from chromosome 4 to a 310-kb duplicated section. A diagnostic PCR for this structural variant (Kitpinz) was created for the simple genotyping of carriers and confirmed the presence of the variant allele in all Pinzgauer and most white spotted Tux-Zillertaler cattle. The introgression of the Kitpinz variant confirms admixture and the reported historical relationship with Austrian Tux-Zillertaler of these short-headed breeds and suggests a mutation event that occurs before breed formation (Kuttel et. al., 2019).

Ivan Pavlík et. al. (2014) researched genetic variation in the Pinzgau breed in Austria and Slovakia. A total of 12,442 individuals were used in the sample reflecting the reference population and have been studied four sub-populations. The mean inbreeding coefficient (five generations taken into account) was 0.0186, 0.0242, 0.0151 and 0.0126 for Austrian dairy products (AD), Austrian beef (AB), Slovak dairy products (SD) and Slovak beef (SB) respectively. The effective size of the population varied from 122.5 (AD) to 809.4 (SB).

Genetic evaluation represents an important tool in breeding and cattle selection, Romanian Pinzgau being a part of active breeds adapted to local conditions with local origin. Currently, in Romania, Pinzgauer cattle breed is included in a genetic program of conservation.

CONCLUSIONS

Pinzgau cattle breed represents a valuable genetic reserve for livestock of Romania. This is more strategically necessary than ever to maintain as much livestock variety as possible and to ensure a prompt and timely response to the needs of future generations.

The Romanian Pinzgau must be regarded as a part of national genetic wealth, owing to its rusticity, tolerance to the unique hilly and mountainous climate, with a remarkable endurance, an indication of its genetic distinction from other breeds.

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RELATIONSHIP BETWEEN METRITIS AND ANIMAL NUTRITION

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Abstract

Turkey is one of the leading countries of Europe in terms of animal existence. Today, yield per animal is important, not the number of animals. Reproduction is one of the indispensable items of dairy businesses. Most dairy cows do not achieve their reproductive performance goals, and economic losses occur at significant rates. The purpose of breeding cattle health is to ensure that cows become pregnant again until the best time after freezing and thus keep the time between the two raisins within economic limits. Reproductive performance is closely related to the baby's prenatal and postnatal health. Being pregnant again after birth is the most important factor for profitability. Immunosuppression is suppressed in long time, undernourished or stressed animals and the incidence of mastitis and metritis in animals is increasing. Economic losses due to this case take place. Considering the economic dimensions of these losses in our country, studies on 155 enterprises were made in Burdur, Kırklareli and Konya provinces. Within these studies, disease related loss was 1.258 turkish liras for clinical metritis, 697 turkish liras for retention.

Key words*: breeding, health, immunosuppression, metritis, reproduction.*

INTRODUCTION

Our country is one of the leading countries in the world in terms of animal existence. Today, the productivity per animal is important, not the number of animals.

Reproduction is one of the indispensable elements of dairy farms. In many dairy cows, reproductive performance targets cannot be achieved, significant economic losses occur. The purpose of reproductive herd health is to ensure that the cows become pregnant again in optimal time after calving, and therefore to keep the time between the two calves within economic limits. Reproductive performance is closely related to the health of the cow in the weeks before and after birth. The conception of the cow as soon as possible after birth is an indispensable factor for profitability (Kocaarslan, 2013).

The immune system is suppressed in animals that cannot become pregnant for a long time, malnutrition or under stress (Foldi et al., 2006) and the incidence of animals in cases such as mastitis and metritis increase (Görgülü, 2011). Economic losses occur due to these cases. Considering the economic dimensions of this loss in our country, studies were conducted in 15 enterprises in Burdur, Kırklareli and Konya provinces. Within the scope of these studies, the loss due to disease was determined as 1.258 TL and 697 TL per animal for clinical metritis and retentio secundinarum, respectively (Yalçın, 2008). The purpose of this review is to identify uterine infections, their causes, and their relationship to animal feeding.

POSTPARTUM PHYSIOLOGY AND UTERINE INVOLUTION

Processes for cows to conceive again after birth include uterine involution, regeneration of the endometrium, elimination of bacterial contamination of the uterus and resumption of cyclical activity of the ovary. Uterine involution; physical shrinkage, laxation of the karsts and regeneration of the endometrium (Sheldon et al., 2008). At birth, uterus weight is approximately 10 kg, whereas postpartum falls to 5 kg on day 6, 2 kg on day 12, 1kg on day 25 and 0,7 kg on day 50 (Öcal, 1997; Kocaarslan, 2013). Caruncula forms lochia flow and also contains blood from fetal fluids and broken umbilical veins in lochia (Figure 1). Initially endometrial regeneration occurs in the intercarpal region and then continues with the proliferation of cells to cover the sacrococcygeal regions (Sheldon et al., 2008)

The thickness of the cervix in uterine involution drops from 30 cm to 2 cm on postpartum day 7 cervix. Lochia is red brown in color and has no odor, sometimes is more fluid and yellowish-white. Postpartum day 14-23. the lochia is cut (Sheldon et al., 2008). In the first 15 days of cervical involution, the uterus is slower to involution and normally the diameter of the cervix is larger than the diameter in corns of uterus. Within 25-47 days of the postpartum, the cervix and uterine involution are substantially completed in terms of physical size (palpation-induced involution). A complete microscopic involution lasts longer and is resulted in around the day 25-50 postpartum. Although the physiological role of prostaglandin F2 alpha (PGF2α) within the first month of the postpartum is not clearly defined, it is believed that it plays a role in the induction of uterine contractions and thus contributes to uterine involution (Sheldon et al., 2008).

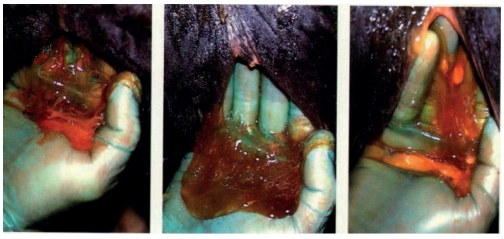

Figure 1. Normal lochia flow (Güler, 2015).

DIAGNOSTIC METHODS IN UTERINE DISEASES

Uterus; mechanically preserved anatomically by bacterial contamination by the vulva, vestibular sphincter and cervix (Bondurant, 1999). Immunologically, the defense mechanism consists of two parts, cellular and humoral. Cellular defense is largely composed of polymorphonuclear leukocytes (PML), and the humoral defense mechanism consists of globulins (Hussain, 1991). When bacteria reach the uterus, the most important part of the defense of the uterus towards bacteria is the nonspecific phagocytosis of neutrophil leukocytes (LeBlanc, 2008). Neutrophil leukocytes come to the inflammatory zone with the induction of various peptides produced by microorganisms in the inflammatory zone. Neutrophils come to the surface of tissues, cavities and microorganisms. During the periods when progesterone is dominant over the defense mechanism, the immunoreaction is reduced, whereas when the estrogen is dominant, the immunity is stimulated (Lewis, 1997).

PATHOGENESIS

The uterus is sterile before birth. During the birth and postpartum period, the cow's feces and skin are contaminated with bacteria from the surrounding environment and the uterus becomes contaminant (Sheldon and Dobson, 2004). During the first two weeks of the postpartum, the cow's uterus is in contact with a wide variety of bacteria ranging from 80% to 100% (Lewis, 1997; Azawi, 2008). The presence of uterine bacteria at this period is normal (Foldi et al., 2006). More than 90% of the cows are contaminating with uterine bacteria within the first 15 days after birth, while the rate of uterine bacterial culture gradually decreases within 2-4 weeks of the postpartum as the involution process progresses. On the 45th day after birth, this rate is 9% or less (Bondurant, 1999). In short, the bacterial contamination of the uterus in the first two weeks after birth does not necessarily mean that there is a clinical inflammation. That is, a distinction must be made between uterine contamination and uterine infections. Interstitial infection means the formation of the uterine-related disease following a series of events such as attachment of pathogenic bacteria to the mucosa, proliferation and colonization, penetration of colonized bacteria into epithelial tissue, and release of bacterial toxins (Sheldon et al., 2006; Azawi, 2008).

The postpartum uterine lumen environment promotes the multiplication of aerobic and anaerobic bacteria. Most of these bacteria contaminates the uterine lumen and then the bacteria are removed with the aid of the uterine defense mechanism. In addition, *Arcanobacterium pyogenes* (*A. pyogenes*), *Escherichia coli* (*E. coli*), *Fusobacterium necrophorum* (*F. necrophorum*) and *Prevotella spp.* Bacteria often play a role in the formation of uterine diseases. Indeed, *A. pyogenes, F. necrophorum and Prevotella spp.* it has been determined that they act synergistically to increase the likelihood of uterine disease. It has

also been found that these bacteria increase the likelihood of clinical endometritis and the severity of infections. Numerically, the most common pathogens are *E. coli* and *A. pyogenes*. E. coli infections are already formed and provide the basis for A. pyogenes infections (Sheldon et al., 2008).

UTERINE İNFECTIONS

Metritis is uterine cavity, inflammation of the superficial and deep layers. Edema, leukocyte infiltration and myometrial degeneration are seen. Endometritis is only the endometrium, that is, the mucous membrane. If this involves inflammation; that is serosal perimetritis. If it also contains ligaments, it is called parametritis (Sheldon et al., 2006; Sheldon et al., 2008).

ACUTE PUERPERAL METRITIS

It usually occurs within 21 days following birth, and as shown in Figure 2, it usually occurs 4-10 days after birth (Foldi et al., 2006; Sheldon et al., 2006; Sheldon et al., 2006).

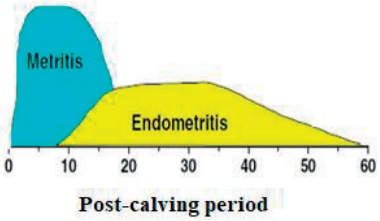

Figure 2. The period in which postpartum metritis species were active (Sheldon et al., 2008).

Acute puerperal metritis is characterized by a foul-smelling, watery uterine content with tissue rashes in it, enlarged and thin-walled uterus due to delayed involution, 39.5℃ fever, loss of appetite, stagnation, A. pyogenes, *E. coli* and *F. necrophorum, Bacteriodes spp., Provetella spp.* (Tuna, 2010) In the diagnosis of Puerpural metritis, rectal heat is observed in the first 10 days after delivery and a vaginal discharge is observed every 3 days until the postpartum day 21 is reached. The vagina make to be palpation. Vaginal discharge is mucopurulent, purulent, and putrified (Sheldon et al., 2004; Huzzey et al., 2007).

ENDOMETRITIS

Endometritis refers to the inflammation of the uterine endometrium layer. It is not deeper than the inflammatory in stratum spongiosum (Sheldon et al., 2006) and is the inflammatory response to all kinds of bacterial, viral, mechanical, thermal, chemical and toxic effects that affect the uterus itself. it is possible to mention many factors that cause endometritis and cause predisposition. Among these, dystocia, retention, secundinarum, and attempts to help birth follow infusion of irritant substances into the uterus (Youngquist, 1997). In vitro study, bovine herpesvirus (BHV-4) was claimed to play an important role in endometritis (Donofrio et al., 2007). Dystocia is an important cause of endometritis predisposition. First, the probability of retentio secundinarum and placental disposal is higher in those who have power delivery than in those who have normal birth (Table 1).

Table 1. Excretion times of postnatal membranes (Youngquist 1997).

Birth after	Membranes
Time, hour	Excretion rates, %
3	16.0
6	77.3
9	88.7
12	94.6
15	96.2
18	97.8
21	98.5
24	100.0

In addition, obstetric assistance and maternal tissue injuries during power delivery increase the incidence of uterine pathogens. Acute puerperal metritis is these factors that cause predisposition. also cause chronic endometritis in the healing process (Paisley, 1986; Correa, 1993; Noakes, 2001). In addition, inflammatory reaction leading to the separation of the caruncula and cotyledons in the postpartum period and lack of uterine contractions cause the placenta to be ineffective and thus endometritis (Guard, 1999). Microorganisms that cause endometritis often reach the uterus vaginally during mating, insemination or postpartum period. However, in some cases, transmission

through the blood (hematogenous) may also be possible. In majority of cows, post-natal bacterial contamination of the uterus is mentioned, but in normal conditions this flora is rapidly eliminated. Endometritis can't perform uterine elimination of bacteria along with the inflammatory reaction of the endometrium in the shaped animals. Although the rate of bacterial contamination of the uterus is important in the development of endometritis, the pathogenesis of the disease is linked to the ability of the animal to eliminate infection rather than to bacteria (Kaya, 2008).

CLINICAL ENDOMETRITIS

Postpartum 21 days or more in the next days, purulent, mucopurulent exudates or is characterized by inflammation of the vagina. Cervical thickness is more than 7,5 cm at postpartum 21 days or after 26 days. In this time period, the vagina purulent uterine exudate is enough for diagnosis (Gilbert et al 2005, Sheldon et al., 2006). The most important symptom of clinical endometritis in cows is increase the enlarged uterus and vaginal discharge with cervix-wide (LeBlanc et al., 2002; Kasimanickam et al., 2004; Gilbert et al., 2005).

SUBCLINICAL ENDOMETRITIS

It is the inflammation of the endometrium without clinical symptoms and usually the uterine flow is not visible on the external side (Sheldon et al., 2006). In subclinical endometritis cows, there is a marked reduction in reproductive performance despite the absence of a clinical symptom (Sheldon et al., 2009). Endometriosis refers to an excessive infiltration of neutrophil leukocytes, and this increase can only be determined by endometrial cytology, not by the purulent structure of the cervical fluid (Foldi et al., 2006; Sheldon et al., 2006). The frequency of subclinical endometritis coincidence ranges from 37-74% (Sheldon et al., 2009), with a great deal of time depending on the time of diagnosis and postnatal transplantation. The main distinguishing feature between endometritis and metritis is that endometritis cows are not clinically disease (Güler, 2015). Subclinical endometritis is a major cause of infertility in cattle.

PYOMETRA

Postnatal functioning is a uterine inflammation characterized by accumulation of purulent or mucopurulent contents in the uterine lumen in the presence of a functional corpus luteum and its progesterone, and the resultant expansion of the uterine lumen (Sheldon et al., 2006). Although a functional corpus luteum is present, the cervix is not always fully closed and the pus flows mainly through the cervix to the vaginal space (Sheldon et al., 2006; Sheldon et al., 2008). Piyometra should be separated from clinical endometritis. The pylorus is less common than clinical endometritis. Pyometra is less than 5% less than the uterine clinical fever (Sheldon et al., 2008).

HIPOCALCEMIA AND METRITIS RELATIONSHIP

Postpartum period hypocalcemia causes dysfunction of bone, muscle and nerve tissue (Hayirli and Çolak, 2011). As shown in Grummer (1996), normocalcaemic cattle are more balanced in terms of energy balance and lower in concentration of NEFA than hypocalcemic cattle.

Subclinically hypocalcemic cattle have lower concentrations of neutrophils in the first 3 days of postpartum. The uterus health deteriorates due to the resulting immunosuppression. The incidence of metritis is parallel to the calcium concentration. Metritis has been reported development in 75.3% of hypocalcemia cattle (Martinez et al., 2012) (Figure 3).

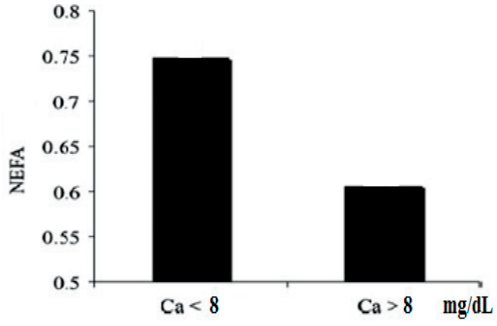

Figure 3. Calcium and NEFA between relation (Martinez et al., 2012)

RELATIONSHIP BETWEEN NUTRITION AND UTERUS HEALTH

In the periparturient period, negative energy balance arises if the energy requirement can't be met by feed consumption to meet body needs, growth of fetus and maintain lactation (Figure 4). Adipose tissue is used for energy needs, depending on the lack of enough energy source in the environment. Mobility of triglycerides in the adipose tissue results in nonesterified fatty acids (NEFA). In animals with high weight condition scores (WCS ≥3.5), the amount of nonesterified oil acid (NEFA) used to meet energy needs increased in circulation (Holtenius, 2003; Mashek, 2003; Arslan and Tufan, 2010). According to Theilgaard (2002), stressors such as epinephrine and norepinephrine increase lipolytic stimulation more as the amount of unfamiliar fatty acid (NEFA ≥ 0.6 mEq / L) increases (Ingvartsen, 2006).

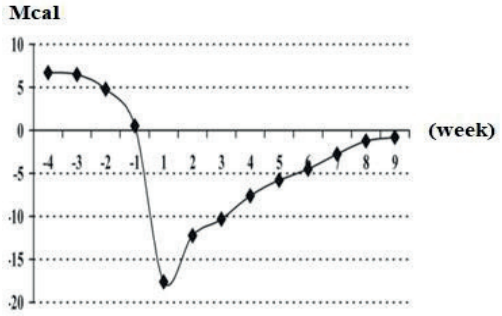

Figure 4. Peripartum energy balance (Hayirli and Çolak 2011)

Approximately 50% of these fatty acids (NEFA) are oxidized to ketone bodies in the liver or re-esterified to triglycerides. Re-esterification of triglycerides and ketogenesis is carried out in hepatic tissue when NEFA is too abundant in the medium, and it is also converted to BHB, which is the predominant ketone body in a part of NEFA (Emery, 1992; Butler 2003). Since triglycerides can't be removed sufficiently by the liver as low-density lipoprotein (LDL), a significant portion is stored here (Rukkwamsuk, 1999). The increase in ketone bodies with the accumulation of triglycerides in the liver results in the formation of metabolic disorders such as fatty liver syndrome and ketosis. In addition, oocytes, granulose and immune cells undergo oxidative stress due to the high concentration of ketone bodies. Anovulation is prolonged due to negative energy balance (Hoeben et al., 1997; Suriyasathaporn, 1999; Bisinotto, 2012).

Increasing NEFA due to suppression of dry matter consumption in 2 weeks before birth and myeloperoxidase activity of suppressed neutrophils are associated with negative energy balance. The increase of both NEFA and BHBA in 2 weeks after birth may decrease the possibility of conception of the animal in early lactation (Hammon et al., 2006; Walsh et al., 2007; Ospina et al., 2010).

NEGATIVE ENERGY BALANCE AND HORMONAL BALANCE

Dairy cattle are hypo insulinemic in the early lactation period. The lower plasma insulin concentration in the mammary gland stimulates the increased oxidation of fatty acids and reduces glucose uptake and oxidation in insulin-responsive tissues (Butler, 2003). These hormonal changes lead to a decrease in dry matter consumption in the dry period and to the mobilization of fat deposits in postpartum period (Drackley, 2005; Ingvartsen, 2006). Insulin and Growth hormon-Insulin-like growth factor-1 (GH-IGF-1) play an important role in regulating metabolic activity during negative energy balance (NEB) of lactation in leptin and thyroid hormones (Chilliard et al., 2005). Negative energy balance (NEB) can delay ovarian activity by affecting the release of Luteinizing hormone (LH). When glucose and insulin levels decrease, it lowers the release of LH and limits the response of the ovary to gonadotrophins and endogenous opioids and other lactation hormones are provided by the inhibition of the pituitary gland of LH release necessary for the follicular development of the ovary (Butler, 1989).

Insulin-like growth factor-1 (IGF-1) is a factor affecting reproductive activity. IGF-1 is defined as a hormonal stimulant of pregnancy in cattle. It has been reported that IGF-1 significantly influences hepatic adaptation (carbohydrate and lipid) and many types of immune system during the transition period (Clark, 1997; Heemskerk, 1999; Van Dorland et al., 2009). The circulating levels of IGF-1 in the cows are gradually

decreased in the subsequent lactation and lower during delivery to obtain maximum concentration during the uterine involution period. Cattle with endometritis produce less cytokines before inflammation. These cytokines are necessary for the inflammatory response and regulate the proliferation and function of the neutrophils in the infected uterus (Ahmadi et al., 2015).

OXIDANTS AND OXIDATION MECHANISM

Reactive oxygen species produced in excessive amounts in organism for any reason interact with nucleic acids, lipids, proteins, enzymes and carbohydrates, causing harmful effects resulting in cell damage and death. (Halliwell, 1993; Yerer and Aydogan, 2000). Free radicals make the most important effects on lipids. This effect is called lipid peroxidation. This reaction takes place continuously as a requirement of aerobic metabolism. Unsaturated fatty acids found in the cell membranes are the most susceptible to free radical damage due to the excessive number of double bonds (Kocaarslan, 2013). There is an increase in the amount of reactive oxygen species (ROS) in cows associated with the metabolic needs of pregnancy, labor and lactation, and this amount of ROS produced triggers stress due to oxidative oxidation in the animal (Sordillo, 2005). These ROS's initiate lipid peroxidation and start to damage the cells. In particular, the cells responsible for the immune system are much more susceptible to oxidative stress. Because their cell walls contain a high amount of polyunsaturated fatty acids (PUFA) and therefore they are much more susceptible to peroxidation. They also produce very large amounts of ROS at the same time when their immune cells are stimulated. The oxidative stress experienced during the transition period makes the animals more susceptible to other diseases. Dry matter consumption is significantly reduced in the few days before birth and the animal's immunity system is very weak (Kimura, 2002; Goff, 2006; Hammon et al., 2006). Alterations in the levels of sudden estrogen and progesterone during the last days of birth and a very serious increase in plasma cortisol levels during labor are another factor affecting the suppression of the immune system (Goff, 1997; Ingvartsen, 2006). Changes in the cellular level in the immune system occur (Mallard et al., 1998) and lymphocyte functions, antibody responses, cytokine production. (Cai et al., 1994, Kimura, 2002).

ANTIOXIDANTS AND TRACE MINERALS

Zinc, copper, selenium, manganese, molybdenum are micro-level substances essential to the body. Trace minerals are found at low levels in the tissues of the body. It is difficult to use in terms of skeletal development, immunological response and reproductive performance (Figure 5).

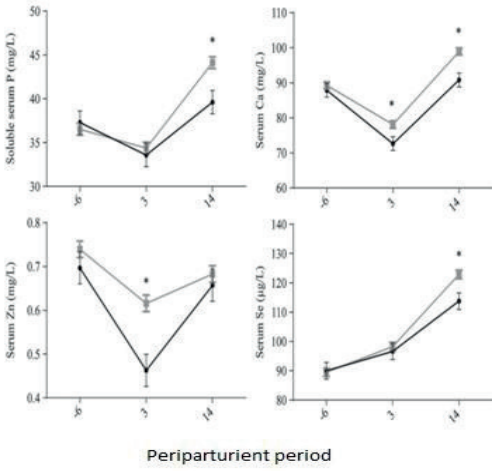

Figure 5. Concentration of trace minerals in endometritis and healthy cows in the periparturient period (Bicalho et al., 2014)

Zinc and copper serve as cofactors for components and enzymes of metalloenzymes. Enzymes that are copper chelated in cattle are important for survival (Underwood and Suttle, 1999). Xin et al. (Pogge et al., 2012) Zinc is one of the important elements for the health and growth of the animal. It has been reported that the neutrophils have a SOD (superoxide dismutase) and bactericidal capacity in the patients with copper deficiency (1991). Inadequate growth, deep anomalies, reproductive problems (Suttle, 2010).

During the transition period, the trace mineral consumption decreases due to the decrease in animal feed consumption. The alternative way of tracing mineral reinforcement is injection

method. Induction of mineral traces of Cu and Se increased the concentration of liver by injecting 15 days of inception and increased the plasma concentration of Zn and Mn at several hours. In a study, 2 doses in the dry period were reduced with injections containing zinc, selenium, manganese and copper, as shown in table 2 (Machado et al., 2013).

Table 2. Percentage in relation to diseases of trace minerals used against uterine diseases (Machado et al., 2013)

Uterine Health	Control (%)	Micro mineral (%)
Dead birth	6.1	4.3
Endometritis	34.2	28.6
Metritis	11.5	11.8
Retensiyo Sekundinaryum	6.7	6.8
Abomasal displacement	2.6	1.3

VITAMINS AND OTHER SOURCES

Colostrum contains vitamin E and vitamin A at high levels. Ruminants in the postpartum period suffer from the inadequacy of these two vitamins by removing the colostrum (Goff, 2002).

In addition to changes in beta-carotene, vitamin A and vitamin E energy balance, vitamin sources that regulate antioxidant concentrations fall during birth (Goff, 2002). These vitamins play an important role in the immune function. Low concentrations of these vitamins are associated with suspicion of falling fertility and diseases in dairy cattle. Concentration of beta-carotene and vitamin E in circulation is lower in healthy cattle than in cattle with retention secondary cultures (LeBlanc et al., 2004).

ANTIOXIDANT EFFECT OF VITAMIN E-SELENIUM

Selenium deficiency is a negative effect on reproduction in all species. This problem has been widely reported in cows and sheep and selenium insufficiency is the direct cause of abortions, and selenium-related problems have increased susceptibility to diseases, and it has been stated that infertility is observed by complicating the excretion of the placenta. Vitamin E and selenium have been reported to complete involution in 8 days. Blood selenium flow was associated with high selenium in the ovary and this increase was reported to significantly increase GSH-Px activity in the follicular fluid and luteal tissue (Duraisamy, 2010). In animals fed vitamin E as feed additive, circulating NEFA and cortisol fall during frosting (Pontes et al., 2015). Addition of selenium and vitamin E increases the concentration of selenium in the serum and colostrum at the end of the dry period (Moeini et al., 2009). Vitamin E and selenium have also increased pregnancy rates in cows and sheep (Segerson, 1977; Segerson et al., 1981). According to Segerson (1981), this effect is attributed to the increase in sperm transport (Kocaarslan, 2013).

Addition of vitamin E and selenium to feeds reduces the incidence of retentio secundinarum (Segerson et al 1981). Arechiga et al., (1994) reported that a single prepartum injection of Vitamin E and selenium increased fertility. Vitamin E and selenium are reported to increase the incidence of juvenile dysplasia and have a positive effect on uterine health. Arechiga et al. (1998) reported that the number of inseminations per pregnancy decreased significantly in cows treated with vitamin E and selenium, and the pregnancy rate increased significantly.

Vitamin E and selenium are applied enough in well fed sheep and cows. Segerson (1977) suggested that selenium and vitamin E increase uterine contractions by affecting uterine muscle functions. In sheep, when vitamin E and selenium were applied, the fertilization rate was higher than the other group. In female sheep treated with vitamin E and selenium, the data of oestrus showed more total uterine contraction and more correct transduction in oviducts. Studies conducted in previous dairy cows suggest that selenium and vitamin E have a positive effect on reducing uterine muscle function and decreasing the incidence of retinococcal secretion (Duraisamy, 2010). Selenium, vitamin E and combinations of these have been reported to increase fertility in some studies (Segerson, 1977; Segerson et al., 1981; Arechiga, 1994), but not in others (LeBlanc, 2002), when assessed for fertility effects.

CONCLUSIONS

Metabolic changes in the puerperal period are negative for uterine health. There are applications to minimize the negative effect of these metabolic changes caused by negative energy balance on uterine health. In this period, glycogenic and lipogenic diets are recommended. The gluconeogenic adducts, including propylene glycol, glycerol and propionic acid, are glucose precursors and increase serum glucose concentration. Increased serum glucose concentration lowers fat mobilization due to increased insulin levels and low energy demand and thus increases reproductive efficiency in terms of uterine health. Glyconeogenic nutrient supplements have a stimulatory effect on insulin secretion. Propylene glycol appears to be a very effective glyconeogenic substance and reduces plasma NEFA and BHB concentrations. Propylene glycol and its metabolites stimulate insulin secretion.

Cattle feed consumption is low in early lactation and dietary fat supplement changes energy status in the early postpartum period. Increased caloric concentration with fat added to the ration improves the reproducible parameters. This improvement depends on the composition of the fatty acid. Polyunsaturated fatty acid (PUFA) positively affects the balance between the hypothalamus, pituitary and ovarian. Linoleic acid stimulates uterine involution (Gábor ve ark 2016).

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POSSIBILITIES FOR USING PLANT EXTRACTS IN THE COMBINED FORAGE FOR THE SUCKING AND WEANED PIGS

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Abstract

The purpose of the research was to establish the possibilities for using plant extracts as an alternative of the nutritional antibiotics for growing pigs. Under the conditions of the performed experiment we established that the average live weight at birth was 1,512.4±24.6 g. The average weight of the experiment pigs was 1,459.6±42.9 g at birth, which is almost 80 g less, compared to the control animals. At the end of the sucking period, a higher average live weight by 2.2% is registered for the experiment animals, despite the unreliable differences in the groups. Putting in the biologically active plant supplement increases the average daily gain of the pigs by 4.8% in comparison with the control animals, and the overall gain by 4.6%. The inclusion of the biologically active complex of dry extracts from the plants Cichoria intybus L, Cotinus coggygria L. and Tanacetum vulgare in the mixes of the growing pigs leads to significant decrease of the number of the unwanted microorganisms in the digestive system of the animals – aerobic mesophiles, coliforms, staphylococci and micrococci, and enterococci, etc.

Key words*: antibiotics, faecal samples, microbiological analisys, plant extracts, sucking pigs.*

INTRODUCTION

After the complete ban of the subtherapeutic doses of antibiotics by the EU, a lot of controversial opinions appeared, originating from the sudden change in the productive indicators of the animals, the occurrence of persistent diarrhoea in the young animals, the economic losses in the industry.

A lot of antibiotics supporters appeared, due to an increase in the morbidity rate of the pigs, respectively an increase in their therapeutic use, as well as a productivity decrease of the different animal categories, especially with the weaned pigs (Casewell et al., 2003; Phillips et al., 2004; Vigre et al., 2008).

Plant and herbal extracts are a new direction in the search for a new alternative of nutritional antibiotics for the nutrition of young animals.

The use of herbs and their healing effect have been known since ancient times. According to Trifunschi et al. (2017), medicinal plants like *Viscum album* L. and *Allium sativum* L. are an appropriate alternative for the medication of different diseases. New methods are being investigated to highlight bioactive compounds from these authors.

In our country there are unique and valuable herbal plants, which allows the production of forage supplements for the needs of stockbreeding, and which can successfully replace the subtherapeutic doses of antibiotics, used as growth stimulators for years.

Studying various herbal mixtures in appropriate concentrations and doses would find practical application in solving some health problems when the pigs are being weaned (Ivanova-Peneva and Kanev, 2014).

All this gave us a reason to establish the effect of the biologically active plant product VemoHerb on the growth indicators of the sucking pigs and its significance for the improvement of the microbiological status of faecal samples of growing animals.

MATERIALS AND METHODS

In order to establish the effect of plant extracts on the growth indicators and the health status of the pigs, two experiments were conducted under production conditions in a pig located near the town of Plovdiv, Bulgaria (42.35°; 24.733°). During the first experiment, a total of 91 sucking pigs were used, originating from the farrows of

10 mothers (Large White x Landrace), divided in two groups of 5 mothers – control one (n=44 sucking pigs) and experiment one (n=47 sucking pigs). The mammals from the first group received standard combined forage without plant extract supplement or a nutritional antibiotic, while a herbal extract with the trade name VemoHerb was added to the combined forage of the experiment group, which contains a combination of dry extracts from chicory (*Cichoria intybus* L.), tansy (*Tanacetum vulgare*), and sumac (*Cotinus coggygria* L.) in a dose of 150g/t of fodder. The VemoHerb supplement is standardized using analytical indices, which are included in the company quality specification of the product (Valchev et al., 2009; Zapryanova-Boeva, 2011). The mother pigs were fed with standard combined forage for the category.

The second experiment was conducted with growing pigs in the period with weight from 6 to 20 kg. The nutritional antibiotic – Flavomycin was used as basis of comparison for the control animals, in a dose of 5 g/t fodder, and the experiment animals received combined fodder with the addition of VemoHerb in a dose of 150 g/t.

The animals from both experiments were bred in boxes on the floor, the feeding was without restraint with fodders with balanced nutritional ingredients in accordance with the category of the animals. The pigs from the first experience were fed from the fifth day after their birth, as the sucking period continued 29.4±0.5 days.

All animals had free access to drinking water.

During the first experiment with sucking pigs, the following indicators were controlled: live weight at birth and at weaning, individually; composition of the combined fodders – per components and end product, health status of the animals – daily.

In addition to control of the growth (Zapryanova-Boeva, 2011), at the beginning and the end of the second experiment microbiological analyses were performed of faecal samples for content of pathogenic and conditionally pathogenic microorganisms in a gram of sample. Samples from the excrements of the pigs were taken from each box, in sterile test tubes, which were sent for analysis in microbiological laboratory on the same day.

The trials are a part of a series of experiments which help to establish the effect of the tested biologically active plant supplement as a growth stimulator for growing pigs and the possibility to replace the nutritional antibiotics in pig breeding.

The received results were processed statistically with software product SPSS, v24, IBM.

RESULTS AND DISCUSSIONS

The data from the growth performance of sucking pigs from the first conducted experiment is shown in Table 1.

The average live weight at birth was 1,512.4±24.6 g, which is within the normal range for this indicator, which appears as an important factor, determining the survival rate and productivity of the pigs through their sucking period (Milligan et al., 2002).

The average weight at birth of the experiment pigs was 1,459.6±42.9 g, which is almost 80 grams less in comparison with the weight of the control animals.

At the end of the sucking period, a live weight which is 2.2% higher on average is registered with the experiment animals, despite the unreliable differences between the groups.

On the basis of a relatively low growth intensity, generally for the animals of this category, the addition of the biologically active plant supplement VemoHerb increases the average daily growth of the experiment pigs by 4.8% in comparison with the control animals, and the overall growth is by 4.6% (Table 1).

Table 1. Growth performance of suckling piglets

Traits	I group		II group		Average	
	LS	±SE	LS	±SE	LS	±SE
Piglet body weight at birth, g	1,545.7	44.4	1,469.6	42.9	1,512.4	24.6
Piglet body weight at weaning, g	6,172.4	198.9	6,307.0	192.5	6,261.5	131.4
Gain						
- Total gain, g	4,626.7	177.7	4,837.5	171.9	4,749.1	117.5
- Average daily gain, g	157.2	6.0	164.7	5.8	161.52	3.9

The research regarding the influence of herbs on the productive indicators of sucking pigs in scientific literature are too multi-directional. Ivanova-Peneva and Kanev (2014) established that better weight development of the pigs from the control group ($p<0.05$) compared to the group with oregano supplement.

Another study of the same category of animals, conducted under similar regimen, Ivanova-Peneva et al. (2010) establish that the use of the same herbs, *Origanum vulgare* and *Potentilla erecta Raus*, in the combined forage for sucking pigs, improves the growth of sucking pigs with 11% ($p<0.01$) for the suckling period (1-34 days), as well as the live weight at the weaning. According to these authors, the lack of enough literature on the issue of the influence of plant extracts on the production indicators of sucking pigs makes it difficult to interpret the acquired results. Meanwhile, data for the application of herbs in growing and fattened pigs is not missing (Toncheva et al., 2004; Valchev et al., 2005; Oetting et al., 2006; Zapryanova-Boeva, 2011), etc.

The factors we studied show that only the mother has a reliable effect on the controlled traits (Table 2). According to Ivanova-Peneva and Kanev (2014), the reliable effect of the box is probably due to the milkiness of the mother, and it is extremely difficult to achieve the equalization in milkiness.

Table 2. The effect of the sex, group and the mother on the growth indicators of sucking piglets

Model	**F- criterion and degree of reability**			
	Traits			
	Piglet body weight at birth	Piglet body weight at weaning	Total gain	Average daily gain
Sex	0.33	0.566	0.876	0.764
Group	1.517	0.237	0.726	0.792
Mother	6.945***	2.033*	2.066*	2.010*
***** P<0.001, *P<0.05**				

The data of growth indicators of pigs from the second conducted experiment is presented in our previous studies (Valchev et al., 2003; Zapryanova-Boeva, 2011), where we establish 18.8% higher average daily growth of the experimental animals compared to the control ones ($p<0.01$). The results for the use of the forage are similar, as adding plant extracts leads to reliably better ($p<0.05$) conversion of fodder in the experimental group compared to the control pigs.

The influence of herb extracts on the gastrointestinal microflora was studied by Castillo et al. (2006), in a comparative experiment with early weaned pigs under the following regimen: I control group; II group with supplement of 0.04% of Avilamycin; III group – 0.3% of sodium butyrate, and IV group – 0.05% mixture of Origanum spp., Cinnamonum spp and *Capsicum annuum* extracts. At the end of the experiment, post mortem samples were taken from the contents of the digestive tract in order to define the total number of micro-organisms, as well as the contents of lactobacilli and enteric bacteria in the jejunum and cecum of the pigs. The authors come to the conclusion that the total number of microorganisms in the separate parts of the tract does not change in dependence on the experimental supplements, but they detect increase ($P=0.003$) in the ratio of lactobacilli: enteric bacteria in the cecum of the animals from the group which took herb extracts.

The anti-bacterial activity of essential oils and herb extract were studied by Hammer et al., 1999; Gislene et al., 2000; Burt and Reinders, 2003; Kim and Shin, 2004; Si et al., 2006; Fisgin et al., 2009).

Santoro et al. (2007) studied the effect of essential oils mixture (*Origanum vulgare* – oregano and *Thymus vulgare* - thyme) on the growth and development of *Tripanosoma cruzi*. The results of the observation show that the essential oils from oregano and thyme inhibit the development of trypanosomes for 24 hours at IC50.

Similar results for the inhibitor effect of essential oils on different intestinal parasites were established by Nostro et al. (2004), Moon et al. (2006), etc.

In order to establish the influence of the studied product on the intestinal microflora,

microbiological analyses of faecal samples were taken for contents examination of pathogenic and conditionally pathogenic microorganisms in a gram of sample in them (Table 3).

Table 3. Microbiological analysis of faecal samples, number per gramme

	Average	Min	Max
Aerobic mesophilis	4,390,825x10^3	433x10^5	68x10^8
Coli forms	17,111x10^5	4x10^5	46x10^8
Staphylococci and micrococci	5,755x10^3	4x10^5	123x10^5
Enterococci	251,165x10^2	166x10^3	633x10^5
Proteus	1,590,750	333x10^3	35x10^5
Fungi and yeast	72,065x10^2	166x10^3	186x10^5
Sulfitreducing clostridia	1,725	0	5,000
Salmonellas	ND	ND	ND
Bacteria anaerobic	ND	ND	ND
Listeria monocitogenes	ND	ND	ND

ND- not detectable

Figure 1 presents clear data for the categorical reduction of unwelcome microorganisms at the end of the experiment with the group of VemoHerb supplement. The reduced number of *Aerobic mesophiles* of the control animals is within 20%, and with the experiment pigs – five times more.

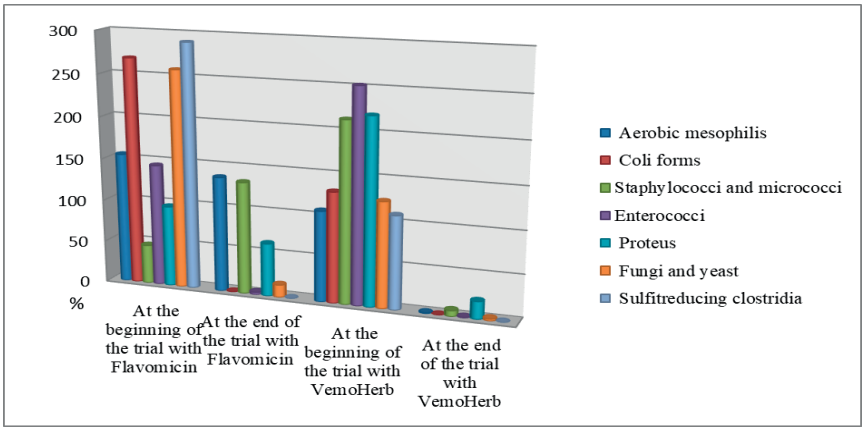

Figure 1. Content of pathogenic and conditionally pathogenic microorganisms in faecal samples (like deviation of mean)

The supplement of nutritional antibiotic and plant extract has an equal effect on the reduction of the population of Coli forms and Enterococci in the faecal samples of pigs. At the end of the experimental period, the number of Staphylococci and micrococci in the group with a supplement of the tested herbal product is around 93% smaller, compared to the average value of this indicator. With Proteus, a decrease is registered from 37 to 79%, respectively with the control and the experiment group.

In comparison with the control samples at the end of the experiment, the Fungi and Yeast group with supplement from plant extract reach 2% from the average for the indicator, which is 13% less than that of the animals, which have been given nutritional antibiotic.

CONCLUSIONS

Under the conditions of the performed experiment we established that the average live weight at birth was 1,512.4±24.6 g. The average weight of the experiment pigs was 1,459.6±42.9g at birth, which is almost 80 g less, compared to the control animals.

At the end of the sucking period, a higher average live weight by 2.2% is registered for the experiment animals, despite the unreliable differences in the groups.

Putting in the biologically active plant supplement increases the average daily gain of the pigs by 4.8% in comparison with the control animals, and the overall gain by 4.6%.

The inclusion of the biologically active complex of dry extracts from the plants *Cichoria intybus* L, *Cotinus coggygria* L. and *Tanacetum vulgare* in the mixes of the growing pigs leads to significant decrease of the number of the unwanted microorganisms in the digestive system of the animals – aerobic mesophiles, coliforms, staphylococci and micrococci, and enterococci, etc.

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THE QUALITY OF FODDERS FROM BIRD'S-FOOT-TREFOIL, *LOTUS CORNICULATUS* L. UNDER THE CONDITIONS OF MOLDOVA

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Abstract

The aim of this study was to determine the green mass productivity, the biochemical composition and the fodder value of a perennial legume – bird's-foot-trefoil, Lotus corniculatus cv. Doru, grown in an experimental field of the National Botanical Garden (Institute), Chișinău. In the third growing season, the bird's-foot-trefoil was characterized by high growth rate and regenerative capacity after mowing, making it possible to mow it four times per season. The annual productivity was 44.50 t/ha green mass or 9.70 t/ha dry matter. It was determined that the quality of the green mass varied depending on the harvest time: crude protein 143.5-194.5 g/kg DM, crude fats 30.1-44.8 g/kg DM, crude cellulose 261.7-357.0 g/kg DM, nitrogen free extract 338.3-456.1 g/kg DM, sugars 37.5-61.0 g/kg, starch 18.1-37.8 g/kg, carotene 48.45-77.00 mg/kg, calcium 10.6-14.9 g/kg and phosphorus 2.2-3.3 g/kg. The biochemical and fodder value of the prepared haylages from first and second cuts were: pH 4.46-4.70, lactic acid 34.1-37.2 g/kg DM, acetic acid 5.7-9.4 g/kg DM, butyric acid 0.2 g/kg DM, organic matter 891.8-902.2 g/kg DM, crude protein 170.9-188.8 g/kg DM, crude fats 41.6-44.6 g/kg DM, crude cellulose 332.2-358.9 g/kg DM, nitrogen free extract 298.6-357.5 g/kg DM, sugars 0.87-1.86%, starch 0.91-1.76%, carotene 14.0-24.0 mg/kg, calcium 10.0-10.8 g/kg DM and phosphorus 2.7-3.1 g/kg DM. The prepared hay from first and second cuts contained 18.89-20.76% crude protein, 2.56-2.74% crude fats, 31.62-32.42 % crude cellulose, 33.86-36.33 % nitrogen free extracts, 10.49-11.01 % ash.

Key words: *biochemical composition, fodder value, green mass, hay, haylage, Lotus corniculatus, productivity*

INTRODUCTION

One of the principal sources of protein of the human diet is of animal origin. The increasing demand for food of animal origin which is caused by the steady growth of the world´s population can and must be met by improving animal performance. Animal husbandry implies a continuous process that requires a constant supply of high quality feed. Feed costs can account for 65% of livestock production expenses. Animal husbandry production is based on natural and cultivated pastures. In recent years, a lot of attention has been paid to improving the quality and reducing the production costs of forages (Marin et al., 2016). In many countries, various fodder species are studied, bred, cultivated and used as sources of protein, essential amino acids, biologically active substances, to provide a balanced diet for animals and thus to increase their productivity and, in addition, to improve soil fertility, to restore degraded grassland and marginal lands (Kshnikatkina et al., 2005). Leguminous plants typically contain more crude protein and less neutral detergent fiber than grasses, which increases the rate of forage digestion, resulting in greater intake. Perennial forage legumes represent one of the highest quality solutions to the constant demand for plant protein in animal husbandry. The inclusion of tanniferous plant species in forage production would be beneficial (Waghorn et al., 1998; MacAdam and Villalba, 2015).

Lotus is the largest genus of the tribe *Loteae*, family *Fabaceae*, comprises 140 species with worldwide distribution. Some of the *Lotus* species show a great potential for adaptation to a number of abiotic stresses, infertile and acidic soils, relevant components of grassland

ecosystems in environmentally constrained areas, can be regarded as pioneer forage legumes and are moderately tolerant to soil salinity (Díaz et al., 2005; Escaray et al., 2012).

Bird's-foot trefoil, *Lotus corniculatus,* is undoubtedly the species considered to have the greatest agricultural importance and the widest distribution. It is a perennial herbaceous plant, similar in appearance to some *Trifolium* species. The plant root system consists of a deep taproot with numerous secondary roots with good lateral spread. Roots can produce new shoots. Growth form ranges from prostrate to erect with numerous stems (10-60 cm) arising from a basal, well-developed crown and branches arising from leaf axils. Primary growth comes from the crown, but re-growth comes from buds formed at nodes on the stubble left after defoliation. Leaves are pentafoliate, alternately on short stalks with the two leaflets at petiole base resembling stipules. The asymmetrical, pointed leaflets are mainly glabrous and more slender and paler green than those of greater lotus. Inflorescences have up to eight yellow flowers and are umbel-like cymes at the end of long, auxiliary branches. The flowering period is indeterminate; consequently, seed develops over an extended period in summer. Seed pods, 2-5 cm long, contain 15-20 seeds attached to the ventral suture; the seeds are released by a sudden split of the pod along both sutures after one to two weeks of ripening, during which the pods change from green to brown. The seeds are round or oval form, greenish yellow or dark brown color. Bird's-foot trefoil is an excellent source of nectar and pollen for honeybees (Dzyubenko and Dzyubenko, 2008).

Bird's-foot-trefoil cultivation started at the beginning of the 19th century, and in the last decades, this species has been studied in many research centers, and thus over 100 cultivars have been created and implemented, including 4 cultivars that have state permission for use in Romania. Worldwide, more than 4 million hectares are planted with *Lotus corniculatus* cultivars. Romanian cultivars of bird's-foot trefoil reached green mass productivity 40-50 t/ha or 9-11 t/ha dry matter, seed yield 400-500 kg/ha (Maruşca et al., 2011). Bird's-foot trefoil is used as an excellent, non-bloating fodder and provides good forage, hay and silage. It is especially good on infertile, poorly drained soils and on soils that are otherwise difficult to cultivate. The overall forage quality under drought conditions is better than that of alfalfa due to a higher leaf-stem ratio, delayed maturity and better quality of all plant components. *Lotus corniculatus* contains condensed tannins and other secondary compounds that reduce protein degradation in the rumen and increase the flow of amino acids to the intestine for absorption, inhibit the formation of enteric methane, prevent bloat in the rumen and reduce internal parasites, has additional benefits for sheep production such as increased wool growth, live weight gain and fecundity (Doran-Browne et al., 2014). The condensed tannins have anthelminthic properties and decrease nematodes in wild and domestic ruminants (Molan et al., 2011).

The aim of this study was to determine the green mass productivity, the biochemical composition and the fodder value of prepared hay and haylage from bird's-foot-trefoil, *Lotus corniculatus.*

MATERIALS AND METHODS

The Romanian cultivar of bird's-foot-trefoil, *Lotus corniculatus,* 'Doru', created at the Research-Development Institute for Grasslands Braşov, Romania, and cultivated in monoculture in the experimental plot of the National Botanical Garden (Institute) "Alexandru Ciubotaru", Chişinău, latitude 46°58′25.7″N and longitude 28°52′57.8″E, served as subject of the research.

The green mass of 3-year-old bird's-foot-trefoil was harvested manually at 5-cm stubble height. The samples were collected in early flowering periods: the first cut (23.05.2019), the second cut (20.06.2019), the third cut (22.07. 2019) and the fourth cut (23.09.2019). The leaves/stems ratio was determined by separating the leaves, buds and flowers from the stem, weighing them separately. The prepared hay was dried directly in the field. The haylage was prepared from wilted green mass, shredded and compressed in well-sealed glass containers. The content of dry matter, protein, fat, crude cellulose, calcium, phosphorus, soluble sugars, starch, ash, lactic, acetic and butyric acids was appreciated in accordance with standard laboratory procedures (Petukhov et al., 1989).

RESULTS AND DISCUSSIONS

Analyzing the agro-biological features of bird's-foot trefoil, *Lotus corniculatus* 'Doru', in the third growing season, it was established that the revival of plants from dormant buds was uniform, generative shoots developed in early April, they were characterized by faster grow and development rates, the flower bud formation of plants started at the middle of May. At the time when the green mass was harvested for the first time, the *Lotus corniculatus* plants reached 55 cm, the yield was 2.50 kg/m^2 green mass or 0.55 kg/m^2 dry matter, characterized by high content of leaves and flowers in the harvested mass (Table 1). Due to the moderate air temperatures during late May and June, and the optimal moisture content of soil, the revival of plants was fast. It was established that during 28 days, *Lotus corniculatus* 'Doru' plants developed shoots that grew about 33-35 cm, and the plants were cut for the second time, obtaining 1.21 kg/m^2 green mass or 0.25 kg/m^2 dry matter, the harvested mass was richer in leaves (71%). In spite of the favorable weather conditions in June-July 2019, with moderate amount of rainfall and optimal temperatures, the bird's-foot trefoil plants recovered well after the harvest, thus, several new shoots developed and, at the end of July, the length of the shoots was 23-25 cm and 0.41 kg/m^2 natural fodder were harvested. The unfavorable meteorological conditions, the lack of rainfall and the very high air temperatures (35-41ºC) during the second half of the summer affected the regeneration and development rate of *Lotus corniculatus* 'Doru' plants. A better growth and development was observed after the rain that fell at the end of August, the formed shoots were prostrate, thin, and over 17-21 cm long. The yield at the fourth harvest decreased in comparison with the previous harvests, and reached 0.33 kg/m^2 green mass, but with higher proportion of leaves (68%) and dry matter content (29%). The annual productivity of *Lotus corniculatus* 'Doru' in the third growing season reached 44.5 t/ha green mass or 9.7 t/ha dry matter.

Table 1. Some agrobiological peculiarities and the structure of the harvested mass depending on the harvest time

Harvest time	Plant height, cm	Stem, g		Leaf + flower, g		Productivity, t/ha		Content of leaves and flowers in fodder, %
		green mass	dry matter	green mass	dry matter	green mass	dry matter	
First cut	56	1.08	0.25	1.70	0.34	25.03	5.33	63.0
Second cut	34	0.52	0.12	0.79	0.15	12.07	2.47	55.6
Third cut	24	0.54	0.14	0.83	0.18	4.13	0.96	56.0
Fourth cut	18	-	-	-	-	3.27	0.94	68.0

Table 2. Biochemical composition and nutritive value of the green mass of depending on the harvest time

Indices	First cut	Second cut	Third cut	Fourth cut
Crude protein, % DM	16.35	19.45	17.87	14.35
Crude fats, % DM	3.91	4.48	4.16	3.01
Crude cellulose, % DM	35.70	31.90	26.17	27.85
Nitrogen free extract, % DM	33.83	33.92	41.14	45.61
Soluble sugars, % DM	3.95	3.75	4.03	6.10
Starch, % DM	1.81	2.00	2.89	3.78
Ash,% DM	10.21	10.26	10.66	9.14
Nutritive units/ kg GM	0.16	0.16	0.21	0.24
Metabolizable energy, MJ/kg GM	1.69	1.69	2.24	2.56
Calcium, %	1.06	1.06	1.49	1.33
Phosphorus, %	0.26	0.33	0.26	0.22
Carotene mg/ kg GM	60.67	48.45	57.00	77.00

The productivity, the quality and the seasonal distribution of forage may be of great importance to the livestock farmers. The quality of the green mass of *Lotus corniculatus* 'Doru' is presented in Table 2. It was determined that the biochemical composition of the dry matter varied depending on the harvest time: crude protein varied from 143.5 to 194.5 g/kg, crude fats – from 30.1 to 44.8 g/kg, crude cellulose – from 261.7 to 357.0 g/kg, nitrogen free extract – from 338.3 to 456.1 g/kg, soluble sugars – from 37.5 to 61.0 g/kg, starch from 18.1 to 37.8 g/kg, ash – from 91.4 to 106.6 g/kg, calcium – from 10.6 to 14.9 g/kg, phosphorus – from 2.2 to 3.3 g/kg. The concentrations of crude protein and fats were high in the green mass obtained after the second harvest and very low – after the fourth harvest. The significantly higher content

of crude cellulose in the first and second harvests, but nitrogen free extract – in the third and fourth harvests. The level of soluble sugars and starch increased substantially in the third and fourth harvests.

Plant carotenoids are precursors of retinol – vitamin A, together with vitamin E and polyphenols, are natural antioxidants in ruminant diets. Higher carotene concentrations in feed contribute to an improvement in the nutritional value of milk products. It was found that, during the third growing season of *Lotus corniculatus* 'Doru' plants, the amount of carotene decreased from 60.67 mg/kg fodder at the first harvest to 48.45 mg/kg fodder at the second harvest, and increased substantially to 77.00 mg/kg in the green mass obtained at the fourth harvest.

The content of nutrients and their digestibility influence the feed and energy value of natural fodder. Therefore, 100 kg of green mass obtained at the first and second harvests contained 16.0 nutritive units and 169 MJ metabolizable energy, at the third harvest – 21 nutritive units and 224 MJ metabolizable energy and at the fourth harvest – 24 nutritive units and 256 MJ metabolizable energy for cattle. The calculated annual feed productivity achieved 7600 nutritive units and 1200 kg digestible protein.

Some authors have mentioned similar findings about the productivity and quality of *Lotus corniculatus*. So, as a result of a research conducted by Nelyubina and Kasatkina (2017) has been revealed that the yield of green mass from two cuts was 20.3-27.2 t/ha, dry mass 4.2-5.6 t/ha, including 18.9-21.4% crude protein, 2.6-2.9% crude fats, 24.9-29.1% crude cellulose, 7.8-8.3 % minerals, 40.6-42.4 % nitrogen free extract, which provided a high concentration of metabolizable energy (9.49-10.35 MJ/kg) and nutritive units (0.76-0.80 kg DM), the concentration of digestible protein 147-154 g/kg; Kshnikatkina et al. (2005) remarked the chemical composition of dry matter bird's-foot trefoil were: 21.0% protein, 3.31% fat, 25.68% cellulose, 7.31% ash; Shlapunov and Karpei (2014), found that in bird's-foot trefoil pure plantations, the annual productivity was 31.4 t/ha green mass, 5.3 t/ha dry mass, 4.9 t/ha fodder units and 1.01 t/ha crude protein, but in mixture with perennial grasses – 29.2-31.3 t/ha green mass, 5.5-6.1 t/ha dry mass, 4.7-5.3 t/ha fodder units and 0.78-0.93 t/ha crude protein.

The wilted silage, or haylage, from legume plants play an important role in the nutrition, wellbeing and productivity of animals. It can help solving some problems in the livestock sector by providing a balanced diet for animals with an appropriate amount of protein and fiber. As for the organoleptic properties, the haylage prepared from *Lotus corniculatus* 'Doru' consists of green leaves and yellowish-green stems; has a pleasant smell of pickled vegetables; the texture of the plants stored as haylage was preserved well, without mold and mucus. The fermentation quality and fodder values of haylage prepared from *Lotus corniculatus* 'Doru' are shown in Table 3. It has been determined that the amounts of organic acids reached 34.1- 37.1 g/kg and pH values 4.46-4.70, most organic acids were in fixed form.

Table 3. The fermentation quality of the investigated haylage from *Lotus corniculatus* 'Doru'

Indices	First cut	Second cut
pH index	4.70	4.46
Total organic acids, g/kg	37.1	34.1
Free acetic acid, g/kg	1.7	4.0
Free butyric acid, g/kg	0.2	0.0
Free lactic acid, g/kg	7.6	6.1
Fixed acetic acid, g/kg	4.0	3.9
Fixed butyric acid, g/kg	0.0	0.0
Fixed lactic acid, g/kg	23.6	18.6
Total acetic acid, g/kg	5.7	9.4
Total butyric acid, g/kg	0.2	0.0
Total lactic acid, g/kg	31.2	24.7
Acetic acid, % total acids	15.36	27.57
Butyric acid, % total acids	0.54	0.0
Lactic acid, % total acids	84.10	72.43

The haylage prepared from green mass obtained at the first cut was characterized by optimal content of lactic acid and low content of acetic acid, in comparison with the haylage prepared from the green mass obtained at the second cut. The butyric acid was detected in fixed form (0.2 g/kg DM) in the haylage prepared after the first cut.

The prepared haylages (Table 4) contained 170.9-188.8 g/kg DM crude protein, 41.6-44.6 g/kg DM fats, 332.2-358.9 g/kg DM crude cellulose, 298.6-357.5 g/kg DM nitrogen free extract, 8.7-18.6 g/kg DM soluble sugars and 9.1-19.1 g/kg DM starch, 97.8-109.1 g/kg DM ash, 10.0-10.8 DM calcium and 2.7-3.1 g/kg

DM phosphorus. In comparison with the initial mass, in the prepared haylages level of crude protein, crude fats, calcium and phosphorus did not change essentially. The amount of carotene decreased substantially to 14-24 mg/kg fodder.
It has been calculated that 100 kg of haylage prepared from green mass obtained at the first cut contained 22 nutritive units, 4.18 kg digestible protein and 265 MJ metabolizable energy, but – at the second cut – 25 nutritive units, 4.93 kg digestible protein and 296 MJ metabolizable energy.

Table 4. Biochemical composition and nutritive value of the investigated haylage from *Lotus corniculatus* 'Doru'

Indices	First cut	Second cut
Dry matter, g/kg	33.89	36.44
Crude protein, %	17.09	18.88
Crude fats, %	4.16	4.46
Crude cellulose, %	33.22	35.89
Nitrogen free extract, %	35.75	29.86
Sugars, %	1.86	0.87
Starch, %	1.79	0.91
Ash, %	9.78	10.91
Nutritive units/ kg	0.22	0.25
Metabolizable energy, MJ/kg	2.65	2.96
Calcium, %	1.00	1.08
Phosphorus, %	0.27	0.31
Carotene mg/ kg	14.00	24.00

Some authors mentioned various findings about the ensilage fodder. Heuzé al. (2015) revealed that the bird's-foot trefoil silage contained 34.7% dry matter with 20.2% protein, 3.8% fats, 38.5% NDF, 30.9% ADF, 9.7% lignin, 5.0% WSC, 9.3% ash, 14.3 g/kg tannins, 65.0% digestible organic matter, 19.2 MJ/kg gross energy, 11.7 MJ/kg digestible energy. Coblentz and Grabber (2013) mentioned that, in the USA, the silages made from bird's-foot trefoil green mass obtained at the first harvest, contained 18.8-20.9% protein, 38.1-41.5% NDF, 31.7-35.7% ADF, 9.3-9.7% lignin, 5.7-8.1% hemicellulose, and the silage made at the second harvest contained 20.2-21.4% protein, 38.5-39.3% NDF, 33.0-33.6% ADF, 8.2-10.0% lignin, 5.5-6.9% hemicellulose.
Hay is valuable feed for farm animals, a rich source of protein, vitamins and minerals, both in winter and throughout the year. The quality of the hay from *Lotus corniculatus* 'Doru' is presented in Table 5. The prepared hay contained 18.89-20.76 % crude protein, 2.56-2.74 % crude fats, 31.62-32.42 % crude cellulose, 33.86-36.33 % nitrogen free extract, 10.49-11.01 % ash. The nutritive value of 100 kg of hay was 51-56 nutritive units, 11.6-13.3 kg digestible protein and 758-764 MJ metabolizable energy.

Table 5. The biochemical composition and the nutritive value of the investigated hay from *Lotus corniculatus* 'Doru'

Indices	First cut	Second cut
Crude protein, %	18.19	20.76
Crude fats, %	2.56	2.74
Crude cellulose, %	32.42	31.62
Nitrogen free extract, %	36.33	33.86
Ash, %	10.49	11.01
Nutritive units/ kg	0.56	0.51
Metabolizable energy, MJ/kg	7.58	7.64
Calcium, %	1.16	1.16
Phosphorus, %	0.29	0.34

Several literature sources describe the bird's-foot trefoil hay quality. According to Medvedev and Smetannikova (1981), the chemical composition of hay was: 14.0-22.3% protein, 1.5-3.6% fat, 6.9-11.2% ash, 22.4-26.0% crude cellulose, 39-51% nitrogen free extract. Kaplan et al. (2009) remarked that hay contained 17.16-20.94% CP, 38.00-43.95% NDF, 30.6238.78% ADF, 7.17-10.17% ash, 2.87 g/kg condensed tannins, 58.69-65.04% digestible dry matter, 9.25-9.59 MJ/kg metabolizable energy, RFV 119.6-159.3. Coblentz and Grabber (2013) found that the concentrations of nutrients in the hay prepared from the green mass first cut ranged from 18.2 to 19.6% protein, 37.6 to 38.7% NDF, 30.7-32.2% ADF, 8.7 to 9.0% lignin, 6.2 to 6.2% hemicellulose and 1.16 to 2.43% condensed tannins, but in the hay prepared after the second cut – from 18.2 to 19.6% protein, 37.2 to 40.1% NDF, 31.8-33.6% ADF, 9.4 to 9.7% lignin, 5.4 to 6.5% hemicellulose and 1.23 to 2.77% condensed tannins.

CONCLUSIONS

The Romanian cultivar 'Doru' of *Lotus corniculatus*, in the third growing season, was characterised by high growth rate and regenerative capacity after being mowed, making it possible to mow it four times per season, reaching a productivity of 44.5 t/ha green mass or 9.7 t/ha dry matter.
The quality of the green mass varied depending on the harvest time: crude protein 143.5-194.5 g/kg DM, fats 30.1-44.8 g/kg, crude cellulose 261.7-357.0 g/kg, nitrogen free extract 338.3-

456.1 g/kg, sugars 37.5-61.0 g/kg, starch 18.1-37.8 g/kg, carotene 48.45-77.00 mg/kg, calcium 10.6-14.9 g/kg and phosphorus 2.2-3.3 g/kg.

The biochemical and fodder value of the prepared haylages was: pH 4.46-4.70, lactic acid 34.1-37.2 g/kg, acetic acid 5.7-9.4 g/kg, butyric acid 0.2 g/kg, organic matter 891.8-902.2 g/kg, crude protein 170.9-188.8 g/kg, fats 41.6-44.6 g/kg, crude cellulose 332.2-358.9 g/kg, nitrogen free extract 298.6-357.5 g/kg, sugars 0.87-1.86%, starch 0.91-1.76%, carotene 14.0-24.0 mg/kg, calcium 10.0-10.8 g/kg and phosphorus 2.7-3.1 g/kg.

The hay prepared after the first and second harvests contained 18.89-20.76% crude protein, 2.56-2.74% crude fats, 31.62-32.42% crude cellulose, 33.86-36.33% nitrogen free extract and 10.49-11.01% ash.

The Romanian cultivar 'Doru' of *Lotus corniculatus* can be used in the Republic of Moldova to restore degraded permanent grasslands, as a component of the mixtures of plants sown to create temporary grasslands, grass-legume strips in vineyards and orchards, and the harvested biomass can be used as natural and conserved fodder for animals.

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THE EFFECT OF FEED ADDITIVES BASED ON THE MYCELIUM OF THE FUNGI *GANODERMA LUCIDUM* AND *LAETIPORUS SULPHUREUS* ON THE PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF POULTRY BLOOD

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Abstract

The possibilities of using the vegetative mycelium of xylotrophic fungi of the varnished tinder fungus (Curt.:Fr.) P. Karst. and tinder funnel of sulfur yellow (Bull.) Murrill as food additives for poultry of the parent flock. These types of mushrooms are known as promising biotechnology producers of compounds of sterol and carbohydrate nature, carotenoids, vitamins and other substances with high biological activity. In the process of growing the mycelium was enriched with trace elements germanium, which can partially stop oxygen starvation in tissues. Powdered feed additives were fed with the main diet and allowed to increase the total blood protein of poultry against the background of fraction growth by 5-7%, and the hemoglobin content - by 7-9%. A positive effect on the immune parameters of the parent flock blood and the antioxidant status of their body were established, and a decrease in the markers of oxidative processes was shown. Feed additives allowed to increase the safety of the parent poultry flock, improve incubation rates of eggs, increase the rates of chickens by 5-6% relative to the control.

Key words*: poultry farming, food additives, medical fungi*

INTRODUCTION

Large-scale poultry farming on different farms is associated with a number of serious problems. A large crowding of poultry causes stress, which entails a decrease in immunity, in addition, in such conditions there is a risk of the rapid spread of infections. In this regard, there is a need for the prevention these diseases, including mass immunization and other approaches to reducing morbidity and mortality.

In recent years, the attention of researchers has been attracted by the methods of non-drug maintenance of the bird's body resistance. Higher fungi are considered as sources of immunomodulating and prophylactic agents (Barneche et al., 2016; Jeong, Park, 2018; Koo et al., 2019).

The anti-inflammatory activity of *Laetiporus sulphureus* and the antioxidant, anti-inflammatory, anti-tumor and antiviral activity of compounds from *Ganoderma lucidum* (Jeong, Park, 2018; Sohretoglu, Huang, 2018; Khalilov et al., 2019) are indicated. Fungi *Lentinus edodes*, *Ganoderma lucidum*, *Pleurotus ostreatus*, and *Cordyceps* sp. they are used to optimize the intestinal microflora of chickens, which helps to strengthen the immunity of birds (Robinson et al., 2018).

Of greatest interest in the context of our research are studies related to the role of higher fungi as inhibitors of oxidative stress in poultry (Li et al., 2019; Teng et al., 2019). It has been shown that fungi and their polysaccharides can play an important role in poultry farming: polysaccharides could act as immunity inducers or immunomodulators and exhibit antibacterial, antiviral, antiparasitic biological activity; fungal phenolic compounds can act as antioxidants; mushrooms in the broiler diet can be used as growth stimulants as an alternative to antibiotics; mushrooms in the diet could improve egg production and egg quality; mushrooms can be used as a safe and effective alternative for the induction of molting and enhancing immunity (Khan et al., 2019).

Growing consumer concerns about food safety and their residues have spurred the search for alternative prevention and control strategies, among which herbal medicine has become attractive due to a renewed interest in natural medicine. In this context, studies on the possibility of using natural ingredients derived

from mushrooms for the prevention and treatment of pathological conditions in productive birds seem relevant (Peek et al., 2013).

In the course of our research, the feasibility of using feed additives prepared on the basis of the mycelium *Ganoderma lucidum* and *Laetiporus sulphureus* in poultry practice was examined.

MATERIALS AND METHODS

The investigations had been carried out on the basis of the Department of Biology, Biological Technologies and Veterinary Sanitary Expertise of Penza State Agrarian University (Russia), the Inter-Faculty Biochemical Laboratory and Kolyshleyskaya Poultry Factory of the Penza Region from 2015 to 2019.

The objects of research were Hisex Brown hens, which make up the parent herd, eggs and young animals obtained in the experiment. The effects of the mycelium of the varnished tinder fungus (*Ganoderma lucidum*) and the sulfur tinder fungus (*Laetiporus sulphureus*) on the biochemical parameters of blood and serum of birds, the biochemical parameters of eggs, the productive qualities of the parent herd, the incubation quality of the eggs and the state of the young were studied.

The most productive fungal strains were used to prepare feed additives based on mycelium. The process of obtaining mycelium included three stages. The first stage was the deep cultivation of mycelium for its subsequent inoculation on solid-phase substrates. At the second stage, the obtained deep mycelium was transferred to solid-phase substrates (mixtures of straw, sawdust and grain), packaged in 4-5 kg packs in 10 l plastic bags or 10 l glass jars. At the third stage of preparation, an air-dry substrate overgrown with mycelium was ground in a laboratory mill to obtain a fine-grained powder (particle diameter of not more than 100 μm). The finished product was mixed with the main diet of the bird at the rate of 0.1%.

After feeding the feed additive in the diet, on the 15th, 30th and 45th day, blood was taken for laying hens for analysis. The calculation of the number of red blood cells and white blood cells was carried out simultaneously in the Goryaev chamber according to the generally accepted method. The calculation of the number of cells was carried out according to generally accepted formulas. Blood hemoglobin was determined by the hemoglobin cyanide method. Statistical data processing was performed using the functions of Excel package Microsoft Office.

RESULTS AND DISCUSSIONS

Feed additives based on the mycelium *G. lucidum* and *L. sulphureus* were fed to the bird of the parent herd for 45 days. The control group of chickens received the main diet. Analysis of the blood of birds on the content of serum proteins (total protein and albumin) was carried out three times during the experiment: on the 15th, 30th and 45th days. A positive effect on the dynamics of serum protein content was established, which is reliably shown for the variant using *G. lucidum* (Figure 1). Under the influence of a feed supplement based on *L. sulphureus*, the content of total protein in serum increases in comparison with the control by the 30th day, but then decreases below the control indicators. The dynamics of the albumin fraction indicates that the marked increase in the concentration of protein in serum occurs due to albumin. The results can be explained by the antioxidant effect of *G. lucidum*, which results in stabilization of protein metabolism in the cell.

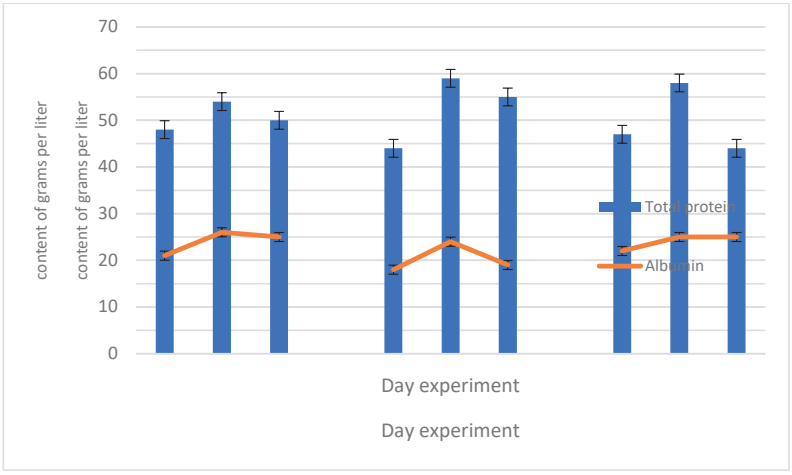

Figure 1. The effect of feed additives based on mycelium on the content of total protein and albumin fraction in the blood serum of birds of the parent herd: option 1 - *G. lucidum*, option 2 - *L. sulphureus* ($p < 0.05$, error bars - average error)

In order to assess the immune status of the organisms of the hens of the parent herd, the serum lysozyme content and serum bactericidal activity were also determined. In our studies, these indicators in the experimental groups receiving feed additives based on the mycelium of the studied species of fungi were significantly higher compared to the control group of hens of the parent herd (Table 1).

Table 1. Indices of natural resistance of hens of the parent herd at the age of 135 days (n = 25), ($p > 0.05$)

Groups	Indicators	
	The concentration of lysozyme in serum, μg / ml	Bactericidal activity in blood serum, %
Control group (normal diet)	24.2±3.17	19.6±1.33
Experimental group 1 (diet with feed additive *G. lucidum*)	41.5±4.33	23.4±1.97
Experimental group 2 (diet with feed supplement *L. sulphureus*	38.2±1.66	15.7±2.31

Thus, the serum lysozyme content and bactericidal activity in the hens of the experimental groups receiving feed additives based on mycelium were significantly higher than in the control group ($p<0.05\%$). A morphological study of whole blood revealed that its composition increased the number of macrophages and mast cells.

Thus, the use of a feed additive based on the mycelium of the studied fungi has a positive effect on the immune parameters of the blood of chickens of the parent herd, stimulating the protective functions of the bird's body.

Indicative results were obtained by studying the effect of mycelial supplements on the antioxidant status of birds. In this case, two indicators were used: the activity of the enzyme glutathione peroxidase and the content of malondialdehyde (MDA) - $CH_2(CHO)_2$ - in the blood serum of chickens of the parent herd. It was found that the activity of the glutathione peroxidase enzyme in the chicken serum of the experimental group receiving *G. lucidum* mycelium averaged 1022.0 μmol glutathione/min, whereas in the control serum it was 861.3 μmol/min. Thus, under the action of dietary supplements, GP activity increased by 18.7%. The feed supplement based on the *L. sulphureus* mycelium did not significantly affect the activity of glutathione peroxidase; the enzyme activity was 965.0 μmol/min (Figure 2).

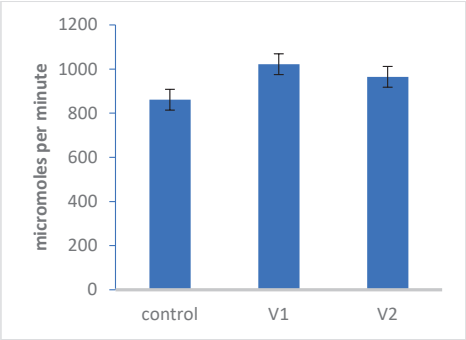

Figure 2. The effect of feed additives based on mycelium on the activity indicators of the glutathione peroxidase enzyme in the blood serum of birds of the parent herd: option 1 - *G. lucidum*, option 2 - *L. sulphureus* (p <0.05, error bars - average error

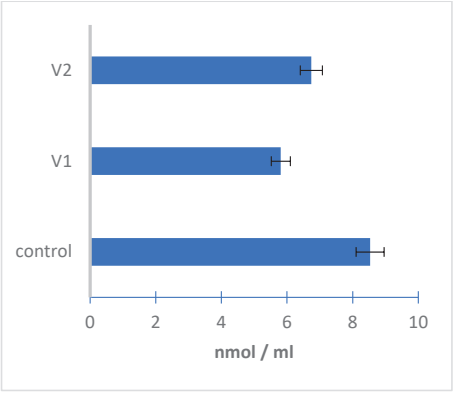

Figure 3. The effect of feed additives based on mycelium on the content of MDA in the blood serum of birds of the parent herd: option 1 - *G. lucidum*, option 2 - *L. sulphureus* (p<0.05, error bars - average error)

MDA is formed in the body during the degradation of polyunsaturated fats by reactive forms of oxygen, and serves as a marker of oxidative stress. It was shown that the substance is able to react with DNA, forming DNA adducts, that is, leading to mutations. The concentration of MDA serves as a marker of the degree of endogenous intoxication.

The change in the MDA content in chicken serum under the influence of a feed additive based on G. *lucidum* mycelium in a positive direction is significant and is probably the most valuable of the noted facts of the effect of mycelium on serum biochemical parameters. The effect of using a feed supplement based on *L. sulphureus* mycelium was less pronounced. In the control serum samples, the average MDA content was 8.53 nmol/ml, and under the influence of the additive in the first experimental group it decreased to 5.81 nmol/ml, i.e. 32%, and in the second experimental group to 6.74 nmol/ml, i.e. 21% (Figure 3).

The effect of feed additives on the egg production of chickens of the parent herd was also studied.

The control group (n = 25) received the main diet, and crushed mycelium was added to the diet of the hens of the experimental groups.

The experiment was carried out for 41 days, however, the eggs were counted for comparison, starting from the 7th day of research, taking into account that the additive had time to act, as well as the adaptation of the birds in the workshop. Statistical analysis allowed us to establish a reliable positive effect of a feed additive based on *G. lucidum* mycelium. Under its influence, the proportion of eggs that went for incubation increased significantly.

If in the control the proportion of rejected eggs reached an average of 14.1%, then in the experiment an average of 9.7%.

On some days in the first experimental group, rejection was not performed at all. The egg production of chickens of the second experimental group receiving a feed supplement based on the *L. sulphureus* mycelium remained at the control level, the results obtained were not significantly different from the control ones (Table 2).

Table 2. Average egg production rates of chickens of the parent herd under the influence of a mycelial dietary supplement (n = 25), (p> 0.05)

Options	Egg production (n=25)		Defect (pcs, %)	Incubation (pcs, %)
	pcs	%		
Control group (normal diet)	20.6±3.2	82.4	3.34±1.05 (16.2)	17.3±1.1 (83.8)
Experimental group 1 (diet with feed additive *G. lucidum*)	23.1±2.6	92.4	2.21±0.77 (9.6)	20.9±0.8 (90.4)
Experimental group 2 (diet with feed supplement *L. sulphurous*	21.2±2.4	84.8	2.34±1.05 (11.0)	18.86±1.1 (89.0)

CONCLUSIONS

The experiments performed have established the positive effect of feed additives based on the mycelium of the fungi *Ganoderma lucidum* and *Laetiporus sulphureus* on the anabolic processes and the immune status of chickens of the parent herd. The growth of indicators of the body's natural resistance has been established. It has been shown that the use of feed additives based on mushroom mycelium eliminates oxidative stress in birds, increases productive indicators and allows to reduce the proportion of rejected eggs, thereby increasing the incubation volume. The data obtained are consistent with the prophylactic potential and biological activity of these types of fungi described in the literature. Thus, the feasibility of using feed additives based on the mycelium *Ganoderma lucidum* and *Laetiporus sulphureus* in poultry practice has been identified.

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EFFICIENCY OF USING *ECHINACEA PURPUREA* IN FEEDING LAYING HENS OF A PARENT FLOCK

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Abstract

The main factor in obtaining high-quality hatching eggs is the full balanced feeding of laying hens of the parent flock. In order to study the effect of Echinacea purpurea on the morphological qualities of hatching eggs, a scientific and economic experiment was carried out on laying hens of the parent flock of broilers (it was named as Kobb-500). The birds of the experimental groups were additionally introduced into the feed mixture 1% dry weight of Echinacea purpurea. Evaluation of Kobb-500 chicken eggs at various age periods showed that all morphological indicators of the eggs corresponded to optimal values: protein index - from 7.6 to 8.4%, yolk index - from 39.0 to 42.0%, form index - from 74.9 to 75.3, Howe units - from 82.5 to 84.4 conventional units. In the experiment, a positive effect of Echinacea purpurea on egg mass, shell thickness, and protein index was noted. In general, studies have shown a positive significant effect of Echinacea purpurea on egg morphology and chicken productivity.

Key words*: cross "Kobb-500", Echinacea purpurea, hatching qualities of eggs.*

INTRODUCTION

The most important element of the technological process in broiler meat production is an efficient system of poultry farming. At the same time, the main factor in obtaining high-quality hatching eggs and increasing the hatchability and viability of young poultry is considered to depend on the balanced feeding (Struk et al., 2013).

Therefore, nowadays it becomes important to increase the reproductive qualities and productivity of poultry with the directed influence of biologically active additives (BAA) on metabolic processes at different periods of poultry breeding. These BAA include various kinds of biological stimulants of plant origin, as well as a number of pharmacological drugs.

In this regard, to obtain premium products, it is safer and more effective to use natural herbal additives (NHA). The positive example of natural herbal additives could be obtained from a plant of the Asteroideae family - *Echinacea purpurea* (Daryin, 2011; Ovchinnikov et al., 2012).

Thus *Echinacea purpurea* was used in our research as NHA to improve the quality of hatching eggs and the productivity of laying hens of the broiler parent flock.

The quality of eggs is one of the main conditions ensuring high incubation productivity. The level of the most important zootechnical indicators (the output of the young poultry and the viability and productivity of the broiler parent flock) depends on the hatching eggs quality (Dyadichkina, 2010). Evaluation of the quality of eggs is the first necessary step for successful incubation.

MATERIALS AND METHODS

Following the aim to examine the effect of *Echinacea purpurea* on the morphological qualities of hatching eggs, a scientific and economic experiment was carried out upon the laying hens of the broiler parent flock from the crossbreed "Kobb-500" poultry in the conditions of the Vasilievskaya Poultry Farm (the Penza region, Russia).

During the experiment the analogue method was conducted and six groups of laying hens with 250 birds in each were formed. In addition, 1% of dry mass experimental groups of *Echinacea purpurea* was added to the daily ratio (compound feed).

In the experiment we used a variety of *Echinacea purpurea* grown in the Scientific

Research Institute of Agriculture (Lunino village, the Penza region, Russia). *Echinacea purpurea* was introduced into daily compound feed by step mixing in a microdoser.

The experimental groups of laying hens were fed a compound feed mixture with the addition of *Echinacea purpurea* from 32 to 45 weeks of age according to the experimental scheme (Table 1). All experimental hens received a plant stimulant with a frequency of 21 days. The first experimental group had been receiving a compound feed mixture with the addition of Echinacea *purpurea* continuously for 7 days, the second - for 6 days, the third - for 5 days, the fourth - for 4 days, the fifth - for 3 days.

Table 1. The experimental scheme

Group	Poultry flock	Feeding Features	Duration of application additives	Frequency, days
Control	250	Main ration (MR)	Constantly	Constantly
1 experimental	250	MR+1 g *Echinacea purpurea* per 100 g of the compound feed mixture	Within 7 days	In 21 days
2 experimental	250	MR+1 g *Echinacea purpurea* per 100 g of the compound feed mixture	Within 6 days	In 21 days
3 experimental	250	MR+1 g *Echinacea purpurea* per 100 g of the compound feed mixture	Within 5 days	In 21 days
4 experimental	250	MR+1 g *Echinacea purpurea* per 100 g of the compound feed mixture	Within 4 days	In 21 days
5 experimental	250	MR+1 g *Echinacea purpurea* per 100 g of the compound feed mixture	Within 3 days	In 21 days

For the experiment, the hatching eggs of the broiler parent flock from the crossbreed “Kobb-500” poultry were used (aged of 32, 38 and 42 weeks of life).

These eggs were evaluated according to the following morphological indicators: egg mass, egg shape index, shell density and thickness, protein and yolk index, Xay index. The definition of these indicators was carried out as follows: the mass of the egg and its components - by weighing on an electronic scale with an accuracy of 0.1 g; density - with the help of saline solutions of various concentrations; shape index - with a trammel; shell thickness – with the special device to determine the shell strain; protein and yolk height – by an altimeter and a trammel, and, according to the data obtained, protein (yolk) index was calculated by dividing its height by the average diameter; the ratio of the protein mass of yolk was obtained by dividing the mass of protein by the mass of yolk; Xay index was defined according to the table using the value of the egg mass (g) and the standing height of the outer dense protein (mm) when pouring the contents of the egg onto a flat glass. The egg production of laying hens was measured daily with the subsequent calculation of egg production for the initial and average layers. The amount of egg mass - was determined as the product of the egg production of the average laying hen and the average egg mass.

The climate parameters of the keeping premises were maintained according to the recommendations for the broiler parent flock from the crossbreed “Kobb-500” poultry. The poultry was kept outdoor using a set of “Roxell” equipment manufactured in Belgium.

RESULTS AND DISCUSSIONS

It is known that the quality of eggs is determined as a result of the total exposure to genetic factors, feeding conditions and poultry keeping.

One of the main indicators of the quality of eggs is their mass. In the experiment, it was noted that as aged the hen as the egg mass increased: at the age of the 32 weeks, the average egg mass in all groups was 59.0 g, at the 38 weeks of age it increased by 4.2 g and at the 45 weeks of age by 7.55 g. The mass of eggs at the beginning of the experiment, at 32 weeks of age, varied slightly from 59.4 to 58.6 g. After six weeks, at the 38 week of age, the mass of eggs from the laying hens of the experimental groups was higher than the same in the control group by 0.36 g. The largest mass of eggs differed in laying hens of the 1st and 4th experimental groups and was 0.8 and 0.9 g, respectively. However, at the 45 week of age, the weight of eggs in poultry groups varied slightly from 66.2 to 66.8 g. Nevertheless, the experimental laying hens retained a tendency to exceed this indicator over the control group by 0.4 g.

It was noted that with the age of the bird and the increase in egg weight on average for all groups of birds, the relative protein content decreased from 63.9% at the 32 week of age to 61.8% at the 38 week and to 61.3% at the 45 week of life. At the same time, the experimental group exceeded the control counterparts by this indicator at the 32 week of age by 0.6%, at the 38 week of age by 0.1% and at the 45 week of age by 1.7%. With a relative decrease in protein content with age of the poultry, the yolk content increased. At the 32 week of age, the relative yolk content was 25.5%, at the 38 week of age - 29.4% and at the 45 week of age - 30.0%.

At the 32 week of life, the highest relative yolk content was observed in the 1st and 2nd experimental groups of 25.7 and 25.8%, which is higher than the control group by 0.3 and 0.4%, resp. ($P<0.05$). At the 38 weeks of age, the highest relative yolk content was also observed in the 1st and 2nd experimental groups of 30.1 and 29.8%, which is higher than the control group by 0.4 ($P<0.05$) and 0.1% resp. At the 45 week of life, the highest relative yolk content 31.7% was detected in the control group. The data obtained indicates that as the egg mass increases as to the yolk content increases and the protein content decreases, which affects their ratio.

Table 2. The ratio of the eggs components from the experimental laying hens, M±m, %

Indicator	Group					
	Control	1 experimental	2 experimental	3 experimental	4 experimental	5 experimental
At the 32 week of life						
The mass of eggs, g	59.2±0.5	59.2±0.5	59.0±0.7	58.6±0.5	59.4±0.5	58.7±0.7
Protein	63.4±0.1	63.6±0.2	63.6±0.2	64.2±0.1	64.1±0.1	64.5±0.2
Yolk	25.4±0.1	25.7±0.1	25.8±0.1	25.2±0.1	25.6±0.1	25.5±0.1
Shell	11.2±0.1	10.7±0.13	10.6±0.1	10.6±0.11	10.3±0.12	10.0±0.12
At the 38 week of life						
The mass of eggs, g	62.9±0.4	63.7±0.4	63.5±0.4	62.4±0.4	63.8±0.4	62.9±0.4
Protein	61.7±0.1	61.3±0.1	61.6±0.1	61.8±0.1	62.1±0.1	62.3±0.1
Yolk	29.7±0.1	30.1±0.2	29.8±0.2	28.3±0.1	29.3±0.2	29.2±0.2
Shell	8.6±0.1	8.6±0.08	8.6±0.09	9.9±0.1	8.6±0.1	8.5±0.09
At the 45 week of life						
The mass of eggs, g	66.2±0.4	66.7±0.4	66.7±0.4	66.4±0.4	66.5±0.4	66.8±0.4
Protein	59.9±0.2	62.2±0.2	61.5±0.2	61.3±0.2	61.4±0.2	61.8±0.2
Yolk	31.7±0.1	29.4±0.1	30.0±0.1	29.9±0.1	29.8±0.1	29.1±0.1
Shell	8.4±0.08	8.4±0.1	8.5±0.1	8.8±0.09	8.8±0.08	9.1±0.1

In the experiment, it was noted that with an increase in the egg mass, the absolute shell mass changed insignificantly. In this regard, the relative mass of the shell with the age of the

poultry and increasing in the mass of the egg gradually decreased. So, at the 32 week of life, the relative shell mass was 10.6%, at the 38 week of age - 8.8% and at the 45 week of age - 8.7%.

The thickness of the shell mainly determines its strength and resistance to mechanical destruction. At the 32 week of life, the thickness of the shell ranged from 355.9 to 362.0 microns. In general, from the experimental flock, the egg shell was 4.1 microns thicker compared to the control group ($P<0.001$). The eggs of the first experimental group of 362.0 μm differed in the largest shell thickness, which is 6.1 μm more than that of the control analogues ($P<0.001$). At the 38 week of age, the thickness of the shell on average for all groups increased by 15.2 microns and amounted to 374.5 microns. The birds of the 3rd and 4th experimental groups differed in the largest shell thickness, while the excess over the control group was 9.5 and 10.5 μm, respectively ($P<0.001$). At the age of 45 weeks, the shell thickness on average for all groups was 362.5 μm, which turned out to be 12 μm lower than the average shell thickness at the 38-week-old bird ($P<0.001$). However, even at this age, the experimental poultry was defined by the largest shell thickness in comparison with the control group. Among all the experimental groups, the largest shell thickness was observed in the 4th and 5th experimental groups, while the superiority over the control group was 15.3 and 17.3 μm ($P<0.001$).

The egg quality is also characterized by the density of the egg, which defines the freshness of the egg and determines the quality of the shell. During the current research, it was noted that the egg density decreased with age. At the 32 week of life, the egg density was 1.081, at the 38 week - 1.074 and at the 45 week - 1.070 g/cm^3. The experimental group had the higher egg density at the 32 and 38 weeks of life compared with the control group of laying hens. However, the similar results were not observed at the 45 week of life. The diameter of the air chamber is negatively correlated with the egg density. Analyzing the experimental data, it was generally noted that with a decrease in the egg density, the diameter of the air chamber increased. At the 32 week of age, the laying hens with the egg density of 1.081 g/cm^3, the diameter of the air chamber was 1.95 mm, at the 38 week of life with the egg density of 1.074 g/cm^3, the diameter of the air chamber was 2.2 mm and at the 45 week of life with the egg density of 1.070 g/cm^3, the diameter of the air chamber is 2.3 mm.

The egg shape is an important quality indicator, as it significantly affects the position of the embryo during its development, as well as the damage of the shell in the process of collection, transportation and hatching. The most accurate indicator of the shape is the shape index, which normally should be 74-78% for non-calibrated eggs. The higher the egg shape index - the more rounded the eggs, and the lower the egg shape index - the more extended and elongated eggs.

At the 32 week of age, the form index for all analyzed groups was 77.08, at the 38 week - 78.41 and at the 45 week - 77.88. In this case, the tendency to increase the shape index with an increase in egg mass was noted.

The quality of the contents of the egg is characterized by the indices of protein and yolk. The yolk index of the hatching eggs ranged from 44.12-44.81% and remained virtually unchanged during the reproductive period. At 32 weeks of age, it was 44.23%, at 38 weeks of age - 44.67%, and at 45 weeks of age - 44.34%.

One of the indicators of the egg quality is the protein index, which in the experiment was characterized by relative stability. This indicator amounted to 6.86% at the 32nd week of life, and 7.16% at the 38th and 45th weeks of life, which corresponds to normative indicators.

Regarding the indicators of the protein quality, the Xay index has the highest relationship with its index, since both of these indicators are determined on the basis of measuring the height of the dense protein layer. The optimal values of the Xay index for hatching eggs are 65-87. Analyzing the obtained data, it could be found out that during the reproductive period this indicator was in the range of 74.3–82.0 conventional units (Table 3). At the 32 week of age this indicator was 74.3, at the 38 week - 78.5, and at the 45 week - 82.0 conventional units.

Table 3. Indicators of egg density, shape index, diameter of the air chamber and shell thickness of the experimental laying hens, M±m

Indicator	Group					
	Control	1 experimental	2 experimental	3 experimental	4 experimental	5 experimental
At the 32 week of life						
The density of the egg, g/cm^3	1.080± 0.001	1.081± 0.001	1.080± 0.001	1.080± 0.001	1.081±0.001	1.087± 0.001
Air diameter cameras, mm	1.95± 0.03	2.00± 0.04	1.97± 0.03	1.99± 0.03	1.80± 0.03	1.99± 0.04
Shell thickness, microns	355.9± 0.5	362.0± 0.7	358.0± 0.6	360.9± 0.5	360.0± 0.7	359.0± 0.7
Index of the form,%	76.39± 0.43	77.16± 0.17	77.22± 0.18	77.90± 0.43	77.36± 0.17	76.42± 0.18
At the 38 week of life						
The density of the egg, g/cm^3	1.072± 0.001	1.072± 0.001	1.074± 0.001	1.075± 0.001	1.074± 0.001	1.075± 0.001
Air diameter cameras, mm	2.20± 0.03	2.20± 0.03	2.21± 0.03	2.21± 0.03	2.21± 0.03	2.20± 0.03
Shell thickness, microns	368.6± 1.0	372.1± 0.6	373.1± 0.6	378.1± 0.9	379.1± 0.6	376.1± 0.5
Index of the form,%	78.55± 0.23	78.08± 0.25	78.40± 0.24	78.53± 0.23	78.40± 0.26	78.50± 0.24
At the 45 week of life						
The density of the egg, g/cm^3	1.071± 0.001	1.068± 0.001	1.070± 0.001	1.069± 0.001	1.069± 0.001	1.071± 0.001
Air diameter cameras, mm	2.29± 0.02	2.29± 0.02	2.31± 0.02	2.29± 0.02	2.30± 0.03	2.29± 0.02
Shell thickness, microns	350.8± 0.8	362.1± 0.9	363.1± 0.9	364.9± 0.8	366.1± 0.9	368.1± 0.9
Index of the form,%	77.15± 0.18	77.86± 0.18	78.06± 0.18	77.82± 0.17	77.93± 0.19	77.94± 0.18

The most important indicator of laying hens of the broiler parent flock is the egg production. The highest egg productivity for the entire accounting period from 32 to 45 weeks of age was observed in the poultry of the experimental groups, which averaged 1374 eggs, which is 64 eggs higher than the data of the control group (P<0.01). The poultry of the 2nd and 4th experimental groups had the highest egg productivity, while the superiority over the control group was 72 and 74 eggs resp. In the calculation of the average laying hen, the egg production in the control group was 74 eggs, in the 2nd and 4th experimental groups there were 3.7 and 4.6 eggs more (P<0.01).

Table 4. Productivity and safety of laying hens in the experimental groups

Indicator	Group					
	Control	1 experimental	2 experimental	3 experimental	4 experimental	5 experimental
Egg laying of the initial laying hen, eggs	73.4	76.2	77.4	77.3	77.5	76.4
Egg laying of the average laying hen, eggs	74.2	77.9	77.9	77.8	78.8	77.1
Gross egg collection by group	1310.9	1360.3	1382.9	1380.6	1384.4	1363.8
Preservation,%	97.77	95.54	98.63	98.91	96.23	97.89

CONCLUSIONS

Thus, the assessment of eggs upon the laying hens of the broiler parent flock from the crossbreed "Kobb-500" poultry at different age periods showed that all morphological indicators of the eggs corresponded to the optimal values: protein index - from 7.6 to 8.4%, yolk index - from 39.0 to 42, 0%, egg shape index - from 74.9 to 75.3, Xay index - from 82.5 to 84.4 conventional units. In the experiment, a positive effect of *Echinacea purpurea* on the egg mass, shell thickness, and protein index was noted. Also revealed a significant effect of *Echinacea purpurea* on the egg productivity of the experimental poultry.

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PERSONALIZED NUTRITION-CURRENT TRENDS AND CHALLENGES

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Abstract

At present at national, European and international level, there is a real tendency for personalized nutrition, known to be that a food can have a positive effect for one person and negative for another and has emerged as an alternative to solving various health problems. Personalized diet recommendations are based on data on food habits and risk behaviours, physical characteristics and genetic profiling. The genetic code is similar for all people, the small variations that differentiate us can determine the effect, different responses of nutrients and how each individual metabolize food, as they interact with the genes. In the pioneering phase, the challenge is to opt out of global protocols in different conditions and paradigm shift. The nutrition of the future will be based on the identification and analysis of the genetic profile, and according to the existing data base, will be developed personalized therapeutic nutritional and pharmacological plans corresponding to the identified genetic profile. The purpose of this article is to capture the current level of knowledge about genetic factors that will allow for customized diet and broadening of the horizon of gene nutrition.

Key words*: personalized nutrition, genetic profile, genetic nutrition*

INTRODUCTION

The first definition about individual nutrition were mainly based on discussions about "genes", frequently used as a synonym for personalized approach. The nutritional advice given according to the individual characteristics and behaviors is future, but it takes a lot of work to be able to make known key messages, which will lead to a change in the dietary preferences of people (Ordovas et al., 2018). Despite what has been said, this thought has been reconsidered to encompass the notion of levels.

Any of us comes into the world with a genetic dowry inherited from parents who adapted appetite, gives response to stimuli, chemical and biological transform food components and cause various diseases. Eating disorders are on the rise. Even if it is a big problem, we can say with certainty that most of the growth occurs due to the unhealthy diet based on already existing risk behaviors and we refer here to the consumption of alcohol, coffee or smoking. Conclusive clues accredit the idea that it is possible to be more secure in adopting an adequate lifestyle drug treatments to prevent illnes in individuals predisposed to disease (Knowler et al., 2002). Personalized nutrition is being discussed because it is promising now and it will definitely be the nutrition of the future. The purpose of this article is to bring information that gives another dimension to personalized nutrition: information about the reciprocal action of nutrients with the genome, about the different response of humans to interventions on eating habits, about how genes and their variants can influence absorption and metabolizing nutrients from diet. The diet involves, firstly, the balancing of some deficiencies existing in the nutritional processes of assimilation and dissimilation in the body through the food assembly, and secondly, through the appropriate nutritional processes, the ability to use the nutrients indispensable to an affected organism (Natea, 2008). We would like to present views from more recent or older works in the field of personalized nutrition, noting relevant issues that alter the particular response to lifestyle or eating habits. Because at the molecular level two identical conditions will not be encountered, each of them having a unique genetic profile, after identifying the particularities of the profile, personalized nutrition must be effective in prevention by specifying the predisposition to a certain disease because, the existence of a characteristic gene or some mutations, in most situations, this is the premise of a particular disease. If the genetic resource is to be expressed as a condition, it is

due to the interaction of the whole genome-environment-behavior (Fenech, 2007). Personalized nutrition should ultimately lead not only to prevention, but also to limiting the number of illnesses or reducing the suffering period compared to general nutritional recommendations. Once the genetic profile is identified, the condition can be treated by an individualized protocol so that it is necessary to select the nutrients according to their composition in the nutrients that defend the genome (Fenech, 2007).

Nutrigenetics and Nutrigenomics

Nutrigenetics and nutrigenomics are defined as knowing the efficiency of "genetic" transformations on hereditary nutritional challenges and biologically active nutrients in manifesting a functional nucleotide sequence (Simopoulos, 2010; Corella and Ordovas, 2009; Trujillo et al., 2006; Ferguson, 2009; Kaput, 2008; Ordovas and Corella, 2004). Harnessing the information about the genome, associated with wide-ranging omics techniques, has created the possibility of obtaining new information, which aims to achieve an efficient perception of nutrient-gene interference depending on genetic endowment, having as final aim the development of the processes individualized nutrition, to obtain that physical, mental and social well-being as well as disease prevention (Simopoulos, 2010; Corella and Ordovas, 2009; Trujillo et al., 2006; Ferguson, 2009; Kaput, 2008; Ordovas and Corella, 2004). The development of nutrigenetics bears a new nutritional paradigm, hinting at the possibility of personalizing the foods corresponding to the individual genetic structure. We hypothesize that in the next 25 years there will be a radical transformation of the technology that will encompass fields such as biotechnology, nanotechnology and genomics (Coronado et al., 2007). In the report of a special significance that resulted from research food-gene interference the condition of achieving intra and transgenerational effect is epigenetics (Jirtle and Skinner, 2007; Sharma et al., 2010). The accepted definition of epigenetics refers to researching the transformation of the function of inherited mitotic and/or meiotic genes that do not require remodeling in the DNA sequence (https://ncbi.nlm.nih.gov/pubmed/11498582) and is epigenomics refers to the research of epigenetic transformations in a cell or the whole organism. Epigenetic transformations act on norm growth and development DNA methylation globally, plays a role in Nogenesis cells because, in many hypo- and hyper-methylation researches, DNA has been associated with cancer. Because nutrition can readily adjust biological chemistry directions by transforming the phenotypic effects of genes, the nutrigenomics domain has the potential to boost the knowledge of the interference between food and the epigenomics (chemical transformations at the level of DNA and histone proteins) in the development of human diseases (de Luca et al., 2017). Recent examples of the role of epigenetics have been encountered in obesity (Bordoni and Capozi, 2014; Capozi and Bordoni, 2013) and in the predisposition to develop type 2 diabetes. Nutrigenetics and nutrigenomics are based on several essential elements. First, nutrition can influence health outcomes, by directly harming gene expression in biochemical and energy transformations in organism biology. It may indirectly affect all genetic mutations either at the chromosomal level or at the base sequence level, and may further produce changes in quantitative determination and gene expression. Secondly, the effects of nutrition on the health of nutrients and their combinations (nutrients) are based on different hereditary genetic aspects which may change the absorption, biochemical and energy transformations of the nutrients. A deficiency or excess of nutrients can affect genome stability, cause mutations or gene grading contrary to normal rules, and gene expression may result in other phenotypes in different life stages. Good health results can be obtained if the nutritional needs are addressed on a personal level, taking into account the hereditary genetic profile, the acquired genetic characteristics, conditioned in turn by their lifestyle, traditions and eating habits, risk behaviors and health status. Of particular importance is to give better meaning to information from epidemiological and clinical epidemiological intervention studies regarding the impact on health of dietary factors that may help to revise recommendations for personalized nutrition (Ordovas and Corella, 2004; Simopoulus and Ordovas, 2004).

It is becoming easier for an individual's genome to be identified, to provide information on a broad spectrum of (single-nucleotide, polymorphism SNP, insertion-deletions, inversions, or variants of number of copies) in critical genes involved in metabolism, nutrients and pathways that require micronutrients as cofactors (Frazer et al., 2009).

Nutrigenomics is one of the most developed fields of research; includes studies on the impact of dietary components on genome functioning in terms of gene expression patterns and epigenetic modifications, such as DNA methylation and histone modifications (Bordoni and Gabbianelli, 2019). Most of the nutrigenomic research has been carried out on human nutrition and the effects of nutrients on the etiology of the disease (Reddy et al., 2018).

The purpose of nutrigenomic studies is to achieve personalized nutrition (Bouwman, 2008) considering who could use personalized nutritional products, associated tips and what are the limitations of providing potential users, provided extremely specific information about individual health risks and the benefits of specific habits. Nutrigenomics investigates the ways in which nutrients can act as chemical signals to influence gene expression, thereby altering protein synthesis and functioning of different metabolic pathways (Martinez, 2007). Nutrigenomics determines the effects of ingested nutrients and other dietary components on gene expression and regulation that is, they study diet-gene interaction to identify dietary components that have beneficial or harmful effects on health (German, 2005; Miggiano, 2006). Our genes include a myriad of hereditary alternatives that are aware of the transformations regarding nutrition some of them through poorly understood systems (van Ommen et al., 2017). It will also determine the nutritional needs based on the person's genetic makeup, the association between diet and chronic diseases, such as cancer, type 2 diabetes, obesity and cardiovascular disease (CVS) (Miggiano and DeSanctis, 2006).

Studies with reference to diet-gene interactions

According to various database searches, three levels of personalization are available to provide personalized nutrition (Gibney and Walsh, 2013; O'Donovan et al., 2017). The pan-European Food 4 Me study, the largest randomized controlled study recently illustrated, analyzed the performance of personalized nutrition (Celis-Morales et al., 2017). The study was conducted in seven European countries, was developed as a clinical study with the participation of 1600 individuals, divided into four intervention groups. The classification referred to dietary recommendations at the personal level as follows: - level 0 - control group that received non-personalized recommendations; level 1 - the group received individual recommendations based only on food intake; level 2 - the group received individual advice based on dietary intake and phenotype; level 3 - received individual recommendations based on dietary intake, phenotype and genotype. There was also a shift from individuals to groups of nutritional recommend-dations and they are called "stratified" or "adapted" (Ordovas et al., 2018). Other studies have investigated, how genes influence food preferences, affecting their signaling pathways rewards, or homeostatic energy (Garcia-Bailo et al., 2009). Developing a genetic basis for food tastes could provide the opportunity for the development of new nutritional products, targeting characteristic genotypes or ethnic population, and may explain the inconsistencies between studies related to foods with chronic disease (El-Sohemy et al., 2007).

To date, the diversity of diet-gene interference analyzes has hampered the transposition of the purpose of analyzes into practical applications of the guidance based on personalized nutrition. The tests used indicate the precariousness of a patient with lactose and fructose intolerance or gluten intolerance. A conclusive sample is that the nutrient mutation 13910 C/T, is at a distance of about 14 kilo bases above the LCT lactase gene and controls lactose transcription. Substitution of thiamine with cytosine in this polymorphism allows permanent digestion of lactose at maturity (Enattah et al., 20020. In the diagnosis of hypolactasia, it was shown that C/T (13910) polymorphism with only a single protein component, with 13910 base pairs at the 5' end of lactase, is closely linked to lactase resistance and harms much of the statical collectivity, due to lactose intolerance (Rasinpera et al., 2004). In the study of celiac

disease, an appreciation of dietary applicability and the perception of celiac of dietitians could be made, especially in the village environment of Australia 2007-2014. Understandable impediments to appreciating a gluten-free diet, including restricted accesibility to dietitians or autonomy and limited prices of gluten-free preparations (Ludvigsson et al,. 2014) or screening at phenylketonuria have allowed the use of nutritional advice based on genetic makeup (avoiding lactose by limiting the consumption of fresh milk, avoiding gluten and phenylalanine containing products for people at risk). CYP1A2 gene censor caffeine metabolism in coffee. Individuals carrying genetic variation C are at increased risk of myocardial infarction if they consume more than one cup of coffee per day, it is recommended to limit coffee consumption (https://www.genetx.eu/).
The PLIN1 gene controls storage and the release of adipocyte fat. Female persons with the AT or GT haplotype have an increased risk of obesity. The FTO gene contributes to exercising control over hunger, hypothalamus, and eating preferences.

Efficiency of personalized nutrition in cancer

Cancer is a multi-step process in which gene expression, protein function and metabolite begin to function aberrantly (Franco and Reitsma, 2001). The risk of cancer development can be significantly increased if there is a gene-diet interaction (Nutrigenomics: https://www.Diet.com). Nutritional health depends on the interaction between the environmental aspects of supply, bioavailability, consumption and cohesion of dietary components, and genetically controlled aspects of digestion, absorption, distribution, transformation, storage, and excretion by proteins in the form of receptors, carriers, enzymes, and hormones (Berdanier and Hargrove, 1993; Castro and Towle, 1986; Rucker and Tinker, 1986; Williams et al., 1990). The ADH1 gene controls the metabolism of alcohol to acetic aldehyde, the ALDH2 gene controls the metabolism of acetic aldehyde to acetic acid. The two genetic variations in the same individual A (ADH1) and T (ALDH2) confer an increased risk of gastric cancer at an alcohol consumption greater than 5 g/day (https://www.genetx.eu/). The AGTT haplotype is associated with an increased risk of gastric cancer (https://www.genetx.eu/). Personalized nutrition has traditionally been based on adapting food components according to personal needs and options. Today, this thinking has been reinforced by the use of highly successful ascending terminologies, in order to facilitate the knowledge of the molecular systems that are the foundation of a healthy state. This understanding would allow the adaptation of special dietary recommendations to suit the needs of the characteristic patient groups based on the identified genetic profile. The excellent evolution of nutrition directions on the most rigorous researches for the development of health requires a proper assessment of the bioaccessibility and cost-effectiveness of food components. To make these phenomena possible, credible explanations on the description of nutrients, their contributions and their consequences after application are indispensable.

Future practical implications of nutrigenetics and nutrigenomics

Nutrigenetics and nutrigenomics are fundamental aspects in establishing the effect of personalized nutrition on health, and the incidence of nutrients can be assessed objectively and as widely as possible, using human techniques. Four 'omic' domains of agriculture (agronomics) and nutrition (nutrigenomics, nutriproteomics, nutrimetabolomics) have been evoked as effective processes for understanding the diversity of relationships between individuals as a solution to environmental exposure to food (Ozdemir and Kolker, 2016). Progress in 'omic' techniques is constantly shifting the different way of responding to the same lifestyle treatment, to reality. Even if they are attached to the decisive analytical challenges, precisely the exact adaptation of the multiomic research and the introduction of the means and the computerized data banks, will facilitate the reproduction of the clinical results, in positive individual feeding procedures (Braconi et al., 2018). Genetic dowry, way of life and eating habits can exert an influence on personal nutritional needs and depending on the geographical area, the existing social-economic status, religion and traditions. The transposition

of these knowledge into valid recommendations following the identification of the genetic profile at the individual level is used in a few circumstances (phenylketonuria or galactosemia), because the genetic profile is overwhelming on any other factor. Accessibility based on valid studies is the only way that can guarantee that the information provided by nutrigenetics and nutrigenomics are applied accordingly. In Romania, personalized nutrition as a trend, is more at the level of research and very little transposition into practice. The challenge is to transform the knowledge we have into working tools that, individually applied, contribute to the physical and mental well-being we all want. Of course, identifying the genetic profile also raises ethical issues. But as long as this information / knowledge remains within the boundaries of confidentiality and will be used only and only for the restoration and consolidation of health, while respecting the right to image, dignity and personal life, as regulated in national and European law, the benefit is huge. Although few nucleotide sequences have been observed that have the consequence of cancer genesis, there is no well-which greatly diminishes their life expectancy. Cancer and its treatment alter the nutritional status of the patients by altering the metabolic function and by decreasing the dietary contribution (Shahmoradi et al., 2009; Trabal et al., 2006). Studies have shown that under nutrition is a psychological evidence of a predisposition to disease in developed cancer, and it is therefore possible that it has a great significance (Gupta et al., 2005). It is therefore imperative that future studies be conducted to come up with personal nutrition solutions in these situations as well.

CONCLUSIONS

Deciphering gene – nutrient - disease interference will be difficult. Currently, tests are being carried out and nutritional recommendations are applied in practice to avoid lactose, this being the specific carbohydrate, which represents about 90% of the total milk carbohydrates and avoiding gluten (a protein that favors gluten intolerance, currently undiagnosed) by eliminating noodles, not bakery and pasta product. The introduction of high-value standard bring the feeling of tranquility and confidence of those suffering, transforming health care into a true art. Based on the valid clear evidence of a predisposition to a particular disease, we hope that personalized nutritional will be optional in a first phase in the care plan, and the conclusions of future research will go beyond the option indispensable health certainty. Many research is crediting the idea of paradigm shift due to the fact that there are specific treatment option and diets depending on the individual genetic profile, so as to give up global treatments, the same for everyone and to apply differentially. Many research is crediting the idea of paradigm shift due to the fact that there are specific treatment options and diets depending on the individual genetic profile, so that global treatments, the same for all, and differentially applied, are given up.

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USE OF UNCONVENTIONAL FEED FOR BROILER CHICKENS

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Abstract

Studies have been conducted on the effectiveness of the use of fattening broilers at the age of 15-38 days of unconventional grain feed for poultry farming in the amount of 30% of the feed mass used instead of corn grain. It has been established that the use of bare-grain barley and triticale grains in the composition of the fed experimental compound feeds increases the digestibility of dry substances and crude protein in the digestive tract of chickens by 2.4-2.8 and 3.3-3.5%, which is accompanied by an increase in the growth rate of young animals by 7.4-7.8%, respectively, compared with the use of corn in the control group. The use of bare oats is considered not an effective method, as it leads to a decrease in meat productivity of chickens by 3.1% due to a decrease in the digestibility of nutrients. There is a decrease in feed costs for producing 1 kg of poultry meat when feeding barley and triticale grain by 2.8-3.9% and an increase in slaughter yield of 1%.

Key words*: bare oats, triticale, chickens, barley.*

INTRODUCTION

In the structure of the diets of poultry, grain feed accounts for 70-75% and most of them are corn and wheat. At the same time, feed production in the Russian Federation is experiencing a significant shortage of feed corn, the deficit of which is covered by imports.

In order to import substitution, it is necessary to study the possibilities of using non-traditional grain feeds of domestic selection in poultry feeding, which can become an alternative to corn (Winkler et al., 2018).

Grains of traditional barley and oats in compound feeds intended for young birds, due to the high content of crude fiber (4.5-9.5%), glucans, pentosans and a low level of metabolic energy, is rarely used. By peeling it is possible to increase their nutritional value, but at the same time, the cost of grain increases (Winkler et al., 2017).

The solution to this problem in poultry can be the use of non-traditional grain feeds: a new variety of barley grain – "Omsk bare-grained 1" and oats – varieties "Tyumen bare-grained 1". The advantage of filmless varieties is a low level of crude fiber (1.6 to 2.8%) and a high protein content (12.3-19.5%). By energy value, they are slightly inferior to corn, but superior to wheat by 3.4% and film varieties of barley, oats by 10-14.2% (Jacob, 2012).

Of practical interest for poultry farming may be the triticale grain, which is characterized by a high level of crude protein (13-18%) and metabolic energy (285 kcal/100 g), lysine and contains much less growth inhibitors than rye. Therefore, triticale and bare-grained varieties of crops are promising feed for poultry that would reduce the cost of feed by reducing the proportion of corn and high-protein feed.

An undesirable aspect of the use of non-traditional grains feed in poultry farming is the presence of anti-nutritional substances in them, albeit in a limited amount. Therefore, their effective use is possible only when using a wide range of enzyme preparations in animal feed (Alagawany et al., 2018). In the scientific literature there is a limited amount of information on the use of the studied crops, and the results obtained on the effectiveness of their use are contradictory and require further research, especially on the norms for introducing grain into compound feed intended for poultry farming.

MATERIALS AND METHODS

The experiment was carried out in the vivarium of the Penza State Agrarian University (Penza, Russia) on broiler chickens of the cross "ROSS-508" aged 15 to 38 days. The risk of death at day old due to the presence of small amounts of anti-

nutritional factors prompted us to conduct an experiment on older chickens. For the experiment, four groups of chickens were formed with 40 animals each, analogues in live weight.

In the control group, the chickens received loose feed, in which the corn grain content was 30%. In the composition of compound feeds intended for chickens of the first, second and third experimental groups, instead of 30% of the corn grains in the control group, they included a similar amount of bare-grain barley and oats and, accordingly, triticale grains ("Ukro" variety). The norm for introducing bare grain varieties of grain and triticale into mixed feeds for broiler chickens was taken on the basis of published data.

Due to the high crude protein content in the studied unconventional feed compared with corn, to obtain isoprotein feed at the level of 21%, in the experimental groups increased the rate of input of wheat and reduced the content of sunflower meal.

The required protein level in compound feeds was achieved by introducing high-protein feeds such as full-fat soybean, sunflower meal, peas and protein-vitamin-mineral concentrate (bio supplement "WAFI"). This bio supplement with 49% protein content included: soybean meal, fish meal, minerals, vitamins, enzyme complex (Roxazim G2), phytase (Ronozim 5000) and antioxidants.

The studied compound feeds were completely taking into account the norms for introducing individual feeds into the composition of the compound feed and taking into account the periods of growth of the chickens were balanced according to the main nutrients.

Chickens of both groups were in the same conditions of floor keeping on a litter with free access to water and feed. Watering was carried out using group drinking bowls, and feeding - from group feeders.

To study the effect of different types of grain on digestibility and the use of nutrients of compound feed, a balance experiment was carried out at the end of fattening at the age of 35 days on five broilers of the same mass from each group. In the balance experiment for 5 days, the amount of feed consumed, the amount of excreted litter were taken into account, the digestibility of dry substances was determined.

The protein content in compound feeds, litter was determined by the Kjeldahl-method and protein digestibility was found. At the end of the balance experiment at the age of 38 days, a control slaughter of chickens of 10 goals from each group was performed.

RESULTS AND DISCUSSIONS

In the conducted studies, it was found that when weighing chickens at the age of 28 days, the largest live weight and, correspondingly, high growth rates were found in young animals of the first and third experimental groups when glazed barley and triticale were included in the feed (Table 1).

Moreover, the average daily gain in live weight in broiler chickens in these groups was 68.9 and 69.5 g, which was higher by 4.8 and 5.8% ($P < 0.001$) than in the control and 7.2– 8.5% higher compared to the second experimental group.

In the final period of fattening at the age of 28 to 38 days, in general, the best results were also preserved in the first and third experimental group.

Thus, the live weight of chickens when feeding grain of barley and triticale at the end of the experiment averaged 2185 and 2190 g, which was 6.3 and 6.6% higher compared to control and 9.0-9.3% compared to the use of grain of black oats in mixed fodders .

On average, during the experiment, equally high average daily gain in live weight of 75 g was obtained in the first and third experimental groups, which was 7.4-7.8% higher compared to the control and 11% respectively higher than in the second experimental group.

Table 1. The dynamics of the growth rate of chickens

Indicator	Group			
	Control	1 experimental	2 experimental	3 experimental
Live weight, aged, g: 15 days	452±5.6	455±4.3	448±4.9	457±4.8
28 days in% of control daily average gain, g in% of control	1306±1.1 100 65.7±0.65 100	1351±9.6* 103,4 68.9±0.71* 104.8	1281±13.6 98.0 64.1±0.84 97.6	1361±9.6* 104.2 69.5±0.71* 105.8
38 days in% of control daily average gain, g in% of control	2056±18.2 100 74.9±0.72 100	2185±18.1* 106.3 83.4±0.82* 111.3	2001±19.0 97.3 72.0±0.91 96.1	2190±19.0* 106.6 82.9±0.82* 111.1
During the experiment: absolute gain, g daily average gain, g in% of control	 1603±12.2 70.0±0.89 100	 1730±15.2* 75.2±0.98* 107.4	 1553±18.8 67.5±1.08 96.9	 1733±14.6* 75.3±0.98* 107.8

Note: * - P <0.001 to the control and the second experimental group.

The results of our research are partly consistent with the data of other scientists. In particular, it was found that enrichment of compound feeds with the enzyme preparation "Rovabio" (50 g/t) allows increasing the rate of entry of bare-barley from 10-15 to 25-35%, which contributes to an increase in live weight of chickens by 2.5-4.5% and to reduce feed costs per 1 kg of growth by 1.8-3.6%.

The inclusion of "Roxazim G2" enzyme preparation in mixed feeds with different levels of wheat replacement on triticale from 10 to 25% contributed to an increase in digestibility and assimilation of nutrients of mixed feeds, which led to an increase in meat productivity of chickens.

In our studies, the best results were obtained with a 30% introduction of triticale grains into compound feeds, which is probably due to the presence of a modern enzyme preparation (Roxazim G2, Ronozim 5000) in the highly mentioned Bio Supplement, which has a wide spectrum of action, a high level of enzymatic

activity and, in addition, phytases (Ronozyme 5000), as well as the use of a spring grain variety in the experiment.

As a rule, winter varieties of triticale grains, unlike spring ones, contain more anti-nutritional substances in the form of non-starch polysaccharides, which have a negative effect on the physiology of birds, whose effect is reduced due to the use of enzyme preparations and their use in poultry farming is economically justified even against the background of traditional feeds. In the literature, there is information about obtaining positive results on the use of golden oats against the background of enzyme preparations in the diets of laying hens and goslings-broilers in an amount of 20 to 50% (Alagawany et al., 2018).

Ambiguous results were obtained in studies on the glaze of oats in poultry feeding, which is apparently associated with the use of various grades of grain that differ in different nutritional values and, above all, in the content of Non-Starch Polysaccharides (NSPs).

In the experiments conducted by Belarus researchers, it was found that the inclusion of broiler chickens by the growth periods (start, growth and finish): 10, 20 and 30% of the glazed oats of the Vandrovnik variety allows increasing the live weight of young animals at the end of feeding by 3, 5% (Yanochkin, 2016). Nevertheless we did not obtain positive results on the use of bare-grain varieties of oats in poultry feeding.

The problem was that the studied grains of film-free varieties of oats was soft in structure, which indicated a high NSPs content and, during crushing on sieves with a diameter of 5 mm or more, the grain turned into flour. When receiving the powder of the powdery fraction, the primary task is to ensure a granular and homogeneous structure, which can be achieved by granulating the food.

However, the high content of NSPs worsens the granulation process and leads to the disintegration of the granules, which requires additional costs associated with the introduction

of fixing granules. In turn, friable compound feed with a powdery consistency leads to an increase in feed viscosity, sticking of the beak, and the formation of a hard-to-digest sticky mass in the goiter and stomach, which, in turn, reduces appetite and, accordingly, feed intake.

At the same time, the passage of chyme decreases, water consumption increases, the spread of putrefactive microflora in the intestine increases and, as a result, the growth rate of young animals decreases, which could lead to the death of chickens.

Apparently in this regard, in the second experimental group, mortality of bred chickens was observed, where the safety was only 90%, while in the remaining groups this indicator corresponded to 100%. Due to the low content of NSPs, corn is an excellent raw material for the production of animal feed, which is actively used in foreign countries. It has a high energy value due to the high content of starch, but its disadvantage is the low level of crude protein. In a comparative aspect, the quality of grain of bare-grain barley for poultry farming due to its high protein content and low level of NSPs could be considered ideal if anti-nutritional substances were completely absent in it.

Lowering the rate of entry of bare oats in broiler diets using certain enzyme preparations may be more effective than corn.

The indicators of the dynamics of live weight of young animals obtained in our studies indicate that the efficiency of the use of nutrients in broiler chickens from triticale and bare-grained barley for productive purposes was much higher than when using traditional corn and barley oat grain. This is evidenced by the results of the balance experiment, in which it was found that the chickens of the first and third experimental groups consumed dry matter with animal feed 6.9 and 5.1% more than in the control group, and 7.5-5.7% more than in the second experimental group, which accordingly, it was accompanied by an increase in the intake of crude protein with feed (Table 2).

Table 2. Digestibility of solids and protein in the digestive tract of broiler chickens

Indicator	Group			
	Control	1 experimental	2 experimental	3 experimental
Combined feed consumed, g	305.4	326.5	303.7	321.2
Litter highlighted, g	253.2	260.4	263.1	261.4
Solids consumed, g	277.9	298.4	275.0	292.1
Highlighted solids with litter, g	60.9	58.0	66.4	56.1
Digestibility of solids,%	78.1	80.5	75.8	80.8
Crude protein consumed, g	53.1	57.5	52.4	56.2
Highlighted protein with litter, g	9.75	8.62	10.91	8.31
Digestibility of crude protein,%	81.7	85.0	79.2	85.2

At the same time, there was an increase in the total digestibility of nutrients when using barley and triticale as part of the studied feeds, due to an increase in the digestibility of dry substances and crude protein in the digestive tract of chickens by 2.4-2.8 and 3.3-3.5%. The use of bare oats led to a deterioration in the intake and digestibility of nutrients in comparison with the control. High feed consumption led to an increase in feed consumption per head during the experiment in the first and third experimental groups by 5.1 and 3.2% compared with the control and the second experimental group by 5.7-3.8% (Table 3).

Table 3. Feed consumption during the experiment

Indicator	Group			
	Control	1 experimental	2 experimental	3 experimental
Feed consumption per 1 head: during the experiment, total kg	3.52	3.70	3.50	3.65
in % of control	100	105.1	99.4	103.2
including: growth phase	1.51	1.57	1.53	1.55
finish phase	2.01	2.13	1.98	2.1
per 1 head for the entire fattening period, kg	4.02	4.20	4.05	4.15

Feed conversion, kg	1.96	1.92	2.02	1.90
Feed consumption to receive 1 kg of meat, kg	2.81	2.73	2.92	2.70
in % of control	100	97.2	103.9	96.1

Despite this, due to more intensive growth over the entire period of fattening, the highest rates of feed conversion (1.9-1.92 kg) were established in the first and third experimental groups. At the same time, feed consumption per 1 kg of meat when using grain of bare-grain barley and triticale was 2.8 and 3.9% less compared to using corn.

The results of the control slaughter conducted at the end of the feeding showed that due to the higher live weight, the chickens of the first and third experimental groups had, respectively, a high slaughter mass of carcasses (Table 4).

Table 4. The indicator of slaughter chickens

Indicator	Group			
	Control	1 experimental	2 experimental	triticale
Pre-slaughter mass, g	2042±26.3	2180±27.1	2005±30.8	2187±29.7
Slaughter weight, g	1431±15.6	1549±18.3*	1387±18.8	1557±17.6
Slaughter result, %	70.1	71.0	69.2	71.1

Note: * - P <0.001 to the control and the second experimental group.
Moreover, their slaughter result was higher by 0.9 and 1.0% compared with the control group.

CONCLUSIONS

In our studies, it was found that “Omsk bare-grained 1” and “Ukro” spring triticale grain are a good alternative to traditional corn in broiler chicken feeds and it is advisable to include 30% of the feed mass from 18 days of age until completion fattening.

The use of oats variety “Tyumen bare-grained 1” requires further study by determining the optimal input standards with the selection of certain enzyme preparations.

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TABLE EGGS STRUCTURE, FRESHNESS STATUS AND SHELL INTEGRITY TRAITS UNDER THE INFLUENCE OF CERTAIN ADDITIVES USED IN LAYING HENS DIET

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Abstract

Several nutritional approaches were used to warrant the quality and safety of hen table eggs throughout the last decades. Apart from most of the researches focusing on the usage of a wide variety of minerals sources in layers' nutrition, this study aimed to measure the effect of using six different feed additives on the eggs quality, hypothesizing in the beginning on the indirect effects that they might induce in hens' minerals metabolism, in electrolytic homeostasis or in improving the digestive organs capacity to uptake minerals form diet and to transfer them in egg structure. Seven groups comprising each 120 ISA Brown layers, aged 30 weeks, were formed and fed a conventional diet for peak of production at a daily intake of 120 g mashed feed (2750 Kcal/kg metabolizable energy, 17.5% crude protein). Control group (CG) received plain diet, without additives, while the other groups received different rates of additives supplementation: 0.03 % ascorbic acid (ASC group); 1% sodium bicarbonate (SOB group); 0.3% prebiotics as organic acids mixture (OAC group); 0.1% mannan oligosaccharide prebiotic (MOS group); 0.1% synbiotic mixture-probiotic, prebiotic and phytogenic compounds (SYN group); 0.2 % mycotoxins adsorbent (MYC group). The reasoning criteria were represented by: eggs structure (% of edible and non-edible parts); Haugh Index (HU) as freshness trait; eggshell integrity (% of whole eggs yield); eggshell defects and commercial loss (% of whole eggs yield); shell thickness (mm); shell breaking strength (Newton). All analyses were run for one-week eggs yield, using 280 eggs randomly collected from each group (40 eggs per day). Eggs structure varied between 25.17%-25.73% yolk proportion, 64.47-65.00% albumen proportion and 9.34-9.89% shell proportion, with certain levels of statistical significance between tested groups, in terms of shell participation in whole eggs structure. Haugh Index assessment indicated not significant improvement or decrease of freshness and commercial status of the eggs due to feed additives supplementation (88.33-90.38 H.U., commercial class AA). Usage of certain feed additives, such as prebiotics, probiotics, synbiotic and mycotoxins adsorbents significantly improved shell thickness, by 9.94 to 14.04% ($P < 0.05$), while ascorbic acid and sodium bicarbonate usage slightly but not significantly improved the same trait (3.80-4.39%). The most improved integrity trait was the shell breaking strength, subsequently to shell thickness increasing. Thus, it significantly differed versus control ($P<0.05$, 4.19-5.08% better resistance in ascorbic acid and sodium bicarbonate supplementation) and distinguished significantly ($P<0.01$, 10.3 to 14.2% improved). Overall, commercial loss due to shell quality issues decreased from 8.9% of whole yield to 5.0-6.8% in the hen groups whose diet was supplemented with studied experimental factors.

Key words*: table eggs, quality, shell integrity, feed additives.*

INTRODUCTION

Certain feed additives, such as prebiotics as oligosaccharides were suggested to play a certain role in improving eggs quality and shell quality in particular (Youssef et al., 2013; Świątkiewicz et al., 2015; Bucław, 2016; Li et al., 2017). Organic acids used as dietary supplements, either in drinking water of hens or in feed, were reported to improve overall table eggs quality and shell strength (Grashorn et al., 2013; Khan and Iqbal, 2016). Probiotics containing lactobacilli and enterococci strains (Zhang et al., 2012; Abdelqader et al., 2013; Chung et al., 2015; Fathi et al., 2018) were found to improve eggs yield and eggs quality, mostly through their indirect actions on the hens gut beneficial microflora reinforcement and improvement of essential nutrients used in eggs synthesis. Other authors (Özek et al., 2011) signaled eggs quality improvement effects when mixtures of organic acids and essential oils were used in hens diet. Also, usage of polyvalent synbiotic feed additives are known to improve eggs yields and commercial qualities (Awad et al., 2009; Tang et al., 2015). It is known that

most of the feedstuffs have a certain level of mycotoxin contamination, which could interfere with the proper functioning of intestinal nutrient absorptions (Murugesan et al., 2015), leading to defective uptake of some elements essential in egg formation or with the normal reproductive tract functioning in hens, therefore it could affect eggs yield and, subsequently, certain eggs quality traits, such as shell structure (Filazi et al., 2017). Despite the fact that ascorbic acid provided standalone in diet could induce shell traits improvements especially under thermal stress conditions and at inclusion rated up to 250 mg/kg (Khan et al., 2012; Abidin and Khatoon, 2013), in certain situations, such as dietary synergies with certain minerals, the effects are controversial: either improvements of thickness and breaking strength in combination with Zinc (Karami et al., 2018) or decrease of shell density, due to metabolic acidosis installation in hens blood and disruption of acid-base metabolism (Torki et al., 2014). Sodium bicarbonate was one of the nutritional approaches in improving eggshell quality. In certain stressful conditions for laying hens, that affects the electrolyte balance and calcium uptake and transfer into shell by the oviduct uterus glands, such as thermal stress (ambient temperature above 30°C), $NaHCO_3$ seemed to play a protective role on the shell by 1%-19%, especially through improvements on the shell structure, rather than to the amount of calcium deposited in the shell (Balnave and Muheereza, 1997). Also, feed supplementation with 1-1.5% sodium bicarbonate induced 3-6% increase of shell thickness (Gongruttananun and Chotesangasa, 2005). The rate of improvement was higher as the exposure of hens to longer light programme, therefore to feed access and bicarbonate intake was longer throughout the day. Dietary sodium bicarbonate (3 ‰) significantly affected shell thickness, especially through an increase absorption rate of calcium in the gut (Abbas et al., 2019) or re-absorption in nephrons, to become more available for uterus transfer (Jiang et al., 2015). Under these circumstances, we proposed an overview of some comparable original research results issued from the usage of commercially available additives such as prebiotics, probiotics, synbiotic, mycotoxin inhibitors, vitaminic or mineral supplementations in laying hens diets on table eggs quality.

MATERIALS AND METHODS

Seven groups comprising each 120 ISA Brown laying hens, aged 30 weeks, were formed and fed a conventional diet for peak of production at a daily intake of 120 g mashed feed (2750 Kcal/kg metabolizable energy, 17.5% crude protein, based on corn-wheat, soymeal) and a certain supplementation of additives supposed to affect eggs quality:

* CG = Control group, just plain diet;
* ASC group = + 0.03 % ascorbic acid;
* SOB group = +1% sodium bicarbonate;
* OAC group = +0.3% prebiotics as organic acids mixture;
* MOS group = +0.1% mannan oligosaccharide prebiotic;
* SYN group = +0.1% synbiotic mixture-probiotic, prebiotic and phytogenic compounds;
* MYC group = +0.2 % mycotoxins adsorbent.

No commercial brands of the used feed additives are specified because the purpose of the studies was not to differentiate among different suppliers of food additives, but among different types of additives, in terms of their components. In fact, not all additives categories were available from one single seller.

The reasoning criteria were represented by:

* eggs structure (% of edible and non-edible parts from whole weight);
* Haugh Index (HU) as freshness trait (U.H. = 100log (h-1,7 X $G^{0,37}$ + 7,57); Usturoi et al., 2014; measured via ORKA Food Egg tester);
* eggshell integrity (% of whole eggs yield);
* eggshell defects and commercial loss (% of whole eggs yield);
* shell thickness (mm, measured via micrometer; Igic et al., 2010);
* shell dynamic breaking strength (Newton; Hidalgo et al., 2008; measured via ORKA Egg Force reader instrument).

All analyses were run for one-week eggs yield, using 280 eggs randomly collected from each group (40 eggs per day).

Conventional statistical methods were applied to calculate the main descriptors (mean, standard deviation, coefficient of variation=, in accordance with the methodology described by Kaps and Lamberson, 2017. Then, percentage comparisons were run between groups, in order to run to which extent the experimental factors affected the analyzed traits and analysis of variance followed by post-hoc Tukey algorithm was conducted using the SingleFactorANOVA in MsExcel software.

RESULTS AND DISCUSSIONS

Data related to eggs weight compounds dynamics (g) and, subsequently, to eggs structure (%), are presented in table 1. Thus, whole eggs weight varied between 63.27 g (CG group) and 65.40 g (sodium bicarbonate +1% supplementation). Out of this weights, the shell represented 5.91 g in CG (9.34% in egg structure), while, in comparison it reached higher values in experimental treatments: 6.19 g in ASC (9.70%, P<0.05), 6.39 g in OAC (9.77%, P<0.05), 6.38 g in MYC (9.82% of egg, P<0.01), 6.41 g in SYN (9.83% of egg, P<0.01), 6.44 g in MOS (9.86% of egg, P<0.01) and 6.35 g in SOB (9.89% of egg, P<0.01).

Eggs issued from control group had the highest yolk proportion (25.73%), while the lowest one was found in SYN group (25.17%, -2.19% vs.control). Albumen proportion in eggs structure varied between 64.93% in CG and 65% in SYN group. It seemed the synbiotic feed additive, through its cumulative effect of probiotic, prebiotic and phytogenic induced proper functioning of albumen secretory glands in the magnum area of hens oviduct, while those mobilising minerals from bloodstream and building the shell on the organic matrix in the uterus seemed to have higher value than in control hens, however moderate, in comparison with the other effects induced on shell formation by the other experimental treatments.

Table 1 – Dynamics of eggs structure in accordance with the experimental treatment (diet supplementation)

Trait	Descriptor	Treatment						
		CG (regular diet)	ASC (+0,03% ascorbic acid)	SOB (+1% $NaHCO_3$)	OAC (+0.3% organic acids mixture)	MOS (+0.1% mannan oligosaccharides)	SYN (+0.1% synbiotic mixture: probiotic, prebiotic, phytogenic compounds)	MYC (+0.2 % mycotoxins adsorbent)
Whole egg weight	Mean (g)	63.27	63.84	64.22	65.40	65.31	65.24	64.98
	±StDev (g)	7.14	6.44	6.58	6.74	6.54	6.94	6.41
	CV %	11.28	10.09	10.24	10.31	10.01	10.63	9.87
Shell weight	Mean (g)	5.91	6.19	6.35	6.39	6.44	6.41	6.38
	±StDev (g)	0.71	0.70	0.68	0.69	0.68	0.67	0.61
	CV %	12.08	11.38	10.66	10.82	10.63	10.47	9.63
Yolk weight	Mean (g)	16.28	16.41	16.47	16.61	16.59	16.42	16.53
	±StDev (g)	1.83	1.75	1.72	1.73	1.76	1.67	1.66
	CV %	11.25	10.65	10.43	10.39	10.58	10.16	10.02
Albumen weight	Mean (g)	41.08	41.24	41.40	42.40	42.28	42.41	42.07
	±StDev (g)	5.06	4.64	4.72	4.59	4.39	4.45	4.14
	CV %	12.32	11.26	11.39	10.82	10.39	10.49	9.84
Eggs structure and percent differences between groups	Shell %	9.34[a]	9.70[b] *(P=0.036)*	9.89[c] *(P=0.003)*	9.77[b] *(P=0.021)*	9.86[c] *(P=0.005)*	9.83[c] *(P=0.006)*	9.82[c] *(P=0.008)*
	±% vs. CG	-	+3.80	+5.86	+4.60	+5.56	+5.19	+5.11
	Yolk %	25.73	25.70	25.65	25.40	25.40	25.17	25.44
	±% vs. CG	-	-0.10	-0.33	-1.30	-1.28	-2.19	-1.14
	Albumen %	64.93	64.60	64.47	64.83	64.74	65.00	64.74
	±% vs. CG	-	-0.51	-0.71	-0.15	-0.29	+0.12	-0.29

[ab] within the same row, significant differences, $0.01<P<0.05$
[ac] within the same row, distinguished significant differences, $0.001<P<0.01$

Related to eggs inner commercial quality and freshness status, data acquired in our research and presented in table 2 reveal that the usage of feed additives in all experimental treatments positively influence egg weight and mostly thick albumen height, because these two factors influence the Haugh index. However, regardless the feeding of hens, all eggs falled within the

highest AA commercial quality class, with Haugh Unit scores above 88 (Bhale et al., 2003). The differences between treatment groups and control group eggs varied within + 0.33% H.U. in MYC and +2.32% HU in SYN group, suggesting as well, that the magnum glands functioned better in hens group fed +0.1% synbiotic mixture).

In terms of shell development traits, it was found that the thicker gauge occurred as well in the SYN group, where the shell reached 0.390 mm (+14.04% vs. control, P<0.05), while he supplementation with ascorbic acid +0.03% and with sodium bicarbonate with 1% induced +3.8…+4.39% thickening of the shell. In the other groups, 0.2% dietary supplementation with mycotoxins adsorbent induced +9.94% shell thickening, +10.23% improvement in organic acids mixture 0.3% supplementation, respectively +12.87% improvement in MOS group. All these were significantly different from control and ASC, SOB groups.

Table 2 – Eggs freshness trait and shell quality parameters dynamics in accordance with the experimental treatment (diet supplementation)

Trait	Descriptor	Treatment						
		CG (regular diet)	ASC (+0,03% ascorbic acid)	SOB (+1% $NaHCO_3$)	OAC (+0.3% organic acids mixture)	MOS (+0.1% mannan oligosaccharides)	SYN (+0.1% synbiotic mixture: probiotic, prebiotic, phytogenic compounds)	MYC (+0.2 % mycotoxins adsorbent)
Haugh Index	Mean (HU)	88.33	89.72	88.68	89.18	89.21	90.38	88.62
	±StDev (HU)	7.38	8.18	8.19	7.73	8.58	7.72	8.15
	CV %	8.36	9.12	9.24	8.67	9.62	8.54	9.20
	±% vs. CG	-	+1.57	+0.40	+0.96	+1.00	+2.32	+0.33
Shell Thickness	Mean (mm)	0.342[a]	0.355[a]	0.357[a]	0.377[b] *(P=0.038)*	0.386[b] *(P=0.025)*	0.390[b] *(P=0.019)*	0.376[b] *(P=0.047)*
	±StDev (mm)	0.02	0.02	0.02	0.02	0.03	0.02	0.02
	CV %	5.19	6.23	5.74	6.28	7.19	6.13	5.82
	±% vs. CG	-	+3.80	+4.39	+10.23	+12.87	+14.04	+9.94
Dynamic shell breaking strength	Mean (N)	40.12[a]	41.80[b] *(P=0.041)*	42.16[b] *(P=0.036)*	44.69[c] *(P=0.006)*	45.07[c] *(P=0.005)*	45.82 *(P=0.003)*[c]	44.25[c] *(P=0.008)*
	±StDev (N)	2.53	2.89	2.48	3.23	2.92	2.86	3.13
	CV %	6.31	6.92	5.89	7.23	6.48	6.24	7.08
	±% vs. CG	-	+4.19	+5.08	+11.39	+12.34	+14.21	+10.29

[ab] within the same row, significant differences, $0.01<P<0.05$

Shell thickening induced, as well, subsequently increasing effects of dynamic breaking strength, which varied from 40.12 N in control group to: 41.80 N in ASC group (+4.19%, P<0.05); 42.16 N in SOB group (+5.08%, P<0.05); 44.25 N in MYC group (+10.29%, P<0.01); 44.69 N in OAC group (+11.39%, P<0.01); 45.07 N in MOS group (+12.34%, P<0.01) and to 45.82 N in SYN group (+10.29%, P<0.01), eventually. Within these circumstances, it is interesting to follow the dynamics of eggshell faults occurrence due to the action of experimental factors (table 3).

In CG group (regular diet), there were found 25 eggs with unconformities out of the total 280 sampled eggs during the experimental weekly observation (8.93%). Out of these defects, 2.86% represented visible broken shells, 1.79% were found as micro breakages, 2.14% were eggs with rough shells, and 1.07 % each represented eggs with soft shells or with malformed shells. It is interesting to explore, as follow-up the real occurrence of microintegrity defects on the eggshell, knowing we used an ovoscopic method to identify micro breakages, while some other techniques, like ultrasound checking or resonance percution and analysis of acoustic response curve would be more accurate, as other studies reported (Hunton, 2005; Wang and Jiang, 2005).

Usage of different feed supplements induced modifications of shell thickness and breaking strength, which resulted in decreases by 24 – 44% of shell defects, compared to control group (table 3).

In the group receiving ascorbic acid +0.03%, the defects decreased to 6.79% from the total amount of analyzed eggs.

Table 3 – Proportion of shell defects in relation with the experimental treatment (diet supplementation)

Egg production / defects	Treatment						
	CG (regular diet)	ASC (+0,03% ascorbic acid)	SOB (+1% $NaHCO_3$)	OAC (+0.3% organic acids mixture)	MOS (+0.1% mannan oligosaccharides)	SYN (+0.1% synbiotic mixture: probiotic, prebiotic, phytogenic compounds)	MYC (+0.2 % mycotoxins adsorbent)
Total yield/week (pcs.)	280	280	280	280	280	280	280
Eggs with defects (pcs.)	25	19	18	17	15	14	18
% out of total	8.93	6.79	6.43	6.07	5.36	5.00	6.43
±% vs. CG	100.00	-24.00	-28.00	-32.00	-40.00	-44.00	-28.00
• broken shells (pcs.)	8	6	5	5	5	4	5
% out of total	2.86	2.14	1.79	1.79	1.79	1.43	1.79
• micro breakages (pcs.)	5	3	4	3	3	3	4
% out of total	1.79	1.07	1.43	1.07	1.07	1.07	1.43
• rough shells (pcs.)	6	5	6	4	4	3	5
% out of total	2.14	1.79	2.14	1.43	1.43	1.07	1.79
• soft shell/no shell (pcs.)	3	2	2	2	2	3	2
% out of total	1.07	0.71	0.71	0.71	0.71	1.07	0.71
• malformed shell (pcs.)	3	3	1	3	1	1	2
% out of total	1.07	1.07	0.36	1.07	0.36	0.36	0.71

In organic acids supplemented eggs, the decrease of shell defects was of 32%, compared to control group (only 6.07% eggs with shell issues), while in both groups receiving sodium bicarbonate and mycotoxins adsorbent supplementations, the occurrence of shell defects decreased to 6.43%. Better protective effects related to shell integrity were observed in groups MOS and SYN, where the decrease reached 40-44 % compared to control group (5.0-5.4% eggs with shell unconformities, vs. 8.93% defects incidence). Despite the results sounds promising, it would be interesting to run in depth analysis of proteomics and electrolytes of the uterine fluid in each group in order to better understand and correlate the facts, because, apparently, the most resistant shells and the lowest defect rate appeared due to the synergistic effect of prebiotics, probiotics and phytogenics in SYN group and, meantime, the highest proportion of eggs with soft shells appeared in the same group, suggesting that the uterine environment could be in fact disturbed and forced to work above regular rhythmicity in such case. A follow-up of the research should be considered, knowing that 15%-20% of commercial losses due to shell integrity faults (Roland, 1988; Qiu et al., 2020), which could be prevented by hens nutrition.

CONCLUSIONS

Dietary supplementation of regular laying hens feed with sodium bicarbonate, ascorbic acid, mixture of organic acids, prebiotics as oligosaccharides, synbiotics (mixture of probiotics-prebiotics-phytogenic compounds) or mycotoxin adsorbents induced significant effects on shell participation of whole egg structure, shell thickness and shell dynamic breaking strength.

Less eggs with shell unconformities were found in synbiotic +0.1% supplemented group, suggesting thus the positive synergistic effects of the three types of additives provided in the same product either on nutrients absorption in the intestine, re-absorption in kidneys tubular systems and blood to shell transfer in the uterus, the place where shell is synthetized.

However, studies should be followed up by an exhaustive approach on the uterine histological structure, fluid components and interactions in eggshell formations, because some aspects remain controversial (such as the occurrence of some soft-shelled eggs in the same group with the best dynamic breaking strength values).

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INFLUENCE OF HERBAL SUPPLEMENTS ON THE PRODUCTIVITY AND SLAUGHTER QUALITIES IN FATTENING PIGS

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Abstract

A scientific experiment with twenty fattening pigs from the Danube White breed was conducted in Agricultural Institute - Shumen. The animals were separated into two groups of ten, and were fed and reared in individual boxes. The experiment began with an average live weight of 55.300 kg for the control group and 55.800 kg for the trial group. Throughout the rearing period, the animals received compound feed containing 15.5 g Crude Protein (CP), 0.71 g Lysine, 0.67 g calcium and 0.60 g phosphorus. To the feed of the experimental group were added herbal supplements 20 g/per capita daily with the following composition: 50% rosehip flour, 30% nettle leaves, 10% weeds grass, 5% dandelion, 5% hawthorn. The aim of this experiment was to establish the influence of herbal supplements on the productivity, slaughter qualities, chemical composition and physical properties of m. Longissimus dorsi meat in fattening pigs. In conclusion, we established that the herbal supplement has influenced the fat thickness in the shoulder (30.00 mm and 19.40 mm, P=0.027) and back (17.40 mm and 32.00 mm, P=0.016).

Key words*: fattening pigs, hawthorn, herbs, nettle leaf, rosehip, slaughter qualities, weeds grass.*

INTRODUCTION

The use of herbs and their healing effect has been known since ancient times. For a long time, knowledge about them has not only been down to their macro and microscopic description, but to the discovery of the reasons for their medicinal properties, i.e. the study of biologically active substances in them (Boeva, 2011; Marin et al., 2016).

According to the World Health Organization, healing plants are considered those plants, including leaves, stems, rhizomes, peels, flowers, fruits, grains or seeds, which contain substances which can be used for therapeutic purposes (Bohlin and Bruhn, 1999).

Many of them have been used as feed supplements in pig breeding for stimulating animal growth (Huang et al., 2010; Yan et al., 2010; Ao et al., 2011; Yan et al., 2011; Yan et al., 2012).

They have a positive effect on the secretion of digestive enzymes (Wenk, 2003; Cho et al., 2006; Huang et al., 2010).

The interest of numerous scientists in herbs and plant extracts has been connected to their antimicrobial, antioxidant and stimulating effect on the digestive system (Singh et al., 2002).

Urtica incisa contains a significant amount of biologically active compounds. For example, nettle leaves are sources of terpenoids, carotenoids and fatty acids, as well as various essential amino acids, chlorophyll, vitamins, tannins, carbohydrates, sterols, polysaccharides, isolectins and minerals. Extracts from the above ground parts of the nettle are rich sources of polyphenols, while the roots contain oleanolic acid, sterols and sterile glycosides. Due to the variety of phytochemicals and their proportions, nettle shows noticeable activity against both Gram-positive and Gram-negative bacteria. These properties make nettles suitable for a number of possible applications, including functional foods, nutritional supplements and pharmacological preparations (Dorota et al., 2018).

The most important and comprehensively studied bioactive components of the *Taraxacum* are chlorogenic acid (CGA), cichoric acid (CRA), taraxasterol (TS) and sesquiterpene lactones (SEL). These components have great potential as anti-diabetic pharmaceuticals and nutritional products for the regulation of diabetes. They also have the potential to be used as functional food.

The dandelion contains the bitter substance taraxacin, Cetearyl Alcohol, lactocerol, caoutchouc, inositol, choline, saponins, tannins, organic acids, triterpenes, resinous and slimy substances (Fonyuy et al., 2016).

Hawthorn fruits (*Crataegus pinnatifida* Bge. Var. Major) are rich in pectic polysaccharides and are used from centuries in China as food and an herb (Guo et al., 2019). They contain nutrients and antioxidant compounds. The antioxidant activity of the hawthorn fruits is strongly connected to the total content of polyphenols, flavonoids and triterpenoid acids (Liu et al., 2019).

Polygonum aviculare has a wide range of effects. It can be applied in treatment of different types of cancer, vascular and liver diseases. In its fresh or dried state, the herb can be used in treating problems with the gastrointestinal tract.

It has hematopoietic and urinary effects, increases blood pressure and ventilatory capacity of the lungs. It is known for its beneficial effects on respiratory disorders and inflammation in the bladder area. (https://diagnozata.bg/билкова-енциклопедия).

Over the past few years, medical interest in the *Rosa canina* has increased as a result of recent research into the use and treatment of several diseases, including skin, hepatotoxicity, kidney disorders, diarrhea, inflammatory disorders, arthritis, diabetes, hyperlipidemia, obesity and cancer.

The plants' therapeutic potential is based on its antioxidant effects connected to its phytochemical content, which includes ascorbic acids, phenolic compounds, fatty acids, etc. (Ines et al., 2017).

The aim of the experiment was to establish the influence of the herbal supplement (50% rosehip flour, 30% nettle leaves, 10% weeds grass 5% dandelion, 5% hawthorn) on the productivity, health, slaughter qualities, chemical composition and physical properties of *m. longissimus dorsi* meat in fattening pigs.

MATERIALS AND METHODS

A scientific experiment with twenty fattening pigs from the Danube White breed was conducted in Agricultural Institute – Shumen. The animals were separated into two groups of ten (control and trial groups), and were fed and reared in individual boxes.

The experiment began with an average live weight of 55.300 kg for the control group and 55.800 kg for the trial group.

Throughout the rearing period, the animals received compound feed containing 15.5 g crude protein (CP), 0.71 g lysine, 0.67 g calcium and 0.60 g phosphorus. To the feed of the experimental group were added herbal supplements 20 g/per capita daily with the following composition: 50% rosehip flour, 30% nettle leaves, 10% weeds grass, 5% dandelion, 5% hawthorn.

Throughout the experiment (the following indicators were controlled: feed intake – daily; average daily gain – at the beginning and at the end of the experiment, individually; feed consumption per kg gain – for the entire trial period; health – daily.

After reaching the certain slaughter live weight, the animals were slaughtered. After a 24-hour cooling period a slaughter analysis was done and carcass measurements were established according to the Regulations on Breeding Value, Productivity and Ranking of Breeding Pigs (1996).

The meats' chemical composition was determined according to methods by Sandev (1979).

The physical properties of the meat were determined by the following methods: the pH in the meat was determined according to the method described by Pozharskaya et al. (1964), the color of the meat was determined at three places on both sides of the two-centimeter cut, done perpendicular on the muscle fibers (24 h post mortem), after which values averaged.

Color reading was performed with a Spectocolorimeter "Specol" at a wavelength of 525 nm - according to Pinkas (1981). Muscle fiber thickness and weight loss from meat roasting were determined by the methods described by Otto (1959, 1963, 1964). Water holding capacity (WHC) was estimated by the amount of free water by the method of Grau and Hamm (1952).

Results were calculated according to the methods of the variation statistics.

RESULTS AND DISCUSSIONS

Pigs from both groups were fed with compound feed, compliant and prepared according to their weight development (Table 1).

Table 1. Experiment scheme

Group I (Control – 10 animals)	Group II (Trial – 10 animals)
Compound feed	Compound feed
-	Herbal supplement 20 g per day per animal

The trial group was given herbal supplement with their feed (Table 2).

Table 2. Compound feed for pigs from 55 to 110 kg live weight

Components	%
Corn	25.00
Wheat	51.88
Wheat bran	8.00
Bioconcentrate-14	15.00
Synthetic lysine, 98%	0.12
Total	100.00
Content in 1 kg feed:	
OE, MJ	12.52
Crude protein, %	15.5
Lysine, %	0.71
Methionine + cystine, %	0.51
Threonine, %	0.47
Tryptophan, %	0.16
Crude fats, %	1.97
Crude fibers, %	4.11
Ca, %	0.67
P, %	0.60

Both groups practically had the same intake of compound feed, exchange energy, crude protein, lysine and other nutrients (Table 3).

The differences in average daily gain, feed consumption and nutrients per kg of gain between the control and trial groups were so minimal that they can be ignored. This indicated that the herbal combination used had no significant effect on these features. Our results were similar to those of Oh et al. (2007), who also found that when feeding broilers, there was no significant difference in final body weight, weight gain, feed intake and conversion of compound feed containing antibiotics, 1.0% dandelion supplement.

Table 3. Feed intake, average daily gain and feed consumption results for 1 kg of gain

Traits	Groups	
	1	2
Intake per day		
Feed, kg	3.545	3.535
OE, MJ	44.74	44.62
Crude protein, g	64.2	64.0
Lysine, g	3.4	3.4
Average daily gain, kg	1.084	1.050
Consumption for 1 kg of gain:		
Feed, kg	3.286	3.391
OE, MJ	41.47	42.80

Table 4 indicates the clinical analysis results. It shows that the results for the meat and fat indicators in the halves, as well as the yield are practically the same for both groups.

Statistically significant differences were found in the distribution of fat in the carcasses. The pigs in the trial group had less fat at the shoulders (19.40 mm) than those in the control group (30.00 mm, P = 0.027). At the same time, the back fat thickness (BFT) was thinner in control animals - 17.40 mm versus 32.00 mm, P = 0.016. Hanczakowska et al. (2007), in an experiment with 42 fattened pigs, also found that nettle extract in feed had an effect on slaughter indicators.

Correlation coefficients (Table 5) indicate that there is a connection (R=0.78 and R=0.80) between the independent trait – herbal supplement and the dependent traits – back fat thickness and fat at the shoulders.

From the regression analysis, it can be seen that the proportions of the variability of the features have been largely covered by the regression equation, which is evident from the coefficients of determination - R2 = 0.61 and R2 = 0.63; which is a good scope for explaining the differences between the groups. The regression coefficients were B =-10.60 and B =14.60, respectively, with a high degree of statistical significance (P = 0.007 and P = 0.006).

Table 4. Slaughter analysis results

Traits	Groups		P
	1	2	
Live weight, kg	110.00	109.00	0.071
Carcass weight, kg	74.175	72.940	0.171
Half, kg (left)	37.678	37.070	0.289
Half, kg (right)	36.497	35.870	0.350
Half - meat, kg	30.589	29.408	0.179
Half - fat, kg	5.912	6.462	0.157
Slaughter output, %	67.50	66.992	0.288
Shoulder fat thickness, mm	30.00	19.40	0.027
Back fat thickness, mm	17.40	32.00	0.016
Stook x1	24.2	25.6	0.318
Stook x2	23.6	25.6	0.324
Stook x3	18.2	19.0	0.406
Mean (three measurements) C, K, L	23.668	25.666	0.320
Area MLD, cm2	42.10	39.22	0.178
Head, kg	2.618	2.740	0.253
Neck shop, kg	5.213	4.833	0.278
- meat, kg	4.469	4.09	0.261
- fat, kg	0.744	0.743	0.498
Fore ham, kg	6.528	6.232	0.044
- Meat with bone, kg	5.907	5.492	0.040
- Fat, kg	0.621	0.740	0.146
Breast part, kg	4.999	4.924	0.439
- Meat with bone, kg	4.164	4.056	0.401
- Fat, kg	0.835	0.868	0.364
Belly, kg	7.623	7.700	0.420
- Meat with bone, kg	6.186	5.724	0.041
- Fat, kg	1.437	1.976	0.018
Real ham, kg	10.065	9.804	0.180
- Meat with bone, kg	8.312	8.432	0.276
- Fat, kg	1.753	1.372	0.060
Tallow	0.522	0.763	0.008
Loin	0.36	0.42	0.184
Shin, kg	1.187	1.194	0.463

Table 5. Correlation coefficients, determination and regression

Traits	R	R^2	Invariable	B	P
Live weight, kg	0.11	0.01	111.00	-1.00	0.759
Carcass weight, kg	0.31	0.09	75.410	-1.230	0.387
Half, kg (left)	0.22	0.05	38.290	-0.610	0.543
Half, kg (right)	0.17	0.03	37.120	-0.630	0.629
Half - meat, kg	0.39	0.15	31.770	-1.180	0.266
Half - fat, kg	0.43	0.18	5.360	0.550	0.220
Slaughter output, %	0.11	0.01	68.01	-0.51	0.755
Shoulder fat thickness, mm	0.78	0.61	40.60	-10.60	0.007
Back fat thickness, mm	0.80	0.63	2.80	14.60	0.006
Stook x1	0.17	0.03	22.80	1.40	0.638
Stook x2	0.19	0.04	21.60	2.00	0.596
Stook x3	0.10	0.01	17.40	0.80	0.782
Mean (three measurements) C, K, L	0.21	0.05	21.67	2.00	0.552
Area MLD, cm2	0.31	0.10	44.98	-2.88	0.376
Head, kg	0.28	0.08	2.500	0.120	0.426
Neck shop, kg	0.28	0.08	5.600	-0.380	0.442
- meat, kg	0.30	0.09	4.850	-0.380	0.407
- fat, kg	0.01	0.00	0.750	0.00	0.983
Fore ham, kg	0.47	0.23	6.830	-0.300	0.166
- Meat with bone, kg	0.61	0.37	6.320	-0.410	0.062
- Fat, kg	0.46	0.21	0.510	0.110	0.184
Breast part, kg	0.06	0.00	5.080	-0.070	0.868
- Meat with bone, kg	0.10	0.01	4.270	-0.100	0.784
- Fat, kg	0.11	0.01	0.810	0.030	0.756
Belly, kg	0.08	0.01	7.550	0.080	0.823
- Meat with bone, kg	0.64	0.41	6.640	-0.460	0.046
- Fat, kg	0.78	0.60	0.900	0.540	0.008
Real ham, kg	0.27	0.07	10.320	-0.260	0.444
- Meat with bone, kg	0.14	0.02	8.200	0.120	0.698
- Fat, kg	0.61	0.37	2.140	-0.380	0.064
Tallow	0.84	0.71	0.290	0.240	0.002
Loin	0.40	0.16	0.300	0.060	0.253
Shin, kg	0.03	0.00	1.180	0.010	0.925

Fore ham (6.528 kg versus 6.232 kg, P=0.044) and meat from it (5.907 kg versus 5.492 kg, P=0.040) had higher values in pigs from the trial group. The same statistical significance was also observed in the meat (6.186 kg versus 5.724 kg, P=0,041) and fat in the belly (1.437 kg versus 1.976 kg P=0.018). The ratio of meat to fat in the belly between the two groups was different. We observed a significant and strong connection, respectively, between the study factor and the dependent variables - R = 0.64 and R = 0.78. The model generally explains the variability of dependent traits with coefficients of determination - R2 = 0.41 and R2 = 0.60.

Real ham of the pigs in the first group was 10.065 kg, and for the pigs in the second, 9.804 kg. This difference was not statistically proven. The meat in the real ham was 8.312 and 8.432 kg, respectively. A statistically significant difference was found in the fat in the real ham – 1.753 versus 1.372 kg (P = 0.060), respectively, for the 1st and 2nd groups. The results indicated that the lower weight of real ham in the trial group was due to the lower quantities of fat.

Significant differences were not observed between the groups in relation to the physicochemical content of *m. Longissimus dorsi* (Table 6) and the results can be practically considered as identical.

Table 6. Physicochemical composition (% of *m. Longissimus dorsi*)

Traits	Groups			
	1	Sx	2	Sx
Moisture (total)	75.55	1.28	75.24	0.81
Dry matter	24.45	1.28	24.76	0.81
Protein	20.76	0.97	20.98	0.66
Min. substances	1.10	0.04	1.11	0.03
Fat	2.58	0.32	2.67	0.20
Water retention	33.13	0.31	33.11	0.92
Color	23.65	0.42	22.98	0.71

CONCLUSIONS

The herbal supplement has affected the thickness of fat in the shoulder (30.00 mm and 19.40 mm, P=0.027) and back (17.40 mm and 32.00 mm, P=0.016) in fattened pigs in the control and trial groups.

Statistical significance was also observed in the meat (6.186 kg versus 5.724 kg, P=0.041) and fat in the belly (1.437 kg versus 1.976 kg P=0.018).

The herbal supplement (30% nettle leaves, 5% dandelion, 5% hawthorn, 10% weeds grass, and 50% rosehip flour) in a 20 g/day dose had no significant influence on productivity traits, chemical composition and physical meat qualities of *m. Longissimus dorsi* of fattened pigs.

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PROTECTIVE ROLE OF EFFECTIVE MICROORGANISMS AGAINST PESTICIDES RESIDUES TOXICITY ON TOMATO HAULMS AND THEIR EFFECTS ON PERFORMANCE OF DAIRY GOATS

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Abstract

Fifteen lactating Zaraibi does were assigned to study the effect of EM ability to prevent the probably toxicology effect of the residues of pesticides remained in tomato haulms (TH). Animals were fed ad libitum fresh TH (T1), TH silage (T2) and TH silage treated with EM (T3) and concentrate feed mixture (CFM). Treatment with EM (T3) was resulted in less concentrations of pesticides residues compared to T1 and T2. Higher milk yield, milk composition and 4% FCM were followed EM treatment; T1 had less milk fat content than T2 and T3. All pesticides concentrations residues in milk were higher in T1, but it had less degree in T2 and not detected for T3 Treatment TH with EM (T3) had higher concentrations of TVFA's, acetate, gas production, total count of cellulosic bacteria and less protozoa. T1 had higher concentrations of cholesterol, triglyceride, urea, creatinine, AST, ALT and less glucose, albumin and globulin than those for T2 and T3. So, biological (EM) treatment could be advisable to overcome the harmful effect of feeding TH exposure to pesticides.

Key words: *effective microorganisms (EM), pesticides residues, rumen metabolites, nutrient utilization, goats*

INTRODUCTION

Animal production in Egypt is mainly based on smallholder farms. Several by-products have potential value, especially for ruminants, due to their ability to digest fiber. So, use of local resources and crop byproducts as livestock feeds is become a necessary for profitable production. Presently, indoor use of pesticides for pest control is widespread in Egypt. Meantime, as these agrochemicals are used intensively and excessively in the production system; major problems are caused from the contamination of food by pesticide residues, and pollution of environmental ecosystems. Not only that, but it created many problems on human and animal health. Consequently, there has been a growing interest in nature farming and organic agriculture by consumers and environmentalists. However, no accurate information of the types and amounts of Egyptian household pesticide use, or numbers of contamination incidents is available (Hassan et al., 2010). The misuse and excessive use of chemical fertilizers and pesticides had resulted that Feed and fodder offered to animals are often contaminated with pesticide residues (Raikwar and Nag, 2003). However, they have usually found that such problems cannot be solved without using microbial methods and technologies in coordination with agricultural production (Parr and Hornick, 1992). Ways of degrading pesticide residues became to be fundamentally worthy of attention. Studies on microbial degradation of pesticide residues originated in 1940s, and the researchers were paid more attention on the degradation process and degradation mechanism of organic (Akbar and Sultan, 2016). Bacteria in nature could degrade the pesticide residues, with low cost and environmentally friendly and it would not cause secondary pollution, it converts organic macromolecules into small non-toxic molecules, thus avoiding the secondary pollution. Studies have shown that mineralization and co-metabolism were the main mechanisms for the further degradation of pesticides and their intermediate products (Ye et al., 2018). A number of bacteria that could degrade and convert pesticides have been isolated (Ramya et al., 2016).

EM contains selected species of microorganisms including predominant populations of lactic acid bacteria and yeasts, and smaller numbers of photosynthetic bacteria, actinomycetes and other types of organisms. All of these are mutually compatible with one another and can coexist in liquid culture. There are three types of microorganisms which are categorized into decomposing or degenerative, opportunistic or neutral and constructive or regenerative. EM belongs to the regenerative category whereby they can prevent decomposition in any type of substances and thus maintain the health of both living organisms and the environment (PSDC, 2009). Therefore, the EM has great potential in creating an environment most suitable for the existence, propagation, and prosperity of life (Higa and Parr, 1994). The objective of this experiment was to determinate the poisonous effects of pesticides residues in tomato haulm and whether EM is able to prevent the probably toxicological effects of pesticides on rumen degradability parameters in the rumen environment or not.

MATERIALS AND METHODS

Three tomato haulms with capacity of 3 tons (1 ton each) were used to be fed as fresh, silage and silage treated with EM. The Tomato haulms were collected from Noburia area, Egypt; after harvesting, chopped (1 to 3 cm in length). The silage was prepared by filling successive layers of the chopped materials and heavily trod ten before adding the next layer; molasses was added at the rate of 3% at the ensiling time and EM was add (1 liter/ton silage).

Fifteen lactating Zaraibi does (post weaning) in the 2nd and 3rd season of lactation, aging 2.5-3.5 years with 38.50 ±1.37 Kg in average body weights were randomly divided into three equal groups, (5 does each) for an experimental period of 60 days in randomized complete block design . Animals were offered roughage *ad libitum* twice a day at 8.00 and 16.00 plus CFM was fed to supply the CP requirements according to NRC (2007), while, tomato haulm were allowed to be fed ad libitum in each group, the actual amount of tomato haulm fed was recorded. The CFM consisted of 36% yellow corn, 30% wheat bran, 12% soybean meal (44% CP), 13% cottonseeds meal, 5% molasses, 2% limestone, 1.5% common salt and 0.5% vitamins minerals premix (Table 1). Milk yield was individually recorded on two successive days, milk samples were collected twice daily for 4 times in the 60 days through the collection period from all goats according to Galyean (1989). Milk samples (about 0.5% of total milk produced) were taken biweekly from doses of all groups during lactation. Milk samples were chemically analyzed for total solid (TS), protein, fat and ash according to AOAC (2006), while lactose was calculated by difference.

Three experimental rations were composed of concentrate feed mixture (CFM) plus fresh tomato haulm (F) as control ration, CFM + tomato haulm silage (S) and CFM + tomato haulm silage treated with (EM).

Table 1. Chemical analysis and cell wall constituents of the concentrate feed mixture (CFM) and tomato haulm fed to dairy goats (on DM basis)

Item	CFM	Tomato haulm		
		Fresh	Silage	Silage treated with EM
DM	89.14	28.73	29.38	28.91
OM	94.26	92.15	92.03	91.98
CP	15.62	7.71	7.53	7.69
CF	6.31	37.88	33.14	29.87
EE	3.07	1.69	1.65	1.61
NFE	69.26	44.87	49.71	52.81
Ash	5.74	7.85	7.97	8.02
NDF	27.34	64.89	61.19	57.83
ADF	19.33	46.24	43.77	40.74
ADL	4.02	12.21	11.01	10.49
Hemicellulose	8.01	18.65	17.42	17.09
Cellulose	15.31	34.03	32.76	30.25

Three Zaraibi does were used for rumen fermentation and in situ trials. On the last day of the experiment, ruminal fluid samples (200 mL) were collected at 0 and 4 h post-feeding (morning feeding) from each goat. Ruminal fluid samples were collected via a stomach tube connected with a vacuum pump; pH and temperature were recorded using a portable pH and temperature meter (HANNA instrument HI 8424 microcomputer, Singapore). Ruminal fluid samples were then strained through three layers of cheesecloth. The strained fluid samples (10 mL) were collected for measurement of the number of total viable bacteria as well as cellulolytic, amylolytic and proteolytic bacteria by the roll-tube technique (Hungate, 1969). The remaining strained fluid samples were then immediately mixed with 5 mL of 2 M H_2SO_4 to stop microbial activity. Ruminal fluid samples were then centrifuged at 3000 cycle for 10 min

and the supernatant (100 mL) was taken and divided into two portions. The first portion was kept in a plastic bottle where 5 mL of 1 M H_2SO_4 was added and frozen (20ºC) for later NH3-N and VFA analyses. The second portion was kept in a plastic bottle, immediately fixed with 10% formalin solution (1: 9 v/v, ruminal fluid: 10% formalin) (Galyean, 1989) and stored at 4ºC for later measurement of the ruminal microbial populations. Ruminal fluid was analyzed for NH_3-N by using the hypochlorite–phenol procedure (Beecher and Whitten 1970) and VFA by using HPLC, according to the method of Samuel et al. (1997). The total direct counts of bacteria, protozoa (holotrichs and entodiniomorphs) and fungal zoospores were measured using the methods of Galyean (1989) based on the use of a haemocyto meter (Boeco, Hamburg, Germany).

Blood samples were collected twice (the first one was taken before the beginning of the experiment and the other one at the end of the experimental period), from all goats. Blood samples were obtained from the jugular vein of the goats in the morning before access to feed and water. Serum was obtained by centrifugation of blood and was stored at –20ºC until used for analysis. Glucose concentration was determined by the method of Trinder (1969). Serum cholesterol was determined using the colorimetric method of Stein (1986). Serum total protein (TP) was measured as described by the Biuret method according to Henry et al. (1974). Albumin (A) concentration was determined according to Doumas et al. (1977). Kidney function was evaluated by measuring blood urea using the colorimetric methods of Henry and Todd (1974) using commercial kits. Creatinine was measured using the colorimetric method according to Faulkner and King (1976). Liver function was assessed by measuring the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) by the method of Reitman and Frankel (1957).

Pesticides in feed and milk samples

Solvents and other reagents used (acetone, benzene, ethyl acetate, methylene chloride, n-hexane, florisil 60-100 mesh (pre-treated as in the method of Kadenczki et al. (1992); sodium hydroxide, stannous chloride, carbon disulfide, cupric acetate monohydrate, hydrochloric acid, ethanol, diethanol amine were analytical reagent grade. The analytical standards of the tested pesticides were kindly provided by Department of Environmental Studies, Institute of Graduate Studies and Research, University of Alexandria, Egypt. The selected analytical standards are: (a) - Chlorinated hydrocarbon insecticides: HCB, lindane, p,p'-DDD, p-p' DDE and p-p' DDT. (b)- Halogenated pyrothroids: Cypermethrin, lambda-cyhalothrin. (c)- Organophosphorus insecticides: Dimethoate, malathion.

A simple multi-residue method according to Kadenczki et al. (1992) was applied to extract several pesticides (chlorinated hydrocarbon, halogenated pyrothriod insecticides and organophosphate) from tomato haulms and milk. The principle of this method is based on having a homogeneous sample pulp adsorbed on the surface of activated florisil to obtain a free-flowing powder, which is extracted in a glass column with methylene chloride-acetone (9:1, v/v). The gas chromatograph (GC) used was HP-5890 Series II. Polychlorinated biphenyls (PCBs) was determined by gas chromatograph according to Willett and Hess (1975).

Statistical Analysis

Means were calculated for all variables by goats within period. Data were analyzed using the MIXED procedure of SAS (SAS, 2000). Differences were tested using the PDIFF option in SAS (SAS, 2000) using protected ($P<0.10$) LSD test. Differences were declared significant at a $P<0.05$; and trends were discussed at a $P<0.15$, unless stated otherwise.

RESULTS AND DISCUSSIONS

The concentration of pesticides residues of the tomato haulms are presented in Table 2.

Table 2. Concentration (mg/kg) of pesticides residues of tomato haulm

Items	Tomato haulm		
	Fresh	Silage	Silage treated with EM
Deltamathrin	0.92	0.58	0.21
Aldrin, Dieldrin	1.11	0.69	0.25
Malathion	0.78	0.35	0.11
Cypermethrin	0.96	0.44	0.07
Permethin	0.83	0.40	0.16
HCB	0.33	0.17	0.04
Lindine	0.28	0.11	0.02
PP DDE	0.17	0.07	0.01

The tomato haulms silage treated with EM showed lower values of pesticides residue compared with the fresh and silage form.

Brajesh and Allan (2006) reported that the biochemistry of organophosphorus compound degradation by most of the bacteria seems to be identical, in which a structurally similar enzyme (organophosphate hydrolase or phospho-triesterase) catalyzes the first step of the degradation.

Catherine et al. (2002) found that organo-phosphorus hydrolase is a bacterial enzyme that has been shown to degrade a wide range of neurotoxic organophosphate nerve agents. However, the effectiveness of degradation varies dramatically, ranging from highly efficient with paraoxon to relatively slow with methyl parathion. Plants have evolved interactions and association with micoorganisms that can accelerate breakdown or transformation of certain pollutants in the plant root zones to products that no longer pose environmental hazards (Brimecombe et al., 2001). Sharaf et al. (2006) reported that understanding pesticide metabolism in plants and microorganisms is necessary for pesticide development, for safe and efficient use, as well as for developing pesticide bioremediation strategies for contaminated soil and water.

Data concerning milk yield and its composition of lactating goats fed the experimental rations are presented in Table 3.

Table 3. Milk yields and milk composition for lactating goats fed the experimental rations

Items	Tomato haulm				
	Fresh	Silage	Silage treated with EM	SEM	*P Value*
Milk yields (g/d)	563.25[c]	899.33[b]	955.18[a]	0.31	0.042
4% FCM (g)	479.55[c]	817.08[b]	880.67[a]	0.47	0.019
Fat, (g/d)	16.95[c]	30.49[b]	33.24[a]	0.39	0.001
Protein, (g/d)	15.49[c]	28.42[b]	31.43[a]	0.51	0.004
Milk composition (%):					
Total solids	13.07[b]	13.81[a]	13.85[a]	0.15	0.032
Solids not fat	10.06[b]	10.65[a]	10.56[a]	0.14	0.027
Fat	3.01[b]	3.39[a]	3.48[a]	0.11	0.005
Protein	2.75[c]	3.16[b]	3.29[a]	0.07	0.016
Lactose	6.35	6.68	6.43	0.31	0.744
Ash	0.96[a]	0.81[b]	0.84[b]	0.05	0.004

[a,,b, and c] *Means within rows with different superscripts are significantly different (P<0.05).*

The milk yield and fat corrected milk (FCM) were significantly increased (P<0.05) for goats fed tomato haulm silage treated with EM compared with the other experimental groups (tomato haulm silage and tomato haulm fresh). Milk fat and protein yield were also significantly increased (P<0.05).

Concerning milk composition and milk produced from animals fed tomato haulm silage and tomato haulm silage treated with EM had significantly (P<0.05) higher contents of fat, protein, total solids (TS) and solids not fat (SNF) compared with the tomato haulm fresh group. While milk lactose was not significantly differed (P>0.05) among experimental groups.

The concentration of pesticides residues (µg/kg on fat basis) in goat's milk are presented in (Table 4). The pesticides residue in the milk of goats fed tomato haulm silage and tomato haulm silage treated with EM showed low values of pesticides residues compared with tomato haulm fresh.

Table 4. Concentrations of pesticides residues (µg/kg on fat basis) of the milk goat's samples

Items	Tomato haulm		
	Fresh	Silage	Silage treated with EM
Deltamathrin	0.18	0.04	ND
Aldrin, Dieldrin	0.21	0.09	0.02
Malathion	0.11	0.01	ND
Cypermethrin	0.20	0.05	ND
Permethin	0.13	0.01	ND
HCB	0.09	ND	ND
Lindine	0.09	ND	ND
PP DDE	0.05	ND	ND

ND, not detected.

DebMandal et al. (2008) reported that microbes (fungi, bacteria, and other microorganisms) could degrade or breakdown the pesticides whereas they used them as food source. Quintero et al. (2008) reported that white-rot fungi species have demonstrated a high capacity to degrade organic pollutants such as the insecticide lindane (y-HCH). Also, pesticides (malathion, delatmethrin, cypermethrin, and permethrin, etc.) were used in some dairy farms to protect the animals from house flies and ticks. Some farmers were facing pesticide resistance problem in vector control which is also reflected by Singh et al. (2014) in the region of the present study. Additionally, in some farms those pesticide containers were kept in animal feed storage sites which may result into accidental spillage on feed. Moreover, while spraying pesticides on crops adjacent to dairy farms, the drift and volatilization of these pesticides may result in their deposition on the non-target sites which may include feed, fodder and water.

Data of ruminal pH, NH_3-N and VFA's concentrations are shown in Table 5. The ruminal pH was significantly (P<0.05) higher in both silage groups and increased linearly with silage treated with EM than in fresh tomato haulm group, but there were no significant differences among the both silage groups. Ruminal NH_3-N concentration was significantly (P<0.05) higher in both silage groups and increased linearly with silage treated with EM than in fresh tomato haulm group, but there were no significant differences among both silage groups. Total ruminal VFA concentrations and molar proportions of ruminal acetate were significantly (P<0.05) higher in silage treated with EM group than in fresh tomato haulm group. There was no difference among treatments for molar proportions of propionate and butyrate.

Table 5. Rumen liquor parameters of lactating goats fed the experimental diets

Items	Tomato haulm				
	Fresh	Silage	Silage treated with EM	SEM	*P Value*
pH	6.14[b]	6.53[a]	6.58[a]	0.06	0.038
NH_3. N(mg/$dL_)$	11.83[b]	13.66[a]	13.14[a]	0.52	0.021
TVFA's ((mM)	9.47[c]	11.38[b]	12.42[a]	0.19	0.029
Acetate (mol/100 mol)	61.75[c]	66.48[b]	68.77[a]	0.89	0.011
Propionate (mol/100 mol)	21.97	22.37	22.41	0.53	0.684
Butyrate (mol/100 mol)	8.52	8.66	8.68	0.27	0.773
Acetate: propionate ratio	2.81	2.97	3.07	0.23	0.693
Gas production volume at 24h, ml	28.8[c]	35.2[b]	37.1[a]	0.41	0.001
CO_2, %	46.82[a]	44.37[b]	43.29[c]	0.25	0.029
CH_4, %	28.79[a]	24.33[b]	22.89[c]	0.16	0.001

[a,,b, and c] *Means within rows with different superscripts are significantly different (P<0.05).*

It is interesting to note that the ruminal pH of fresh tomato haulm group was below 6.2, whereas both silage groups were in the normal range (6.53–6.58) and stable. Firkins (1996) stated that the pH range for optimal ruminal microbial digestion is 6.5–7.0. The low ruminal pH of the fresh tomato haulm group may have led to lower NDF digestibility and reduced bacterial populations compared with both silage groups (Table 5).

The higher NH_3-N concentrations of both silage groups may be reflected in greater numbers of rumen microbes. The greater number of rumen microbes in the both silage groups may be explained by the fact that pesticides in fresh tomato haulm group may be detoxified by the EM and pesticides may be adsorbed by the inorganic components, resulting in the removal of the suppression on microbial growth and activity. These microbes in turn ferment more feed, and generate more VFA's and a greater supply of microbial N for both silage groups. This also allows both silage groups to consume more feed as it is disappearing from the rumen faster. The experimental diet contained urea which is used as N source for microbial protein synthesis in the rumen, and as more feed was consumed by both silage groups, this lead to a higher ruminal NH_3-N concentration. The present results show that the EM supplementation increased the NH_3-N concentration, with NH_3 being the main N source for growth and protein synthesis by ruminal bacteria to achieve maximum fermentation. These results are in agreement with those of Dänicke et al. (2005) who reported an elevation in rumen NH_3 concentration and a reduction in duodenal flow of microbial protein in cows fed deoxynivalenol.

Table 6. Effect of the EM supplementation on rumen microorganism population

Items	Tomato haulm				
	Fresh	Silage	Silage treated with EM	SEM	*P Value*
		Rumen microbes			
Bacteria ($\times 10^{12}$ cells/mL)	6.7[c]	8.9[b]	11.4[a]	0.69	0.001
Protozoa ($\times 10^{5}$ cells/mL)	4.3[a]	3.1[b]	2.6[c]	0.25	0.015
Fungal zoospore ($\times 10^{7}$ cells/mL)	3.2[c]	4.8[b]	5.9[a]	0.16	0.004
		Viable bacteria (CFU/mL)			
Total ($\times 10^{9}$)	3.6[c]	7.7[b]	9.5[a]	0.47	0.027
Cellulolytic ($\times 10^{9}$)	2.1[c]	5.9[b]	7.2[a]	0.38	0.013

[a,,b, and c] *Means within rows with different superscripts are significantly different (P<0.05).*

Ruminal bacterial counts and fungal-zoospore counts were significantly higher with both silage groups and increased linearly with silage treated with EM, whereas protozoa counts were lower,

and decreased linearly with the silage treated with EM than in fresh tomato haulm group. Total viable-bacterial and counts cellulolytic bacterial counts were significantly higher in both silage groups and increased linearly with silage treated with EM than in fresh tomato haulm group. It is interesting to note that the populations of total bacteria, total fungal zoospores, total viable bacteria and cellulolytic bacteria increased, while protozoa population decreased with both silage groups and increased linearly with silage treated with EM. These results show that the EM played an important role in changing ruminal microbial populations. A possible explanation for the greater numbers of rumen microbes in both silage groups and their subsequent effects have already been discussed previously. Lower populations of ruminal bacteria in fresh tomato haulm groups in the current study were possibly associated with a fall in ruminal pH below 6.2 (Rode 2008).

Table 7. Blood serum parameters for lactating goats fed the experimental rations

Items	Tomato haulm				
	Fresh	Silage	Silage treated with EM	SEM	*P Value*
Glucose (mg/dl)	65.95^{b}	68.55^{a}	68.95^{a}	0.63	0.036
Cholesterol (mg/dl)	78.88^{a}	74.66^{b}	73.64^{b}	0.93	0.021
Triglyceride(mg /dl)	64.47^{a}	60.27^{b}	59.72^{b}	0.88	0.016
Total Protein (g /dl)	5.92	6.89^{a}	6.97^{a}	1.46	0.006
Albumin (g /dl)	3.06^{b}	3.47^{a}	3.44^{a}	0.07	0.001
Globulin (g/dl)	2.86^{b}	3.42^{a}	3.53^{a}	0.11	0.001
Urea (mg/dl)	43.85^{a}	38.66^{b}	38.83^{b}	0.32	0.004
Creatinine (mg/dl)	1.42^{a}	0.89^{b}	0.91^{b}	0.04	0.001
AST (U/L)	33.64^{a}	29.58^{b}	29.48^{b}	0.15	0.006
ALT (U/L)	22.84^{a}	19.46^{b}	18.66^{b}	0.89	0.09

$^{a, and\ b}$ Means within rows with different superscripts are significantly different (P<0.05).

Data of blood serum parameters for lactating goats are presented in Table 7. Data showed that silage and silage treated with EM rations caused a significant (P<0.05) increase in glucose, total protein, albumin and globulin levels than ration containing fresh silage. While fresh ration had significant (P<0.05) increase in cholesterol, triglyceride, urea, creatinine, AST and ALT than silage and silage treated with EM rations. The changes in carbohydrate metabolism induced by pesticides can be correlated with the effects of these chemicals on the activities of hepatic enzyme system which are intimately involved in glucose production, storage and metabolism and/or correlated with the endocrine activity of the pancreas (insulin activity). Pesticides exposure could cause hyperglycemia which could be a result of glycogenolysis in muscle and liver causing a significant increase in blood glucose level. This disturbance in carbohydrate metabolism may be responsible for the toxic action of pesticides (Ferrando and Andreu–Moliner, 1991).

The reduction of serum proteins, particularly albumin, in animals fed fresh TH contaminated with pesticides could be attributed to changes in protein and free amino acid metabolism and their synthesis in the liver (Rivarola and Blegno, 1991).The increased in blood urea and creatinine concentrations revealed in the present study should be due to pesticides effect. Elevated blood urea is known to be correlated with an increase in protein catabolism in mammalian body or it could be resulted from more efficient conversion of ammonia to urea as a result of increased synthesis of enzyme involved in urea production (Rodwell, 1979).The increased of serum AST and ALT activity indicated liver damage and disruption of normal liver function (Shakoori et al., 1994). The increment of the activities of AST and ALT in plasma are mainly due to the leakage of these enzymes from the hepatic cytosol into the blood stream (Navarro et al., 1993), which gives an indication on the hepatotoxic effect of lindane which leads to the liver damage.

CONCLUSIONS

It could be concluded that biological treatment with fungi or bacteria (silage) could be advisable in order to overcome the harmful effect of TH exposure to pesticides. However, more studies are needed in this respect.

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APPLICATION OF DRY-MIX-MANURE LAYER ON PRODUCTION OF *BRACHIARIA MUTICA* CULTIVATED IN UNRESTRICTED SUNLIGHT AREA

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Abstract

A development of ruminant farms needs to be accompanied by various efforts including the development of livestock feed forage production that adapts well around the farm. One of such effort is to develop B. mutica forage in open land by utilizing livestock waste. Ruminant animals such as cows and goats have a good palatability in consuming this forage. This preliminary experiment is a part of our research that aimed to examine the effect of an application of Dry-Mix-Manure (DMM) as planting media layer on production of B. mutica which is cultivated in an unrestricted of sunlight area. In this study B. mutica cultivation was carried out on a planting bed coated with a DMM 500 dose applied to m^2 surface as planting layer with 10 cm thick and the distance one hole to another was 50 cm along 10 meters. Four rows as a replication of planting. The parameters were: fresh production and dry weight of the forage. Harvesting was done at the age of 8 weeks after planting. A descriptive analysis was realized to the data obtained. The results showed that the mean of fresh production in this unrestricted sunlight areas was 12,527 kg / ha. The conclusion was that fertilize of Dry-Mix-Manure with a dosage 500 in the andosol soils was adaptable to get a good production of B. mutica cultivation under a full day sunlight during 12 hours about 6AM to 6PM.

Key words*: forage, cultivation, Brachiaria mutica, organic fertilizer, bio-waste*

INTRODUCTION

The development of ruminant farms is determined by various factors, one of which is related to the forage of animal feed.

The wet tropical climate is a characteristic of Indonesia climate along the year. The diverse vegetation included the forages consumed by the cattle. Normally the intensity of solar radiation in the mid-year period between July and September in this area is higher than in the period of October to May.

B. mutica is one of the forages suitable to be developed in these climatic conditions because this grass species can adapt to various climatic and soil conditions (Rumokoy and Toar, 2014). This condition is favourable for forage plants in carrying out photosynthesis and the process of forming roots, stems and leaves. In this condition, nearly 50% of the land has not been utilized properly. In reality in various locations have relatively a lot of potential agricultural lands but not yet utilized optimally, or some are even left unused.

Naturally humid climate conditions are an important factor in cultivating and producing forage production in order to maintain or increase ruminant livestock production.

As an alternative solution is to revitalize the land which is based on the activities of researching and developing the use of dry organic mix-manures as a top layer of raised planting media on open land, by cultivating the *B. mutica* forage.

For this reason, we have conducted a research aimed at answering the challenges and opportunities mentioned above by conducting a study of *B. mutica* forage production using DMM 500 fertilizer.

MATERIALS AND METHODS

This research was conducted at the Lotta Agricultural Center (SAL), in the agricultural

area at coordinates 1°25'05.8 "N 124°50'33.2" E, altitude around 150 meters.

Figure 1. Image of Research Location map (Google Map, accessed: 10 November 2019)

B. mutica forage was obtained and selected from SAL coconut plantations. This selection aims to obtain uniformity from *B. mutica* which will be planted in this study. Furthermore, *B. mutica* will grow optimally on various influences such as light intensity, and environmental conditions that related to a reported of Abraham et al (2014)

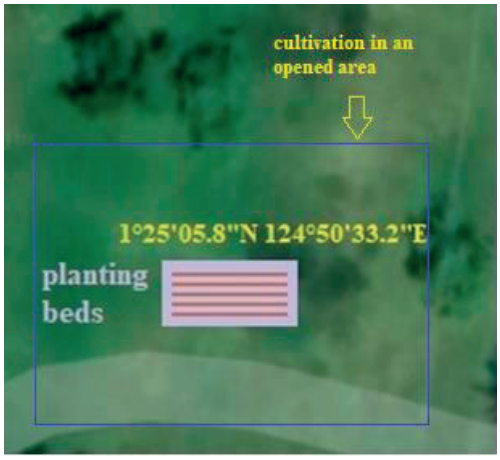

Figure 2. Location of open research land for *Brachiaria mutica* experiment cultivation Figure adapted to the real map (Google Map, accessed: 10 November 2019)

Before planting, the land used is cleaned and then loosened with a tractor, and then cleaned from unused materials such as rocks, gravel and so on, after that bed was made for a planting preparation.

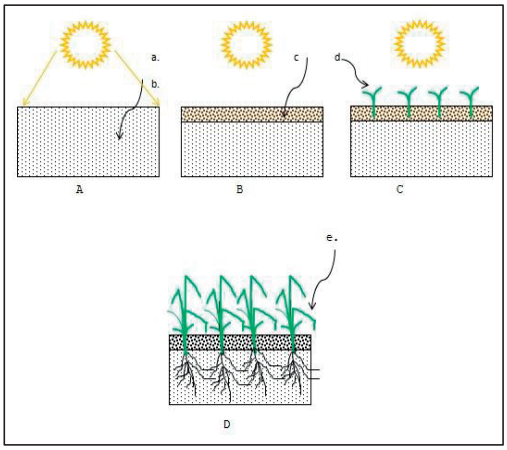

Figure 3. Scheme of a planting system in opened area by using fertilizer of DMM without mechanic restriction of sunlight transmission along the experiment

Notes: A. Field with full day sunlight transmission (a. Sunlight, b. plantation bed); B. DMM layer (c) on the top of the plantation bed of *B. mutica*; C. Plantation of *B. mutica* selected; D. Growth of the experiment forage on the soil fertilized with DMM.

Dry-Mix-Manure layer material was composed of a combination of dry manure material of pigs and cattle farms which was used as a top layer of beds with a dose of DMM 500 grams for dimensions of length and width of 100 cm2 with a thickness of 10 cm.

The planting media beds were incubated for 1 month, after that *B. mutica* was planted with a distance of 50 cm from one hole to the next 10 meters along with 4 rows of planting replications.

To get optimal plant growth, weeding is done to eradicate weeds. Manual weeding is done every 5 days during the planting period. Every day the plants were watering in the morning around 8 and the afternoon at 3. After the plants are 8 weeks old harvesting is done. Samples were collected from each subplot by cutting the plant 10 cm from the soil surface.

Figure 4. A manual sampling of *B. mutica* through a defoliation by a collector

The fresh plant samples were weighed and then put into plastic bags and then dried under the sunlight without shade.

RESULTS AND DISCUSSIONS

Climatic conditions that occur during the observation were sunny without rainfall. The sun's rays started to appear from 6 am to 6 pm, without any cloud obstructions. The type of soil at the site of experiment was andosol (Dien et al, 2018), and could be related to the influence of volcanic dust from Mount Lokon which is about 7 km away. Lokon eruption recorded in the last 20 years happened many times. The dust had affected the agricultural land around Minahasa including in Lotta Pineleng (Kurniawan, 2014). The fresh production results presented in the table 1 could be influenced by the environmental conditions which were favourable for growth of the experiment grass. Environmental factors that influence a growth of *Brachiaria* include: climate (Brouder and Volenec, 2008) water condition (Cameron and Lemcke, 2008), altitude (Asmare et al., 2017), type and fertility of the soil (Luce et al., 2016) as a growth medium, the intensity of sunlight (Lopes et al., 2017) which is very important for photosynthesis in plant's chlorophyll (Young and Smith, 1980; Gomez et al.,), as well as the temperature, humidity of the environment. A good maintenance could be an important factor of grass quality production improvement (Lima et al., 2018), included the factor of genetic (Volenec et al., 1996) and the manner of harvesting (Adnew et al., 2019; Tamele et al., 2017).

Table 1 shows a fresh production of *B. mutica* planted using DMM layer fertilizer was 12.5t / Ha, the minimum yield reached 9,880 kg / Ha while the maximum fresh production was 15,002 kg / Ha. This production rate could be linked to the availability of nutrient elements of the growing media enriched with DMM organic fertilizer. The utilization of manure connected to the quality of soils has been reported by Soelaeman and Haryati (2011).

Table 1. Fresh Production (kg) per ha in opened area

Replication	T01	T02	T03	T04
1	13,600	8,720	13,880	9,880
2	13,080	10,560	14,800	15,000
3	10,360	13,680	12,840	11,640
4	12,480	12,880	11,480	13,560
$\sum$	49,520	45,840	53,000	50,080
$\bar{X}$	12,380	11,460	13,250	12,520

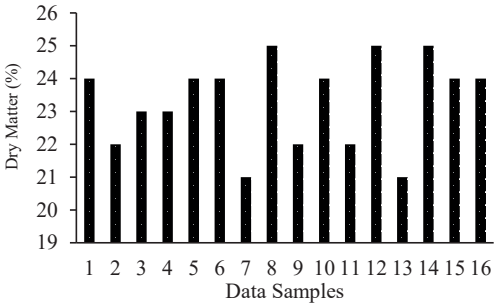

Figure 5. Production of Dry Material of *B. mutica*

Figure 6. Weighing of samples of fresh production of *B. mutica* harvest

The use of the DMM dose was able to reach the average production value which was still higher than the forage production as the previous observations (Rumokoy and Toar, 2014). This showed that a role of DMM fertilizer provided a positive response in producing fresh forages from the above grass species, especially for those grown on andosol soils with full light transmission conditions without obstructions.

Figure 4 showed an average dry matter production of *B. mutica* in the test field (around 21% to 25%).

The role of nutrient release from manure will help produce high quality forage (Suarna and Budiasa, 2016). The utilization of surrounding resources could be applied to the development of forage production and also toward the development of animal husbandry with an integrated system pattern as described by Carvalho et al, (2019) and Rumokoy et al, (2018).

The sunlight radiation has an important role to the process of biomass synthesis in forages planted in opened sunlight areas (Lopes et al., 2017) compared to the location with shade.

Experiment realized by Rumokoy et al, (2014), in a different place and type of soils, showed a variation of fresh production of this grass according to the level of sunlight restriction areas under several ages of coconut as presented in Figure 7. Similarity, Anis et al., 2015 confirmed that sunlight restriction influenced the production of grass production. This situation affected also the nutrition value of the grass plants (Abraham et al., 2014).

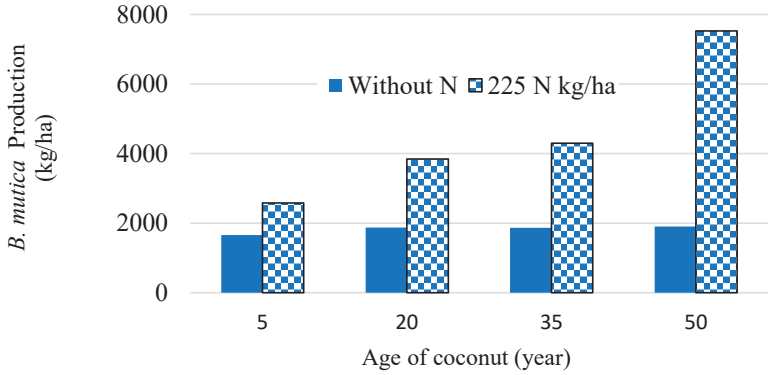

Figure 7. Production of *B. mutica* grass under canopy of coconut trees
Sources*: Rumokoy et al, 2014 (data were processed)*

CONCLUSIONS

B. mutica grass adapts to locations that receive continuous sunlight from 6 am until 6 pm at noon. These results of grass production signals that wetlands in the tropics that have not been utilized optimally can be revitalized by developing *B. mutica* crop production.

The conclusion of this research is that the DMM layer as an organic fertilizer is suitable for application in the production of forage B. mutica in andosol fields without sunlight restrictions. Thus, the availability of forage problem for ruminant animals can be overcome by developing *B. mutica* production even though in a season with high sunlight intensity.

This research can be continued to find out the role of DMM fertilization on B. mutica production after defoliation.

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ROOT PATTERN DEVELOPMENT OF *Brachiaria humidicola* AND *Imperata cylindrica* AND CHANGE OF BOTANICAL COMPOSITIONS OF PASTURE IN COCONUTS PLANTATION

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Abstract

Brachiaria humidicola is one of perennial forage grass with creeping growth habit, having stolon, grown well and tolerant under shade environment, persist regular defoliation and in rotational grazing produce more and long vigorous roots. Imperata cylindrica is considered as the worst weed of over the agricultural land in most tropical regions. This type of weeds impact the lost of soil water via plant evaporations. The aims of this research was to studied the ability of B.humidicola to transform I. cylindrica field to be a good pasture in coconut plantation. In this experiment B. humidicola was planted in the area grown by I. cylindrica but already slashed 5 cm above ground level. Treatments consisted of planting distance of B.humidicola PD-1 = 30 x 30 cm, PD-2 = 50 x 50 cm and PD-3 = 100 x 100 cm apart (A factor), and cutting frequencies CF-1 = 15 days, CF-2 = 30 days and CF-3 = 45 days (B factor). Cutting of B. humidicola together in the same time with I. cylindrica. Treatments were put as factorial arrangement based on completely randomized design. The variables measured were botanical composition, below ground (BG) dry matter, forages production and quality. Data analysis was using ANOVA followed by HSD. The results showed all variable measured were significant higher at the treatments interaction of planting distance of PD-1 and cutting frequencies CF-3. At this interaction botanical composition and below ground dry matter production were dominated by B. humidicola component. Based on these results it could be concluded that B. humidicola as forage cover crops potential and useful as biological herbicide to control I. cylindrica and transformed it to be a good pasture in coconut plantations.

Key words*: botanical, brachiaria, coconuts, imperata, root development.*

INTRODUCTION

Cogon grass (*Imperata cylindrica* Beauv.), family Poaceae, is an invasive, rhizomatous, aggressive C_4 perennial grass that has become one of the most serious invasive species all over tropical and subtropical region. It does not tolerate shaded environments because it assimilates carbon via the C4 photosynthetic pathway (Patakh et al., 2018). It is a strong competitor for growth factors such as water, nutrients, and light because it sprouts and grows more rapidly than most crops (C3 plants). This sensitivity to shading can be exploited in its control by the use of fast growing cover crops, shrubs, or species of grasses with prostrate growing habits (Macdicken et al., 1997; Chikoye et al., 2001). The best way to control *I. cylindrica* is by implementing an integrated approach that employs a variety of options, for example can be suppressed by flattening, tillage or chemical control followed by planting competitive cover crops. *Brachiaria humidicola* cv. Tully is one among several species recommended as well adapted forages in shade environment underneath coconuts plantation in North Sulawesi (Kaligis and Sumolang, 1990) and pesistent under free grazing system in mixed pasture (Kaligis, 1998; Anis et al., 2015). Negative effects of defoliation frequency decrease dry matter production and growth of forage, inhibit develop and even cause death of the rooting (Mousel et al., 2005) because it inhibits nutrient absorption and the effectiveness of photosynthesis. However, recent research results show that the increase in the frequency of

defoliation contrary raised the concentration of TNC in the crown and root (Gittins et al., 2010) and even earlier it was reported that heavy grazing of yaks up to 2.9 heads/ha produced root/shoot ratio biomass higher than the lighter grazing pressure (Gao et al., 2007).

MATERIALS AND METHODS

Study Area

This experiment was conducted in the BPPT research station located 67 meter above sea level, at 01^{0} 30' N and 124^{0} 54' E. in North Minahasa regency, province of North Sulawesi, Indonesia. The climate of the area is tropical and humid which receives an annual average rain fall of 2700 mm. Rainfall distribution is fairly even, except for a period of lower rainfall 100-150 mm per month from July to September (Anis et al., 2015). The pH of the fertile, sandy loam soil is around 6. Light transmission (PAR) at the site under mature tall coconuts averages 73% at 10.00 a.m. on a sunny day. Study area are dominated by *Imperata cylindrica.*

Treatment

In this study involved two kinds of grasses *I. cylindrica* as weeds, and a tropical grass *Brachiaria humidicolla* as competitor to be evaluated was planted in the area grown by *I. cylindrica* but already slashed 5 cm above ground level. Area has been divided to 45 plots by 5x5 meter or 25 meters square each. *I. cylindrica* plant populations were varies between 65 – 90 plants per meter square of experimental area. Treatments consisted of planting distance (PD) of *B. humidicola* PD-1 = 30 x 30 cm (average 16 plant per meter square), PD-2 = 50 x 50 cm (average 8 plants per meter square), and PD-3 = 100 x 100 cm apart (average 4 plants per meter square) as A factor. Cutting frequencies (CF) as factor B consist of CF-1 = 15 days, CF-2 = 30 days and CF-3 = 45 days were applied to both species of *B. humidicola* together in the same time with *I. cylindrica.* Treatments were put as 3x3 factorial arrangement based on completely randomized design with 5 replications. Tiller of *B. humidicola* previously planted in poly bag and grown in a shade house. After grown 30 days in poly bag, then transplanted in experimental plots according to treatments of plant distance being evaluated. The plant grown without any fertilizer applied.

Measurement

Above ground (AG) green material yield of each species was obtained by cutting the central area of each plot containing of different number of *B. humidicola* plant depending on treatment of planting distance. Sampling was used quadrant of 50 x 50 cm lay down randomly two times in the middle of each plot. Below ground (BG) yield i.e., roots and rhizome were sampled from the same quadrants of 50x50 cm after the green materials has been take away. Sample of BG materials was taken used a soil core with inner diameter 5,5 cm up to 25 cm soil depth. Soil sample collected were washed carefully to remove adhered soil with roots and rhizome (Pathak et al., 2018). The samples of surface and BG collected were oven dried at 70 ^{0}C up to constant weight to obtain the biomass. The variables measured include dried weight (ton/ha) of AG dry matter of green materials, and BG dry matter yield of roots and rhizome. In this report we have been used data from 5^{th} harvest of each treatment, considered it is enough to show the morphological change of both plants under the treatments being evaluated. Data were then statistically analyzed by using analysis of variance (ANOVA) by means of MINITAB (Version 16). Honestly Significant Difference (HSD) was applied to determine the difference among treatments, and the differences were considered at $P<0.05$.

RESULTS AND DISCUSSIONS

The effects of treatments of planting distances and cutting frequencies on surface or above ground green material dry matter production showed in Table 1. Planting distance PD-1 has a Bh:Icy ratio at 3.96 point significantly higher than PD-2 and PD-3 treatments 1.93 and 1.42 respectively. This data was attribute by the higher content of *Brachiaria humidicola* in this PD-1 treatment. Even though in the beginning the plant population of *I.cylindrica* in each plot of all planting distance was dominant but due to the aggressiveness growth of *B.humidicola* where this species has a growth habit exponential (Abdullah et al., 2009; Anis et al., 2013) enable to compete with *I.cylindrica*. More

over regular cutting treatment influenced significantly the root growth and produced vigorous new roots of *B. humidicola* (Anis et al., 2015).

Tabel 1. Above ground green material DM production (tone/ha)

Cutting Frequencies (CF)	Planting Distance (PD)									Total-CF
	PD-1			PD-2			PD-3			
	Bh	Icy	Total	Bh	Icy	Total	Bh	Icy	Total	
CF-1	1.57	0.36	1.93	1.01	0.87	1.88	0.87	0.65	1.52	1.78[c]
CF-2	2.04	0.48	2.51	1.73	0.79	1.52	1.28	0.98	2.26	2.43[b]
CF-3	2.30	0.76	3.06	2.10	0.85	2.96	1.65	1.02	2.67	2.90[a]
Total-PD	5.91	1.60	2.50	4.84	2.51	2.45	3.80	2.65	2.15	
Ratio Bh:Icy	3.69[a]			1.93[b]			1.42[c]			

Notes: Bh = *Brachiaria humidicolla.* Icy = *Imperata cylindrica*

Tabel 2. Below ground roots and rhizome DM production (tone/ha)

Cutting Frequencies (CF)	Planting Distance (PD)									Total-CF
	PD-1			PD-2			PD-3			
	Bh	Icy	Total	Bh	Icy	Total	Bh	Icy	Total	
CF-1	0.34	0.10	0.44	0.29	0.16	0.45	0.20	0.20	0.40	1.29
CF-2	0.39	0.12	0.51	0.30	0.17	0.47	0.25	0.19	0.44	1.50
CF-3	0.45	0.11	0.56	0.37	0.15	0.52	0.29	0.21	0.50	1.58
Total-PD	1.18	0.33	1.51	0.96	0.48	1.44	0.74	0.60	1.34	

Bh = *Brachiaria humidicola.* Icy = *Imperata cylindrica*

Below ground dry matter production including roots and rhizome influenced by those treatment as shown in Tabel 2 below. Below ground DM production was dominated by Bh at treatment PD-1 interact with cutting frequencies CF-3 at 0.45 ton/ha, then decrease sharply to PD-2 at 0.37 and PD-3 at 0.29 ton/ha respectively. The higher yield of bellow ground of Bh due to content of crown as the source of energy for re-growth is heavier than the biomass of roots itself (Anis et al., 2015). Nevertheless, in general data show that DM yield below ground of *Brachiaria humidicola* decreased following the increasing of planting distance from 1.18; 0.96 and 0.74 respectively. Contrary, *Imperata cylindrica* yield tend to increase sharply. These results is in the same trend with the earlier reported (Pathak et al., 2017) that production of root and rhizome of *Imperata* was 3.8 times higher than the surface litter production.

CONCLUSIONS

Based on the results it could be concluded that *Brachiaria humidicola* potential and useful as biological herbicide to control *Imperata cylindrica* and transformed it to be a good pasture in coconut plantations.

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EFFECT OF INCLUSION OF COCONUT MEAL IN THE DIETS ON NUTRIENT DIGESTIBILITY OF GROWING PIGS

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Abstract

Although its protein content is less than that of conventional ingredients commonly used as protein sources, copra meal represents the largest quantity of locally available feed protein in many tropical areas, such as Indonesia. It is, therefore, important that information about the nutritional value of copra meal is available. The present study was conducted to test the effect of inclusion of copra meal in growing pigs diets on nutrient digestibility. Twenty-four castrated Landrace Yorkshire pigs (3.5 – 4.0 months of age, ranged from 33 – 40 kg of body weight) were kept in individuals pens. Four treatments were arranged as follow: R1 = 95% basal diet + 5% copra meal; R2 = 90% basal diet + 10% copra meal; R3 = 85% basal diet + 15% copra meal; R4 = 80% basal diet + 20% copra meal, with six replications as blocks based on body weight. The present study was arranged in a 4 x 5 Completely Randomized Block Design with four treatments and five blocks. After a significant F test, Tukey's test (where necessary) was used to inspect differences among treatment means. Differences between treatment means were considered significant when $P < 0.05$. Variables measured were: daily feed and water consumption, nutrient digestibility (energy, crude protein, fat, crude fiber, calcium and phosphorus). The result showed that there was no significant effect ($P>0.05$) of copra meal inclusion in diet on feed and water consumption, as well as nutrient digestibility (energy, crude protein, fat, crude fiber, calcium and phosphorus). It can be concluded that inclusion of copra meal up to 20% in growing pigs diets did not affect nutrient digestibility as well as performance of the pigs. Further study on higher level of copra meal inclusion in pig diets is needed.

Key words*: copra, pigs, consumption, digestibility*

INTRODUCTION

The demand for feed is increasing and co-products from the tropical food industries are increasingly used in diets fed to pigs. These co-products include copra meal and copra expellers (Stein et al., 2015). The coconut palm (Cocos nucifera) is widely distributed throughout Indonesia, especially in the Province of North Sulawesi. The production of coconut oil and copra meal in Indonesia is about 995.00 metric tons and copra meal are about 515.000 metric tons (BPS - Statistics Indonesia, 2017).

Coconut meal or copra meal is produced from the ripe fruit (nut) of the coconut palm (Cocos nucifera). The nut is split and the kernel is removed and dried below 6% moisture. This meal is called copra meal and still contains the oil. Coconut meal (copra meal) is a coproduct of the production of coconut oil. Copra meal is produced by expeller sometimes referred to as coconut meal or coconut oil meal. Copra meal is often fed to monogastric animals such as poultry and pigs (Sundu et al., 2006; Diarra et al., 2004; Jaworsky et al., 2014; Rodjan et al., 2017; Park et al., 2012) and it could be deposited in adipose tissues (Rumokoy, 2012). Although the amino acid profile and digestibility in copra meal are less favourable than in soybean meal, it can provide significant protein and energy in swine diets and may be used to reduce feed costs (Stein et al., 2015).

The protein concentration of copra meal is between 14 and 22% and the protein has a low biological value and a very high Arg:Lys ratio (Stein et al., 2015). Somkune et al., (2013) reported that dried coconut meal contains about 26% crude protein and 26% crude fiber. Copra meal is high in fiber and the energy value is relatively low when fed to pigs. Its high fiber content is an important factor that limits its used in pigs diets (Kim et al., 2011) as it reduces digestibility of nutrients and efficiency of energy utilization (Guttieres et al., 2014; Marin et al., 2017). High fiber co-products from the copra industries are by-products of the production of coconut oil. After entering and circulating in animal's body, this oil could be

part of intramuscular substances composition (Rumokoy and Toar, 2014). Although its protein content is less than that of conventional ingredients commonly used as protein sources, copra meal represents the largest quantity of locally available feed protein in many tropical areas, such as Indonesia. It is, therefore, important that information about the nutritional value of copra meal is available. The present study was conducted to test the effect of inclusion of copra meal in growing pigs diets on nutrient digestibility.

MATERIALS AND METHODS

Animals, diet, and management: twenty castrated Landrace X Yorkshire crossbred grower pigs aged 3.0 to 3.5 months old were assigned to four treatments as follow: T1 = 95% basal diet + 5% copra meal; T2 = 90% basal diet + 10% copra meal; T3 = 85% basal diet + 15% copra meal; and T4 = 80% basal diet + 20% copra meal. The present study was arranged in a 4 x 5 Completely Randomized Block Design with four treatments and five blocks. The initial body weight of animals was 28 to 37 kg. Pigs were divided into five blocks as follow: I = 28 to 29 kg; II = 30 to 31 kg; III = 32 to 33 kg; IV = 34 to 35 kg, and V = 36 to 37 kg. Pigs were placed in individual metabolic crate to facilitate the separate collection of urine and faeces. The diet, based on yellow corn, rice bran, soybean meal, bone meal, salt, and pigmix (premix), was formulated (Table 1) to meet or exceed all requirements for growing swine as defined on a percentage basis by the NRC (2012).

Table 1. Composition of basal (control) diet

Ingredients	%
Yelow Corn	50,0
Rice Bran	25,0
Soybean Meal	22,0
Bone Meal	1,0
Salt (NaCl)	1,0
Mineral(Pig Mix)	1,0
Total	100
Nutrient Assyed	
Dry matter (%)	18,31
Protein (%)	15,35
Crude fiber (%)	6,32
Ether extract (%)	5,87
Calcium (Ca) (%)	0,70
Phosphorus (P) (%)	0,68
Zinc (Zn)(mg/kg)	107
Digestible Energy (DE) (kcal/kg)	3436,0

Table 2. Composition of Experimental Diets

	Treatmens			
	T1	T2	T3	T4
	%			
Control diet	95	90	85	80
Copra meal	5	10	15	20
Total	100	100	100	100
Nutrient Assayed:				
Dry matter (%)	84,31	84,31	83,22	84,94
Protein (%)	15,35	15,37	15,66	16,00
Crude fiber (%)	6,32	6,56	7,01	7,87
Ether exract (%)	5,87	5,88	5,92	5,97
Calcium (%)	0,70	0,71	0,72	0,72
Phosphorus (%)	0,68	0,69	0,70	0,72
DE (kkal/kg)	3436,20	3437,10	3445,20	3425,30

*) Provided per kg of diet: vitamin A, 4800 IU; vitamin D3, 470 IU; vitamin E, 22 IU; menadione, 2.2 g; riboflavin, 2.7 g; niacin, 22 g; d-pantothenic acid, 1.5 g; thiamine, 550 mg; d-biotin, 150 mg; vitamin B12, 20 mg

The diet was offered to the pigs as a mass. Fresh water was provided in the trough ad libitum until the time of feeding. Feed was provided and after 30 min, the uneaten portion was removed and weighed. The trough was refilled with water until the next feeding period. Feed and water intake were recorded during the total experimental period. The design of the trough in the metabolism crate essentially eliminated wastage of feed or water. Trays placed below the crates revealed insignificant wastage. Total (24 h) faecal output was collected beginning at 08.00 h on day 70. Total samples were frozen and retained for later assay of dry matter, nitrogen, calcium, and phosphorus. Feed samples were collected during the experimental

period and retained daily for later analysis of dry matter, energy, protein, fat, crude fiber, calcium and phosphorus, according to methods defined by the A.O.A.C (2016). All data were analysed using the GLM Procedure (SAS Institute, 1989). A Completely Randomized Block Design (Steel and Torrie, 1997) was used to examine the treatment differences. After a significant F test, Tukey's test (where necessary) was used to inspect differences among treatment means. Differences between treatment means were considered significant when $P<0.05$).

RESULTS AND DISCUSSIONS

The results of the performance indices and nutrient digestibility values of the experimental pigs are presented in Table 3. Feed and water intake, daily gain, nutrient digestibility (energy, protein, fat, crude fiber, calcium and phosphorus) of the pigs were all not affected ($P>0.05$) by treatments. Increasing copra meal inclusion in the growing pig's diets had no significant effect on all parameters measured in the present study.

A report based on unpublished research by Jaworski et al., (2017) of University of Illinois at Urbana-Champaign, USA on research conducted from 2008 to 2017, indicated that growth performance declines with increasing inclusion of copra meal at day 20 of experimental period. Average daily gain, average daily feed intake, and gain:feed ratio all showed a linear decrease ($P=0.05$) as the inclusion rate of copra meal increased. The inclusion of copra meal must be less than 25% in diets for growing-finishing pigs (Stein et al., 2015).

The differences in nutrient digestibility among experiments may be due to differences in nutrient composition, drying procedures, oil extraction procedures, and the degree and duration of heat processing that is used during oil extraction. Quality problems may be attributed to the high moisture content of copra during drying and storage (Stein et al., 2015). Copra meal may be included in diets fed to growing and finishing pigs by up to 30 % without affecting growth performance (Stein et al., 2015), but negative effects of increasing levels of copra meal in the diet have been reported. Results with copra meal have been improved if diets either were semi-purified diets or if they were formulated based on digestible amino acid rather than based on crude protein (Jaworski et al., 2017).

Copra meal is incorporated in pig diets as a source of plant protein. On the other hand, copra meal has some limitations due to its high fiber content and its low protein quality that can affect its nutrient digestibility when incorporated into pig diets at high level. As was proposed in the present study that the inclusion of copra meal up to 35% in the growing pig diets could affect digestibility of energy and protein as well as the performance of the pigs. Indeed, the inclusion of copra meal from 5, 10, 15, and 20% in the diets did not give a significant ($P>0.05$) results in all parameters measured. It seems that as the level of copra meal inclusion increased in the diets has not given a major change Palatability of the diets did not change and so that feed consumption did not give a significant difference ($P>0.05$). As a consequence, digestible energy (DE) in the present study among treatment did not give a significant difference as well due to the energy spent in fiber digestion was similar. Increment of copra meal among level was not too big so that that fiber content did not change much. This could also be the one that contributed to the insignificant result on energy spent in fiber digestion, although fiber content of copra meal is high enough. NRC (2012) stated that ingredients or diets high in fiber always followed by low energy content. In that matter, the increased in feed consumption will be followed by fiber consumption that increased energy used in digesting nutrients and reduced digestible energy per se of the feed as well as its feed efficiency. Copra meal contains between 10 and 16% crude fiber and approximately 47% total dietary fiber (Jaworsky et al., 2014). Concentrations of β-mannans, galactomannans, arabinoxylogalactan, and cellulose are relatively high and the water binding capacity of copra meal is high. Water binding capacity is an estimate of the amount of water that a fiber can absorb and hold after an external force has been applied to it via centrifugation. High water binding capacity will usually result in reduced feed intake of animals because of swelling in the intestinal tract. The relatively high concentrations of fermentable fiber in copra ingredient may result in increased needs for

dietary Threonine because dietary fiber increases the endogenous losses of amino acids, and therefore increases the loss of Threonine. Protein levels of copra meal and copra expellers typically range from 20 to 26% (Jaworsky et al., 2014). The concentration of gross energy in copra meal is greater than in corn, but because of the high concentration of fiber in copra meal and copra expellers, concentrations of digestible energy (DE) and metabolizable energy (ME) are less than in corn (NRC, 2012; Sulabo et al., 2013).

CONCLUSIONS

Inclusion of copra meal up to 20% in growing pigs diets did not affect nutrient digestibility as well as performance of the pigs. Further study on higher level of copra meal inclusion in pig diets is needed.

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IMPLICATIONS OF USING SOME PHYTOADDITIVES IN BROILER NUTRITION - A REVIEW

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Abstract

This study aims to discuss the various applications of phytoadditives (basil, thyme, sage) in broiler nutrition. Nowadays, commercial development of plants as natural sources of antioxidants or antimicrobials both to enhance animal health, performance and nutrient digestibility is of current interest. Plants as basil, thyme and sage have been studied countless times for their chemical composition, being rich in bioactive compounds, vitamins, minerals, etc. Consequently, in recent years, these plants have gained attention in their use in the diet of animals, including birds. This review showed that plants as basil, thyme and sage are valuable feed supplements for broilers that can improve the performance, nutrient digestibility, carcass and meat quality, gut health and overall health.

Key words*: basil, thyme, sage, broiler, diet.*

INTRODUCTION

Among the livestock sectors, poultry production systems have the most intense development, especially in the fields of nutrition, disease control, breeding, management and organization of nutritional requirements, together with the pressure of increasing demand for poultry products and the risk of exposure to pathogens. Therefore, this sector is in great need of research in the field of nutrition and sustainable production, based mainly on plants, due to advantages such as low costs, increased bioavailability, no residual effect, without causing antibiotic resistance, etc.

Moreover, highlighting plants with an important role in increasing production performance in the context of banning antibiotics as growth promoters is a strategy that will attract the attention of poultry specialists. The information will be useful to increase production in the poultry sector and to protect the health of birds in a more effective way than traditional ways and to promote and popularize the use of plants among poultry producers.

Many plants have been identified as excellent feed additives for poultry, including basil (*Ocinum* spp.) rosemary (*Rosmarinus officinalis*), thyme (*Thymus vulgaris*), marjoram (*Origanum majorana*), sage (*Salvia officinalis*), oregano (*Origanum vulgare*), mulberry (*Morus alba*), sweet wormwood (*Artemisia annua*), etc (Botsoglou et al., 2013; Rahal et al., 2014; Olteanu et al., 2015; Criste et al., 2017; Panaite et al., 2018; Saracila et al., 2018).

Basil (*Ocimum spp., Lamiaceae*) contains a wide range of essential oils rich in phenolic compounds (Simon et al. 1990; Phippen and Simon 2000) and a higher capacity to inhibit DPPH radicals than that of essential oils (Ahmed et al., 2019).

Thyme (*Thymus vulgaris* L.) contains minerals and vitamins that are essential for health. Its leaves are one of the richest sources of potassium, iron, calcium, manganese, magnesium and selenium (Sharangi et al., 2013). Thymol is the main phenolic component that is primarily responsible for its antioxidant activity (Alireza et al., 2015).

Sage (*Salvia officinalis*) is also a natural source of flavonoids and polyphenolic compounds (e.g., carnosic acid, rosmarinic acid, and caffeic acid) that possess strong antioxidant, radical, and antibacterial activities (Baranauskiene et al., 2011; Hamidpour et al., 2014). Based on

the evidence available in the literature, this plant has anticancer, anti-inflammatory, antioxidant, antimicrobial, hypoglycemic, hypolipidemic effects (Ghorbani and Esmaeilizadeh, 2017).

The present review aims to discuss the effects of phytoadditives (basil, thyme, sage) in the broiler diet on maintaining or improving performance, nutrient digestibility, carcass quality and animal by-products obtained from them.

1. The use of basil in the diet of broiler chickens

There are currently a number of studies focused on the effects of using basil in the diet of broiler chickens. In this regard, Gurbuz and Ismael (2016) reported that the inclusion of 0.5%, 1.0% and 1.5% basil in Ross 308 diet improved the performance and feed conversion rate. The level of inclusion of 3 g/kg basil seeds in the diet of broiler chickens led to a significantly higher body weight ($p < 0.05$) than those who were fed with a conventional diet (Abbas, 2010). The carcass characteristics did not differ significantly but a significant reduction in serum cholesterol was reported compared to the control diet. However, the results of the study led by Sheoran et al. (2017) support the premise that adding basil powder to a level higher than 1% in chicken feed can improve the growth performance and immune status of chickens, by increasing the immune response mediated by T cells and thus protecting them from disease.

Osman et al. (2010) reported that the inclusion in the diet of chickens of 1.0 g./kg sweet basil in the growing and finishing periods led to a better ratio of protein efficiency and performance index values. The use of sweet basil levels (0.5 or 1.0 g/kg diet) in broiler diets can improve productivity, immunological status and carcass characteristics.

On the contrary, Ulupi et al. (2015) showed that the addition of 1, 2 and 3% basil flour in the chick feed did not have an effect on feed consumption, body weight and feed conversion rate, but the production of NH_3 and H_2S detected in manure was well below the safe level recommended for the health of the chicks and the environment. This result is important in the context of a currently growing interest in finding nutritional solutions for chickens in order to reduce the negative effects of N resulting from the decomposition of manure on the environment (Dragotoiu et al., 2010). Air polluted with NH_3 and H_2S can cause health problems for chicks, especially respiratory disorders leading to CRD (Chronic Respiratory Disease). It also increases the activity of the virus that causes Newcastle disease. This contaminated air also pollutes the society around the farm. If the concentration of NH_3 and H_2S in the hall exceeds 0.05%, it can cause the death of the chicks, according to Rahmawati (2000).

On the other hand, Saleh et al. (2020) showed that the addition of basil leaf flour to broiler diets by up to 9% did not significantly increase the weight and length of the digestive organs of the chicks. Thus, the substances contained in the basil leaf flour added to the commercial diets for broilers have not been effective in the growth and development of the digestive organs to a maximum capacity.

Dietary basil seeds had also beneficial effects on broiler health. Thus, Kadhim (2016) reported an improvement in biochemical parameters of the blood, especially total proteins and a decrease in liver enzymes (ALT, AST and ALP) and cholesterol when broilers fed diets including 0.3% and 0.6% basil seeds. The explanation found by Crowell, (1999) was that the active ingredient of plant oil extract inhibits the activity of hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG- CoA) reductase, considered a key enzyme in cholesterol synthesis.

It has also been shown that basil supplementation had beneficial effect on broiler even under heat stress conditions. Thus, Jahejo et al. (2019) reported that basil supplementation at 5 g/kg feed, promotes the growth, improves the intestinal villus size, feed efficiency and immunity of heat stressed broiler chicken.

2. The use of thyme in the diet of broiler chickens

Thyme has been known as a strong growth stimulant and as a good alternative for chemical materials in poultry industry (Alcicek et al., 2004). Thyme as a supplement in the diet of

chickens as a growth promoter or for any other use has beneficial activity on feed use, nutrient absorption, antioxidant status, immune function, carcass quality, performance parameters and odor attenuation and ammonia emission in animal halls (Abd El-Hack et al., 2016). Moreover, it has been found that thymol as an antioxidant potential, protects against cell destruction by decreasing/preventing the loss of mitochondrial membrane potential and inhibiting ROS overproduction due to oxidative stress in liver cells. Exploring the modes of action of thymol, such as pharmacological, nutritional, health and biological benefits can play a vital role in its uses in poultry farms and animal husbandry systems to improve performance parameters (Abd El-Hack et al., 2016).

Cross et al. (2007) reported antibacterial, anticoccidial and antifungal activities of thyme, thus lead to improve the general health of the chicks. The active principles of essential oils, among them also thymol, act as a stimulant of digestibility, balancing the intestinal microbial ecosystem and stimulating the secretion of endogenous digestive enzymes, thus improving the growth performance of birds (Ayoub et al., 2011; Barakat et al., 2016; El-Far et al., 2016). Consequently, thyme can be used as a readily available source of natural antioxidants and antibiotics in food and medicine. In this regard, several studies have been conducted to investigate the antioxidant and immunostimulatory potential of thyme that reflects the health and performance of broilers (Abdel-Ghaney et al., 2017).

The results obtained by Toghyani et al. (2010) suggest that dietary supplementation with 5 g/kg thyme had favourable influences on performance without having negative effects on immune system responses and blood parameters. Similarly, Ahangaran et al. (2019) showed that supplementing the diet of broiler chickens with 0.5 and 1% thyme led to a higher weight gain and decreased feed conversion rate. Thymol and carvacrol concentrations from 100 to 1000 ppm have been shown to have a positive effect on broiler production performance (Hosseini et al., 2013; Pourmahmoud et al., 2013) and on blood metabolites and immune responses (Fallah and Mirzaet, 2016). The difference in results cited in the literature on the effect of thyme on chicken performance may be due to different forms used in tests (oil, powder or various extracts) or different doses, emphasizing the need for further research (Attia et al., 2017).

Shabaan (2012) showed that chickens that were fed a low-energy diet supplemented with a mixture of 0.15% thyme and 0.15% cumin had lower values of total plasma protein, albumin and uric acid and values higher carcass percentage, total edible parts and plasma antioxidant capacity. Hashemipour et al. (2013) reported that supplementing the diet of broilers with mixture of thymol and carvacrol led to improved growth performance, increased activity of antioxidant enzymes and inhibition of lipid oxidation of meat, improved digestive enzyme activity and immune responses. Diet supplementation with thyme essential oil (300 mg/kg) led to an increase in the digestibility of crude protein, and those fed diets without the addition of antioxidants showed an increase in apparent metabolizable energy (Abbasi et al., 2019). Significantly higher ileal digestibility ($P < 0.05$) of crude ash, crude protein, crude fat, calcium and phosphorus was observed in chickens that included in the diet thyme and anise essential oils (150, 750 or 1500 mg/kg), increase directly dependent on the inclusion dose (Amad et al., 2011).

Cho et al. (2014) reported a improved growth performance, reduction of total blood cholesterol levels and also inhibition of *C. perfringens* and *E. coli* proliferation in the small and large intestine have been reported in the diet fed with the addition of phytoadditives compared to the control group and with the batch with the addition of antibiotics after exposure to *Clostridium perfringens.* Moreover, the addition of 0.5 and 1% thyme in the diet decreased the pathogenic microbial population in cecal and meat content (Ahangaran et al., 2019). Thus, the addition of thyme in the diet of broilers can improve the growth rate and hygienic parameters of chicken, essential in terms of food safety. On the contrary, Cross et al. (2007) have reported that mixtures of essential oil of 10 g/kg thyme in the diet of chickens had no effect on the microbial population and its composition in the digestive tract or in their feces (Cross et al., 2007). It was notable that supplementing a

mixture of 0.15% thyme and 0.15% cumin in the low-energy diet improved economic efficiency by 5.79% compared to the control diet (Shabaan, 2012).

Additional studies should also evaluate the safety and toxicity of thyme extracts and essential oils for a new approach in considering their use as a supplement in poultry diets.

3. The use of sage in the diet of broiler chickens

The literature is rich in studies evaluating the effect of using sage in broiler diet. An important idea is that the use of higher doses of sage is not recommended because its essential oil contains tannins, toxic stems and eugenol compounds (Dogan, 2004; Lima et al., 2004). However, the dietary level at which sage extract becomes toxic to poultry has not been established (Yurtseven et al., 2008).

Many researchers have shown that the inclusion of sage in broiler diet improved the performance parameters. For example, Traesel et al. (2011) evaluated performance data following diet broilers supplemented with antibiotics or essential oils of oregano, sage, rosemary and raw pepper extract. The dose of essential oils at the level of 100 mg/kg is suitable for recording a weight gain similar to those observed in broilers fed diets supplemented with antibiotics as growth promoters. Moreover, Hernández et al. (2004) did not observe any difference in feed consumption or feed conversion after administration of 5000 ppm essential oil extract from *Labiatae* plants (sage, thyme, rosemary) in the diet of broilers for 42 days. However, the growth of the broilers was faster than that of the control group. Similarly, Lenuta and Leonte (2011) showed that the addition of sage oil in the broiler chick diet improved body weight by about 1% (diet with 0.5% sage oil), 2% (diet with 1% sage oil) and 8% (diet with 2% sage oil) compared to those fed a conventional diet. The same authors state that sage essential oil is considered to be a potential natural promoter of chick growth.

The results obtained by El-Garhy (2018) showed that supplementing the diet of chickens with sage powder at a level of 8 and 12 g/kg diet led to an increase in body weight of chickens, improved growth rate, consumption and feed conversion, blood parameters, economic efficiency, carcass yield and decreased mortality rate.

Hernandez et al. (2004) proved that supplementation with plant extract of diets for broiler chickens improved the digestibility of nutrients throughout the gastrointestinal and ileal tract. Improvements in the apparent fecal digestibility of dry matter and crude fat were significant after the intake of 5000 ppm of essential oil of sage, thyme and rosemary essential oil in the starter phase, but no effect was detected on the digestibility of crude protein. The extracts led to an improvement in the apparent fecal digestibility of the dry matter and the crude protein in the finishing phase. Faixová et al. (2009) observed that chickens fed diets supplemented with sage essential oil had a reduction in plasma calcium concentrations. Marcin et al. (2016) conducted a 42-day study on 70 one-day Ross 308 puppies. This study aimed to compare the effects of including in the diet of chicks the essential oil of sage (*Salvia officinalis* L.) at a dose of 2.306 g/kg diet on digestive enzymatic activities in jejunum, digestive characteristics and bacterial microflora selected in caecum. The main volatile compounds in sage oil were: eucalyptol 85, alpha-thujon 148, betathujon 72, camphor 149 and borneol 37 g/kg. Supplementing the diet of Ross 308 chickens with sage essential oil (2.306 g/kg diet) led to increased digestibility of crude cellulose and amylolytic and cellulolytic activities in the jejunum chem and decreased number of *E. coli* in cecum (Marcin et al., 2016). The mucus in the digestive tract and the mucus produced could influence the antibacterial protection of the gastrointestinal tract of broiler chickens against *E. coli.* Rasouli et al. (2019) showed in a study performed on Ross 308 broiler chickens fed diet supplemented with different levels of sage extracts (100, 200, 300 and 400 ppm), a decrease ($p < 0.05$) in the levels of total plasma cholesterol, triglycerides and LDL and increases in HDL.

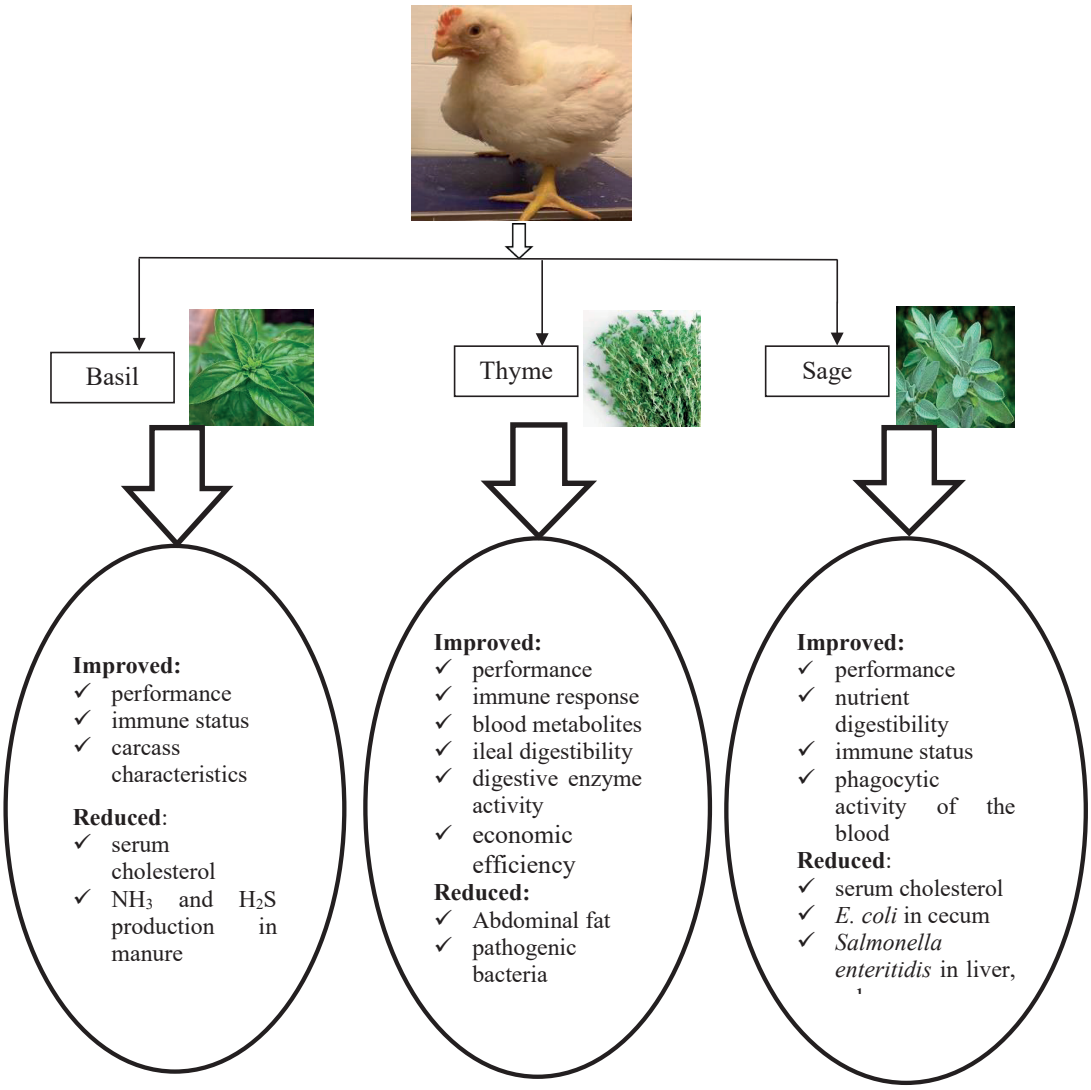

Figure 1. An overview of the effects of basil, thyme and sage inclusion in broiler diet

At the same time, a significant improvement ($p<0.05$) of the broilers' immune response was observed when the concentration of sage extract in the diet increased. The results of the study conducted by Al-Sherify and Al-Alwany, (2016) showed that the addition of 1 and 2% of *Salvia officinalis* leaf powder in the diet of broiler chickens Ross 308 led to a significant improvement ($p < 0.05$) of red blood cell count, PCV concentration and hemoglobin. Piesova et al. (2012) investigated the effects of adding sage extract to the diet on biochemical parameters, the weight of some organs and changes in the number of *Salmonella enteritidis* PT4 (SE) in infected chickens. Compared to the SE group, the sage extract from the SSE group decreased ALP and ALT activities and glucose and bilirubin concentrations on the 4th day after inoculation (p.i.). However, on day 18 p.i., only lower levels of bilirubin and ALT activity were detected. The addition of sage extract to diets decreased the number of *Salmonella* in the liver, spleen and caecum at both sampling periods, along with lower mucus production in the intestines of the chicks. The results of Piesova et al (2012) suggest that adding sage

extract to the diet could be effective in protecting SE-infected chicks. *Salvia officinalis* essential oils (0.05% concentration) were used in a study by Ryzner et al. (2013) in broiler chickens in order to study their effect on antioxidant status and phagocyte activity in the blood. The same authors concluded that sage is an important source of antioxidants that significantly improves the phagocytic activity of the blood. Furthermore, a significant bactericidal effect ($p < 0.05$) of sage extract was detected for *E. coli,* while it was moderate for *Lactobacillus*. Therefore, Rasouli et al. (2019) found that sage extract with positive effects on the parameters of immunity of chicks and antibacterial activity, being strictly related to the level of inclusion in the diet.

CONCLUSIONS

Based on the evidence available in the literature, it can be concluded that phytoadditives such as basil, thyme and sage can be used in broiler nutrition to increase performance, nutrient digestibility, carcass and meat quality, intestinal health and improve overall health. As presented in this review, the results on the applications of phytoadditives (basil, thyme, sage) in the broiler diet varied depending on several factors such as plant variety, form of use (dry plant, essential oil, plant extract), dose of inclusion, bird genetics or diet composition.

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SOME ADDITIVES USED IN POULTRY INDUSTRY FOR ALLEVIATE HEAT STRESS

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Abstract

Due to climate change, thermal stress is becoming more common in poultry. Thermal stress causes changes in the metabolism of birds which is observed in their productive performance. Also, with the appearance of thermal stress and the decrease of the productive performances leads to important economic losses for the producers, but also for the consumers due to the decrease of the products quality. In this review are presented some changes that occur in the body of birds and some methods to alleviate heat stress. Among the cheapest methods of alleviate heat stress is the manipulation of diet. Among these unconventional additives we can mention vitamin A, vitamin E, dehydrated tomato peels and prebiotics such as inulin. Also, the use of unconventional additives in food is much easier to apply, requiring no investment in production facilities.

Key words*: heat stress, oxidative stress, poultry heat stress.*

INTRODUCTION

Heat stress is one of the important obstacles in poultry production. Poultry are the most sensitive heating stresses among all animals, due to its reduced ability to dissipate heat. Therefore, the high ambient temperature adversely affects the production and health of poultry. The importance of animal responses to environmental challenges applies to all species. Heat stress occurs when there is an imbalance between the net amount of energy flowing from the body of the animal in the environment and the amount of thermal energy produced by the animal. (Lara and Rostagno, 2013). Due to its perception, stress, a response to adverse stimuli, is difficult to understand. Stress is the body's nonspecific response to any request (Selye, 1976). Studies conducted so far show that the optimum temperature for hens farming is approximately 20ºC to 25ºC (Tumova and Gous, 2012). After exceeding the temperature of 30ºCelsius, the thermal stress is installed on the poultry (Yardibi and Hoştürk, 2008). When poultry live in a very hot environment they put a lot of effort to maintain their body temperature. In order to maintain their body temperature, the visceral organs function under a greater heat load. As a result of stress, the mortality rate increases, weight gain decreases, feed consumption decreases, and meat quality is also impaired. In the case of laying hens, the thermal stress leads to a decrease in the laying rate and a depreciation of the shell (Lin et al., 2006). Heat stress lead to metabolic and hormonal changes, secretion of inflammatory markers (Etches et al., 2008). It is well know that heat stress disturbs the balance of intestinal microflora, implicitly decreases nutrient digestibility and absorption, leading to a lower feed intake and body weight gain (Feng et al., 2012).

There is increasing evidence that heat stress can have a significant detrimental effect on food, due to a variety of mechanisms but the mechanisms underlying this effect have not been fully elucidated (Rostagno, 2009). Under conditions of high ambient temperature the poultry reduce the feed intake to decrease heat stress, which negatively affects the production performance. Nutritional management is one of simplest and most direct methods used in poultry production to attenuate the adverse effects of heat stress (Sahin et al., 2009). In last

years, plants have come back to people's attention for various reasons (Trifunschi et al., 2017), some of them may be the only treatment for some incurable diseases for this time. Another reason why plants have returned to human attention would be that drugs can have in addition to the active substance and other chemicals that can have adverse effects on human or animal health. As a result of these observations, both in medicine and in the pharmaceutical industry, several plants could be used for curative purposes (Muntean et al., 2017). Many natural compounds such as herbs have been used in animal feed and have shown positive effects on growth performance and various health parameters. Many studies show that the use of herbs leads to a reduction in heat stress, such as: oregano, ginger, Artemisia annua (Panaite et al., 2018; Habibi et al., 2014; Turcu et al., 2018, Marin et al., 2015).The aim of this paper is to provide an presentation of published data on the general applications of herbs or active compounds on the harmful impact of heat stress on the poultry industry.

MATERIALS AND METHODS

Information about heat stress in poultry was obtained from a literature search of electronic databases on oxidative stress, alleviate heat stress, laying hens and broilers reared in high temperature, additives used in poultry diets for to combat effects of thermal stress.

RESULTS AND DISCUSSIONS

Physiological and behavioral effects of thermal stress on poultry:

In conditions of high temperature, birds change their behaviour, this being a reaction of the thermoregulation system that seeks a solution to lower the temperature. Mack, 2013 showed that birds reared in heat conditions spend less time feeding, more time drinking, as well as more time with raised wings, less time moving and more time resting. In order to maintain their thermoregulation and homeostasis when are reared in high ambient temperatures, animals use several adaptation systems such as vasodilation and perspiration (Mutaf et al., 2009). Unlike other animals, birds have an additional thermoregulatory system. The presence of air sacs is very useful during respiration because it promotes air circulation on surfaces thus contributing to increased gas exchange with the environment, and, consequently, heat loss through evaporation (Fedde et al., 1998). Research has shown that heat stress increases the plasma concentration of corticosterone and reduces the level of circulating thyroid hormones (Garriga et al., 2006). It has been found that the enteric nervous system and the central nervous system are connected bidirectionally through the autonomic nervous system, forming an axis between the digestive system and the brain (Eutamene and Bueno, 2007). With the appearance of thermal stress, large amounts of ROS are produced by mitochondria, affecting the energy generation efficiency and implicitly the damage of proteins, lipids and DNA. The mitochondrial membrane is predominantly composed of polyunsaturated fatty acids and proteins, they are particularly affected by oxidative stress (Akbarian et al., 2016).

Below are presented several additives for alleviate heat stress in poultry.

Probiotics used for alleviate heat stress

Sohail (2010) showed that the use of mannan-oligosaccharides and the probiotic-based *Lactobacillus* spp. for 42 day can reduce some of the harmful effects of cyclic heat stress on broilers. Among these improvements we can mention decrease serum cortisol and cholesterol concentrations and increased thyroxine concentration.

Inulin, like other probiotics such as acidifiers, bacteriocins and some phytobiotics support the homeostasis of the digestive system. A probiotic is a selective ferment for certain ingredients of feed, having beneficial effects on the host, acting on one or more beneficial microorganisms that populate the digestive system. In general, probiotics are not digestible by the host organism due to the fact that there is no enzymatic equipment to allow this activity (Gibson et al., 2004). Inulin is one of the most widely used probiotics today (Dankowiakowska et al., 2013). Inulin is a carbohydrate found in many plants. It is found in fruits and vegetables, for example chicory, Jerusalem artichoke, artichoke, onion, asparagus, but also in the stem of cereals such as wheat. The intestinal microflora plays a

special role in the optimal functioning of the gut and implicitly acts positively on the health of the whole organism. (Miremadi and Shah, 2012). The use of inulin up to 2% in the food of laying hens helps to increase the number of beneficial bacteria such as Lactobacillus, helping to improve the rate of food conversion and weight gain. Also, as the number of bacteria in the genus Lactobacillus increases, the absorption of nutrients increases (Wu et al., 2019).

Vitamins used for alleviate heat stress

Regarding the reduction of the negative effects of stress on broiler chicks, vitamin E and vitamin A are used in the diet of poultry, due to the reported benefits of supplementation with vitamin E and vitamin A in birds raised under high thermal stress. This study shows that after the inclusion of 250 mg of vitamin E and 15000 IU of vitamin A per kg of feed for 6 weeks led to a decrease in products such as, MDA in serum and liver, resulting from exposure to heat stress. (Sahin et al., 2001). The use of vitamin E and vitamin A in the feed of chickens raised in thermal stress has led to an increase in the amount of vitamin E in the blood and liver, while also leading to a decrease in the concentration of malondialdehyde (MDA). Also after the additional administration of vitamin E the amount of minerals was affected. Serum copper concentration decreased linearly while, iron and zinc concentrations increased linearly when dietary vitamin E supplementation increased (Sahin et al., 2009).

Minerals used for alleviate heat stress:

Another study showed that using for 6 weeks, separately or together vitamin A and zinc lead to lower concentration of MDA serum, improved feed efficiency and carcass traits. Also supplementing diets with vitamin A (4.5 mg/kg diet) and zinc (30 mg Zn/kg diet) offers a protective potential in preventing the effects of thermal stress on the performance of broiler chickens (Kucuk et al., 2003). Using of 100 mg/kg of Zn decrease mortality and increase activity of the activity of antioxidant enzymes (Ismail et al., 2013).

Using chromium picolinate supplements (up to 800 μ/kg of diet) in poultry feed has been shown a lower cholesterol and glucose concentrations in serum. Chromium is essential for normal glucose metabolism and is a component of the glucose tolerance factor, which works with insulin to move glucose into cells for energy generation (Pogurschi et al., 2019). As the amount of insulin increases, so does the use of glucose, which leads to an improvement in live weight gain, feed efficiency and carcass quality (Sahin et al., 2002).

Another way to combat heat stress, implicitly oxidative stress is to use dietary supplements with antioxidants from plants.

Phenolic compounds present in plants possess antioxidant effects that prevent chronic cardiovascular diseases (Forester and Waterhouse, 2009). These health effects are reported due to the antiradical and antioxidant properties of plant phenolics and plant derivatives (Lurton, 2003).

Some studies show that the use of lycopene in the diets of broiler chickens helps to improve the health of animals. Using lycopene supplements, antioxidant enzyme levels increased and MDA levels decreased in meat and serum (Sahin et al., 2016). A real source of lycopene are dried tomato peels. Lycopene is a non-provitamin A carotenoid which is a powerful antioxidant that provides protection against damage to the cells due to free radicals with a singlet-oxygen-quenching ability (Palozza et al., 2012). The inclusion of 5% dried tomato fruit for 42 days in the diet of broiler chickens raised in heat stress leads to a decrease in HDL cholesterol and triglycerides and an increase in superoxid dismutase (SOD) and glutathione peroxidase (GPx) activity, while the concentration of MDA decreased (Hosseini-Vashan et al., 2016). .

Table 1. Effects of some additives used in poultry for alleviate heat stress

Feed additive	Inculsion	Effects	Reference
Prebiotics and probiotics			
Mannan-oligosaccharides and *Lactobacillus* spp.	0.50%	decrease serum cortisol and cholesterol	Sohail et al., 2010
Vitamins			
Vitamin A	15000 UI/kg of feed	decrease glucose, cholesterol, triglycerides in serum	Sahin et al., 2001
Vitamin E	250 mg/kg of feed		
Vitamin A	4.5 mg/kg of feed	decrease MDA concentration, improve feed efficiency and carcass traits	Kucuk et al., 2003
Zinc	30 mg/kg of feed		
Minerals			
Chromium picolinate (CrPic)	up to 800 µg//kg of feed	decrease serum corticosterone concentration; decreased concentration of glucose and cholesterol in serum concentrations; increased serum insulin concentration.	Sahin et al., 2002
Zinc	100 mg/ kg	increase antioxidant enzyme levels and decrease mortality	Ismail et al.,2013
Plants / plants extracts			
Lycopene	400 mg/kg	increase antioxidant enzyme levels and decrease MDA in serum and meat	Sahin et al., 2016
Tomato peel (dryed)	5%	decrease in HDL cholesterol, triglycerides and MDA in serum; increase superoxid dismutase (SOD) and glutathione peroxidase (GPx) activity.	Hosseini-Vashan et al., 2016
Mulberry Leaf Extract	0.5%	decrease MDA concentration in serum	Gundogdu et al., 2011
Resveratrol	400 mg/kg	decrease MDA concentration in serum; increase egg laying	Sahin et al., 2022

Mulberry Morus is a plant used in traditional medicine for its beneficial effects. The leaves of this plant contain phenolic compounds which has an antioxidant effect (Gundogdu et al., 2011). Mulberry Leaf has been shown to efficiently annihilate free radicals such as NO, superoxides and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radicals. Following the administration of mulberry leaves to laying hens for 12 weeks, the amount of MDA in the serum was lower. With the improvement of the health of the hens, the weight of the shell and its breaking strength increased (Lin et al., 2017).

Inclusion of resveratrol in the diet of laying hens for 12 weeks, led to increased liver antioxidant activity. The amount of MDA also decreased while the percentage of eggs was higher when 400 mg/kg of feed was administered (Sahin et al., 2012).

Al-Juhaimi (2011) showed that parsley contains significant amounts of polyphenols in its composition, thus having beneficial effects on the body due to their antioxidant effect.

CONCLUSIONS

In conclusion, thermal stress causes significant economic losses for meat and egg producers. Some minerals such as chromium, zinc help reduce oxidative products and thus heat stress. There are natural compounds that are easily accessible and can reduce the effects of heat stress.

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POST-FERMENTATION BREWING YEAST AS A FEED SOLUTION FOR LAYING HENS

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Abstract

The paper reveals the potential nutritive impact of the spent brewing yeast as component of feeding supplements for laying hens. The post-fermentation brewing yeast biomass was analyzed for total phenolic content, by applying extraction with methanol as solvent for samples of dried yeast biomass derived from an industrial brewing process. The obtained results suggest that drying process is reproducible and residual brewing yeast could be a source of polyphenols in order to be used for the development of bio-based nutritional feed solutions.

Key words*: brewing spent yeast biomass, total polyphenols, laying hens, feed.*

INTRODUCTION

The valorization of food waste represents the fundament of the sustainable material management and there are sustained efforts in food industry to develop feasible circular economy models that can be multiplied and adapted to the size and diversity of food business operators. According to the EU Commission Council Directive 2018/851 that modifies the EU Commission Council Directive 2008/98/EC, the efficiency of resource use should be improved in order to value the waste in the bioeconomy, including food industry. Therefore, the development and implementation of viable recovery solutions that are able to support the translation from food waste to food by-products are urgently required (Directive (EU) 2018/851; Galanakis, 2015; Rodino et al., 2019; Watanabe et al., 1980).

At EU level, the brewing industry represents a major player in waste generation. The EU countries represent globally the second largest beer producer, after China. Taking into consideration the increasing beer production in EU (i.e. the beer production volumes over 400 million hectoliters in 2018), the volumes of wastes generated by the brewing industry are significantly increasing (link 1; link 2).

In the context of circular economy, the interest for the valorization of agri-food wastes such as the spent brewing yeast (SBY) is increasing. The SBY represents the post-

fermentation yeast that results from the brewing process. It contains a large amount of carbohydrates, vitamins, minerals and proteins, and, therefore, it is used as an ingredient in feed and food supplements (León-González et al., 2018; Mathias et al., 2014; Olaru et al., 2016; Pallag et al., 2018; Podpora et al., 2015; Pogurschi et al., 2019).

Beer is produced from malted cereals and grains (mainly from barley and wheat), along with water, hops and a yeast strain (Humia et al., 2019). Rodino et al. (2019) highlighted two types of use for the application of SBY, namely by using yeast cells that have incorporated various essential elements as feed, as well as by using cell ingredients, especially intracellular components of yeast, as antitumor agents. The study performed by Podpora et al. (2016) proved that a high content of essential amino acids can be found in the yeast extracts and the extracts also have high antioxidant activities in comparison to tea. The yeast strain (commercial *Saccharomyces* and alternative non-*Saccharomyces* yeast strains) together with the original wort content and the spent yeast cropping time have a direct impact over the different components of the yeast extract (Jacob et al., 2019).

The study on the SBY demonstrates that it can be safely used as animal feed for poultry and as protein source in order to replace soya bean (Chollom et al., 2017). Due to the relatively high protein and essential amino acids content, SBY can be considered as an ingredient of feeding supplement for laying hens. The diets rich in antioxidant compounds can greatly improve the performance of hens, but also the quality of eggs.

The oxidative reactions are known to be the basis of numerous biochemical pathways and cellular functions. The imbalance of pro-oxidants and endogenous antioxidant mechanisms in living tissues lead to oxidative stress (Kohen et al., 2002). In animals, the oxidative stress is an important mechanism that leads to pathological disorders and affects the growth of birds (Fellenberg and Speisky, 2006).

The SBY is considered as a valuable source of bioactive polyphenols that can bring added value through its use in the cosmetic, food and pharmaceutical industries (León-González et al., 2018) In addition, some studies have shown the ability of yeast (such as *Saccharomyces cerevisiae*) to act as a delivery system for bioactive molecules based on their capacity to absorb polyphenols during the fermentation process (Jilani et al., 2015, 2016).

The paper introduces the characterization of SBY biomass regarding the content in total polyphenols in order to demonstrate the reproductibility of the drying process, and releves the potential nutritive importance of the SBY as component of feeding supplements for laying hens.

MATERIALS AND METHODS

Spent brewing yeast and chemicals

The SBY samples analyzed in this study, represent inactivated brewing yeast in form of powder resulted from the drying of slurry SBY. These five samples of SBY are collected from the industrial brewing process of different batches of the same brand of beer from the Bergenbier S.A. brewery (Ploiești, Romania), provided by Agsira S.R.L. The brewing yeast employed was a bottom-fermenting yeast.

The liquid (slurry) SBY resulted from the brewing process is the raw material that was subjected to the dying process with the purpose of obtaining dried and inactivated yeast biomass. The spent brewing yeast biomass was dried using a drum dryer (Marinescu et al., 2019) with a drying time applied in order to protect the liquid spent yeast from high temperature exposure (Bărbulescu et al., 2018). The dried yeast is presented in the form of a brownish-brown powder with a bitter-sweet taste, having the specific smell and aroma of yeast.

Five batch samples from five drying processes were tested for dry meter and polyphenols in order to demonstrate the reproducibility of the drying process of liquid spent yeast.

Method for total polyphenols determination

Total polyphenols were analyzed by Folin-Ciocalteau method

About 250 mg of samples were weighted in a centrifuge tube, and 10 ml of water (Milli-Q®

Reference) were added. The extraction procedure was carried out by using ultrasonic bath (VWR® Ultrasonic Cleaner). The ultrasonic treatment took 60 minutes at 18°C.
After that, the samples were centrifuged (Hettich® MICRO 220/220R) at 6000 rpm for 20 minutes.
The supernatant was separated and centrifuged again at 12000 rpm for 15 minutes. The supernatant was separated again, and this was used to measurements.
As polyphenol content is referred to gallic acid, gallic acid standard solutions were used to calibration. These solutions were prepared diluting stock solution of gallic acid reagent (Sigma-Aldrich, Merck KGaA).
The working reagent of Folin-Ciocalteu's phenol was prepared by diluting a stock solution (Sigma-Aldrich, Merck KGaA) with distilled water (1:10, v/v).
Sodium carbonate solution was prepared by mixing 7.42 g Na_2CO_3 (Suprapur®, Sigma-Aldrich, Merck KGaA) with 100 ml distilled water (0.7 M).
Methanol working solution was prepared in ration 4:1 using methanol stock solution (Reanal, analytical grad) and distilled water.
Samples (the supernatant resulted from the second centrifugation) (50 µl) were aliquoted into test cuvettes, 200 µl of methanol solution and 1250 µl of prepared Folin-Ciocalteu's phenol reagent was added.
After a few minutes, 1000 µl of saturated sodium carbonate solution-Na_2CO_3 (7.5% w/v in water) was added. The mixture was then incubated at 50ºC for 5 min.
Afterwards, the absorbance of the reaction mixture was measured at 760 nm using a Spectronic Helios Gamma UV Visible spectrophotometer (Thermo Fisher Scientific).
The polyphenol content of samples was expressed referred to gallic acid.

Moisture determination

Total moisture determination analysis was performed by the method described by León-González et al., (2018). The determination of the moisture content of SBY was performed after heating at 130^0C during 2h using a WTB Binder drying oven (Tuttlingen Germany).

Estimation of total polyphenols

The determination of total phenolic content in SBY biomass was performed using the Folin-Ciocalteu method, which is the mostly used analytical tool for the determination of polyphenols (Ignat et al., 2011; Krumpe et al., 2019; Pallag et al., 2018).

RESULTS AND DISCUSSIONS

Moisture content

The sixth sample is a mix sample was prepared from the 5 yeast sample.
The determined moisture content (%) in the SBY was between 8.73 and 9.31.
By the same drying technology, 5 batches of dry brewer's yeast were obtained.
For each batch of brewer's yeast, the polyphenol content (TPP), expressed in mg, relative to 100 g of gallic acid (TTP mg / 100 g referred to gallic acid), was determined in sextuplicate. The calibration curve for total polyphenols is presented in Figure 1.

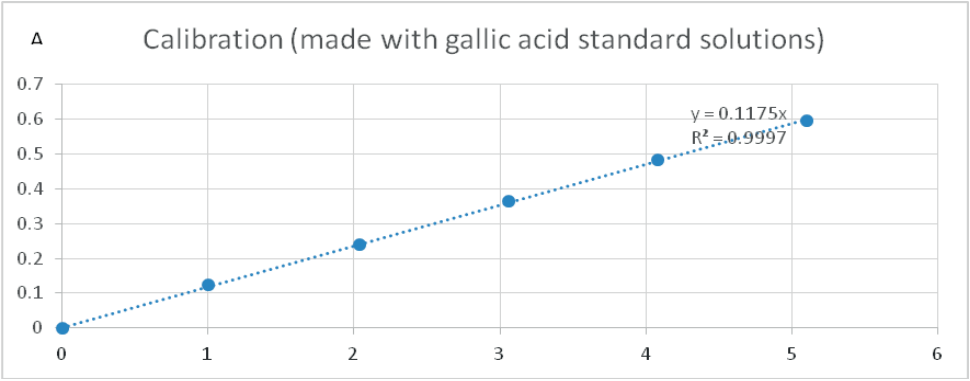

Figure 1. Total polyphenols content in spent brewing yeast extracts under different extraction conditions

Table 1. Results of polyphenol content analysis for each batch and brewer's yeast mixture, respectively

Batch	TTP mg/100 g referred to gallic acid						Average±SD	RSD (%)
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6		
1	408	400	387	409	402	441	**408±18.0**	**4.4**
2	409	419	394	435	440	456	**425±22.9**	**5.4**
3	406	415	438	397	440	445	**423± 20.0**	**4.7**
4	385	378	417	396	389	391	**393±13.4**	**3.4**
5	403	420	416	403	425	414	**413±9.0**	**2.2**
mix (1-5)	397	416	407	389	438	404	**409±17.0**	**4.2**

The analysis of the results listed in Table 1 shows a very good reproducibility of the results for each batch (1-6), supported by the values obtained for TTP mg/100 g referred to gallic acid and the corresponding standard deviation and relative standard deviation respectively. Thus, the results obtained reflect a reduced intralot variability in polyphenol content (TPP), expressed in mg, relative to 100 g of gallic acid (TTP mg/100 g referred to gallic acid) (Figure 2).

TTP mg / 100 g referred to gallic acid vary between 393 ± 13.4 and 425 ± 22.9.

In addition, the results on TTP mg/100 g referred to gallic acid of the mixture of the 5 batches of dry brewer's yeast demonstrate the homogeneity of each batch, but also of the mixture. The value obtained for TTP mg / 100 g referred to gallic acid for the mixture of the 5 batches is 409 ± 17.0 and is within the above range (393 ± 13.4; 425 ± 22.9. These results reveal a low interlot variability of polyphenol content (TPP), expressed in mg, relative to 100 g of gallic acid (TTP mg/100 g referred to gallic acid) (Figure 2).

Practically, both intra- and interbatch variability is reduced, which shows that the method / technology of drying the brewer's yeast samples, respectively the drying conditions were well chosen.

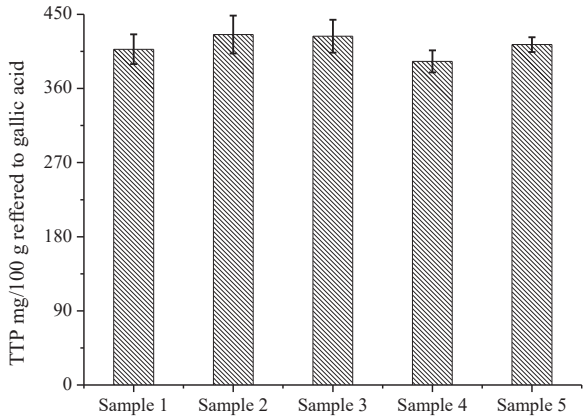

Figure2. Intra- and interbatch variability of polyphenol content per 100 g of gallic acid

The results of this study are in accordance with the study performed by (Amorim et al., 2016) as regarding the physical-chemical characterization and nutritional value of all fractions of yeast biomass. In addition, the results are only partially in accordance with the conclusions of the study performed by León-González et al. (2018) regarding the relationship between the polyphenol content and the type of sample, since in our study the total polyphenol content in the liquid SBY is lower that the values for the dried SBY biomass.

Similarly to the conclusions of León-González et al. (2018) and Rizzo et al. (2006), the different biosorption of polyphenols into yeast could be the explanation for these disparities as well as for the content in total polyphenols vs. the behavior of yeast biomass to separation, drying and extraction procedures.

CONCLUSIONS

The batch-to-batch quality of the spent brewing yeast biomass was demonstrated through the reproductibility of the results obtained for the content of polyphenols.
Based on the performed studies, it is shown that the spent brewing yeast has a relevant content of total polyphenols.
In addition, the different behavior related to biosorption of polyphenols depends on the yeast strain as well as on the brewing fermentation process and the post-fermentation treatments applied to yeast biomass. The reproducibility of drying process was proved.
Our aim in the future is to evaluate different brewing yeast biomasses based on total polyphenol content in order to obtain valuable bio-based ingredient with potential to be used as nutritional supplements for laying hens.

ACKNOWLEDGMENTS

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REPRODUCTION, PHYSIOLOGY, ANATOMY

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REPRODUCTIVE CAPACITY OF MONTBELIARD CATTLE BREED, REARED IN BULGARIA

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Abstract

The aim of the present study is to ascertain the reproductive capacity of the cows of the Montbeliard cattle breed during the adaptation period in the initial stage of the breed introduction in Bulgaria. The study involves 1,222 cows, which is relatively new in Bulgaria, reared in 16 farms. In our country rearing conditions, the Montbeliards reach average first calving age of 31.6 ± 0.19 months. It is observed that the cows born in Bulgaria have calved earlier when compared to those born in France- respectively 30.9 ± 0.32 and 32.7 ± 0.38. The farm, father, year and month of birth have a significant effect on the age of first calving. The average duration of the calving interval of the animals examined is 453±20 days. We have ascertained that of all factors considered, the farm, year and month of calving have the biggest influence on the calving interval. The rank correlation of the farms in terms of duration of the calving interval and the first calving age is positive and high enough (+0.618).

Key words*: calving, lactation, Montbeliard, reproduction.*

INTRODUCTION

The French selection is characterized by its traditionalism and quite high conservatism. Therefore, the selection indexes of the dairy cattle breeds are similar (Launay, 2014) and have not change within ten years. In 2012, however, this happened with the shift to paying more attention to the functional traits, especially those related to longevity. Furthermore, two new priorities- cows fertility and udder health have been introduced, while the milk productivity relative share has been decreased (Govignon-Gion et al., 2016). The reproduction capacity plays a major role among the functional factors. The main factors controlled are the age of first calving, independence, service period and calving interval duration, the total fertility and impregnation after first insemination. In many regions of France, the first calving takes place after two years old. In 2017 the average first calving age of the Montbeliard cattle breed was 27-30 months (Janus et al., 2017). The Montbeliard cows which calved at such age indicated good milk production, longer productive lifespan and higher lifelong milk yield (Janus, 2016). Another study (Zółkiewski et al., 2018) indicates that the average first calving age of the Montbeliard is 29.4 months, and that the breed had fewer ($P \leq 0.05$) lactations when compared to the Simmental whose first calving age was between 24 and 27 months. Amine and Malika (2016) recommend that the Montbeliard first calving age be not earlier than 28.4 months. The first ever import of Montbeliard heifers in Bulgaria was executed in 2005, and by 2012 a total of 1,339 animals were imported (Karamfilov and Atanasov, 2013). The aim of the present study is to ascertain the reproductive capacity of the cows of the Montbeliard cattle breed during the adaptation period at the initial stage of the breed introduction in Bulgaria.

MATERIALS AND METHODS

The study involves 1,222 cows of the Montbeliard cattle breed, controlled by the National Association for Breeding of Montbeliard and Simmental Cattle in Bulgaria in the period 2005-2013. Part of the cows are introduced from France, others were born in Bulgaria after the initial introduction. The cows are reared in 16 farms, situated all over the country. Cows having a calving interval of more than 900 days are not included in its examination. The data are processed via

analysis of variance with the linear models having the following statistical expression:

1. Age of first calving: Yijk=μ+Fi+YBj+SBk+FYij+FSik+YSjk+FY+Sijk+e(M1);Yijl=μ+Fi+YBj+MBl+FYij+FMil+YMjl+FYMijl+e(M2);Yijm=μ+Fi+YBj+Om+FYij+FOim+YOjm+e(M3);Yijkn=μ+Fi+YBj+Sn+FYij+FSin+YSjn+e (M4).

2. Calving interval: Yimnpqr=μ+Fi+Om+Sn+YCp+MCq+Lr+e, where: μ - is the population average YBj, SBk, MBl, Om, Sn, YCp, MCq, Lr are fixed effects of respectively the farm (16); year (9), season (4) and month of birth (4); origin (2- France and Bulgaria); father (237); year (9) and month of calving (12); order of lactation (7); e- residuals. FYij, FSik,YSjk, FYSijk, FMi, YMjl, FYMijl, FOim, YOjm, FSin, YSjn are random effects of a certain factor within the scope of the preceding one (e.g. FY is a random effect of the year of birth in the farm). The statistical processing is performed via SPSS Statistics 21.

RESULTS AND DISCUSSIONS

The heifers of the Montbeliard cattle breed reared in Bulgaria are relatively early maturing. They calve around the age of 31.6 ± 0.19 months. Introduced Montbeliard age of first calving of above 30 months is reported by Vidu et al. (2010) - in Romania- 32 months, Madani (2008) - in Algeria - 33.1 months and others. According to the latter, the optimal first calving age of the breed is 30-36 months. The cows which have calved at an earlier age have exhibited lower milk yield in their lactation periods and lower lifelong milk yield, and the service period between the first and second lactation and the calving interval respectively have been 20 days longer. Similarly to other countries where livestock breeding is well developed, in France there has also been a tendency of decreasing the age of first calving. Thus the duration of the non-productive lifespan is diminished, the breeding efficiency is increased and the costs are reduced (Le Cozler et al., 2009; Cooke et al., 2013). According to LCP (2014) cows which have had initial calving at three years old have yielded 177 liters less milk than those who have calved for the first time at the age of 2 years. The study of the Bulgarian Montbeliard population carried out by us shows that the cows born in Bulgaria have calved earlier than those born in France- respectively 30.9± 0.32 and 32.7± 0.38 months; the difference, however, is improbable (Table 1). The earlier calving has two possible explanations. First, the heifers reared in Bulgaria are impregnated early without waiting for them to gain the necessary live weight. The age is a major factor in most of the farms and the fact that the Montbeliard cows are medium and not early maturing breeds is not taken into account. Karamfilov (2014) claims that the above mentioned is indirectly proved by the fact that at the end of their growth period, the cows born in Bulgaria were ($P<0.05$) lower and shorter than those born in France. The second reason for the difference in the age of first calving of the animals with different origin could be find in the adaptation period which is required by the imported animals.

Table 1. Influence of some major factors on the first calving age

	M1		M2		M3		M4	
Factor	**MS**	**F**	**MS**	**F**	**MS**	**F**	**MS**	**F**
F	121.6	9.239[a]	118.2	11.933[a]	107.9	7.312[a]	63.6	5.446[a]
YB	296.6	22.532[a]	242.8	24.511[a]	258.1	17.496[a]	157.7	13.507[a]
SB^{M1};MB^{M2}; O^{M3};S^{M4}	85.5	6.494[a]	122.5	12.368[a]	14.6	0.987	14.5	1.240[c]
F*YB	75.6	5.746[a]	69.8	7.046[a]	67.0	4.541[a]	41.8	3.584[a]
F $*SB^{M1}$;$*MB^{M2}$; $*S^{M4}$	14.8	1.127	17.3	1.751[a]			22.6	1.939[a]
YB $*SB^{M1}$;$*MB^{M2}$; $*O^{M3}$; $*S^{M4}$	27.8	2.109[b]	29.5	2.981[a]	35.3	2.391[b]	13.6	1.168
F*YB$*SB^{M1}$; F*YB*MB^{M2}	27.2	2.063[a]	26.0	2.625[a]				

F-Farm; YB-Year of birth; SB Season of birth; MB Month of birth; O - Origin; S- father; a<0.001; b<0.01; c<0.05

Part of the heifers were pregnant, others were not. It is possible that the stress during transportation, the new conditions, and the different feeding pattern with new feeds and so on have negatively affected the reproduction functions of the animals.

The adaptation process, however, has not depended so much on the new natural and climatic conditions as on the farming conditions (Table 2). The difference in the age of first calving in the farms ranges from 0.08 to 5.4 months (P<0.001) and the latter is between farms situated in one and the same region. Along with that, in 8 of 14 farms where introduced as well as born-in-Bulgaria heifers were reared, the introduced animals calved at an older age.

The age difference ranges from 0.42 to 7.0 months. The difference regarding the six farms with earlier calving of introduced heifers is within similar scope. In one of those farms, however, the difference is almost a year.

Table 2. Average first calving age of cows in farms from different regions of the country (in months)

Region	Farm	LSM± SE	Region	Farm	LSM± SE
North East	1	32.53±0.323	South	5	31.06±0.602
	6	33.45±0.708	North	7	32.60±1.451
South - West	10	29.82±0.991		8	33.81±1.100
	14	30.20±1.622		12	33.19±0.773
	15	33.56±0.898		13	28.87±0.465
	2	31.25±0.810		4	30.12±0.725
	3	31.10±0.847		16	34.40±0.988
South	11	32.84±0.631		9	29.90±1.451

Along with the farm, the year of birth also has a significant effect on the age of first calving. Through an abundant complex of factors, both the year and month of birth influence the normal course of the ontogenesis during the embryonic and postembryonic stage, the growth speed, development, including of the reproductive organs, the capability of normal fertilisation and pregnancy maintenance. (Allouche et al., 2018). Figure 1 clearly shows that in the course of monitoring, there is a downward trend in the first calving age. During the initial years after the importation of the first animals in 2005, the increase of their number in the herds was due to the import of pregnant heifers but, as we have already pointed out, they calved at an older age.

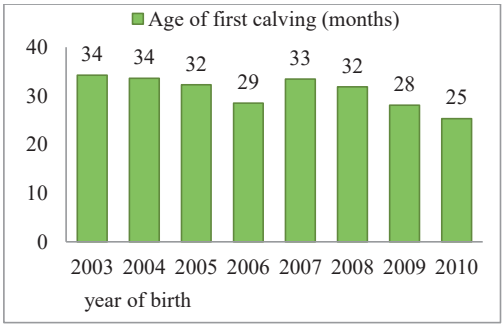

Figure 1. Influence of the year of birth on the first calving age

After the establishment of own reproduction, it is observed that with reference to the animals born between 2007-2010 there was a steady downward trend in the age of first calving.

The optimal first calving age is attained at the initial periods but subsequently, the age continues to diminish and by the end of the 8-year examination period, it is decreased by 9 months (Figure 2).

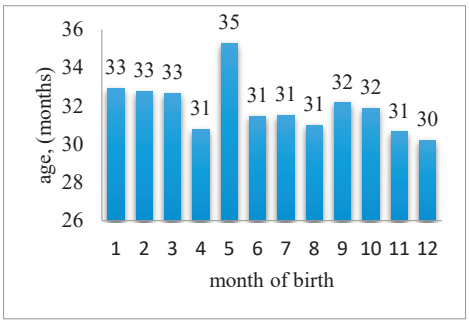

Figure 2. Influence of the month of birth on the first calving age

This cannot be regarded as a favourable tendency when taking into account the time necessary for the heifers of the Montbeliard cattle breed to reach the optimal farming maturity. In most of the farms both in France and in Bulgaria, the Montbeliard cattle breed is extensively and semi-intensively bred.

Therefore, the month of birth, by means of the complex of climatic and feeding conditions, has a significant influence on the course of the embryonic and post-embryonic ontogenesis which in itself is related to the heifers' impregnation ability and their first calving age.

Teke and Akdag (2012) have ascertained that the calves born in the summer attain breeding age later than those born in the other seasons. The later calving generates losses from 123 to 164 USD per head.

Our survey shows that the influence of month of calving needs to be analysed as a priority. Grouping of months into seasons is too provisional and, as it can clearly be seen from table 3, the season is not a reliable source of variation for the first calving age within the farms. Along with that, figure 2 indicates that the heifers born in the period January-March, which are in different seasons, calved at one and the same age. The biggest differences, on the other hand, are observed in those animals born in April and May which are in one and the same season.

Upon analysing longer periods, we ascertained that as a whole, the heifers born in June, July and August calved the earliest, and those born in January, February and March- the latest with a 2-month difference in the first calving age.

The second major controlled factor of the reproductive capacity is the length of the calving interval. This is a complex factor indicating the cows' reproductive capacity because it is connected with the postpartum period, uterus involution, independence and service period length, etc.

The average length of the calving interval of the cows studied by us - 453±20 days is far from the optimal one. With reference to the cows born in Bulgaria it is negligibly longer (456.8±24.6) when compared to that of the cows born in France (449.8±21.1); the origin of the cattle is not a reliable source of variance of the trait (Table 3). When it comes to the Montbeliard cows introduced in Morocco, the average calving interval length was 385.6 days (Boujenane and Aïssa, 2008), in Great Britain- 402 days (NMR, 2009), in Tanzania - 369 (Bouraoui et al., 2009) etc.

Table 3. Influence of major factors on the calving interval length of cows from the Montbeliard cattle breed

Factor	Df	MS	F
Father	250	12,687.722	1.123
Farm	15	53,938.376	4.774a
Origin	1	1,912.541	0.169
Year of calving	7	86,374.902	7.645a
Month of calving	11	24,966.034	2.210i

a - P<0.001; c - P<0.05;

Data of COOPEX (2020) reveal that the Montbeliard has the best reproductive capacity of all diary breeds in France. The cows impregnation from first insemination is

achieved with approximately 1.7 doses of seminal fluid against 2.4 for the Holstein. The success rate of using sexed semen is approximately 28.2 % with reference to the Montbeliard and 14.7 % regarding the Holstein. The calving interval is approximately 392 days against 420 for the Holstein. Nevertheless, a study of Mefti (2016) finds out that the calving interval of cows of the Montbeliard cattle breed was approximately 493 days and the average total fertility of the herd- 69.04% which is definitely indicative of the specificity of the effect of the environmental conditions on the cows reproductive capacity. Of all the factors examined by us, the farm and the year of calving have had the most significant influence on the calving interval. The difference in the length of the calving interval in the farms reaches up to 219.9% (Table 4). Similarly to the first calving age, the rate of the factor is determined not by the natural and climatic conditions but by the farming ones. Thus, the difference in the calving interval in the individual farms is 41.4% in North-East region, 55.0% in South-West and 186.2% in the North region. The only farm with a shortened calving interval (4) was with a relatively early first calving age of the cows (5th rank). The other farms display more significant rank changes but as a whole the rank correlation of the farms with reference to length of the calving interval and first calving age (Table 5) is positive and high enough (+0.618). Such is the correlation between the calving interval and lactation length - +0.697. The first calving age correlates positively with the lactation length. The older first calving age and the longer calving interval are connected to lower milk yield. The decrease in the farms average milk yield per normal lactation is also related to the lactation prolongation.

Table 4. Average calving interval of cows reared in different farms (in days)

Region	Farm	LSM± SE	Region	Farm	LSM± SE
North-East	1	388±24	South	5	473±23
	6	549±26	North	7	525±26
South - West	10	465±28		8	441±29
	14	400±28		12	453±21
	15	434±42		13	484±35
	2	500±50		4	282±87
	3	620±29		16	384±36
South	11	483±27		9	371±67

Table 5. Rank correlations between the farms with reference to the calving interval, first calving age, lactation length and milk yield per normal lactation

Traits	First Calving Age	Length of Lactation	Milk Yield per Normal Lactation
Calving interval	0.618	0.697	-0.447
First calving age		0.476	-0.306
Length of lactation			-0.512

During the first years after the introduction of the Montbeliard cattle breed in Bulgaria, the calving interval varied greatly (Figure 3) which once again can be connected to the adaptation processes running. After 2009 a steady tendency for decreasing the length of the calving interval is observed. The cows which calved in 2012 had approximately 18% shorter calving interval when compared to those which calved in 2009. The lactation length of cows which calved in different years follows a similar pattern. The order of calving is not indicated as a factor which affects the length of the calving interval probably because of the high intra-group variance, however, the trends are clearly expressed (Figure 4). The first calving interval of the population controlled in Bulgaria is the longest and there is a clear downward trend up to the fourth one. The second calving interval is 8.4% shorter than the

first one, the third is 3.7% shorter than the second, and the fourth is 4.6% shorter than the third one.

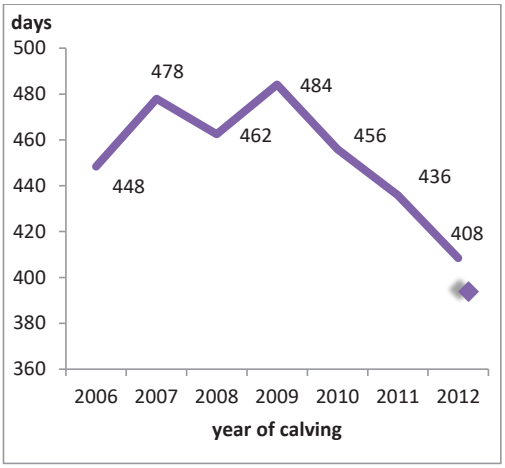

Figure 3. Length of the calving interval of the cows which calved in different years

The total decrease between the first and the fourth calving interval is 15.9%.

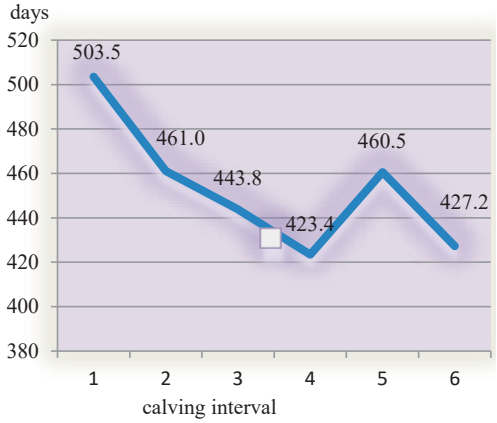

Figure 4. Length of the calving interval depending on the order of calving

The dynamics we observed in the length of the calving interval is different from what other authors have reported. Gadzhev (2005) for example, ascertained gradual increase in the length of the calving interval from 1st to 11th with 5.6% which is explained with the growing insemination problems. Ferris et al (2014) claim that the Holstein cows population in Ireland observed by them had first calving interval of 389 days, the second one was 1 day longer, the third one - 8 days longer, the fifth one - 10 days longer, and the sixth - 2 days longer. A parallel examination of the Norwegian Red Cattle breed, however, shows that the longest calving interval was the first one - 399 days; the second and the third decrease to 379 and 376 respectively, and after that the calving interval increases to 384, 386 and 383 days. Similarly to the population studied by us, the Norwegian cows were imported in Ireland as heifers. Obviously, the explanation for the significantly prolonged first calving interval and its gradual decrease may be found in the adaptation processes. The month of calving also influences the length of the calving interval and the reason for this can clearly be seen in Figure 5. As a whole, the calving from September to January is less dynamic - within 2% but from February to August it is considerable and nonlinear. The cows born in August had the longest calving interval. This is the month when the cows in our country experience the most unfavourable conditions- the high temperatures are combined with the drying of the pastures. When it comes to the preceding months, they are related to the transition from winter to summer feeding and more noticeable fluctuations in the climatic factors. Mouffok et al (2019) have found that the season influences the cows total fertility. In the winter the pregnancy rate was 73.3%, 69.4% in spring, 69,2 in the summer and 63.2% during the autumn.

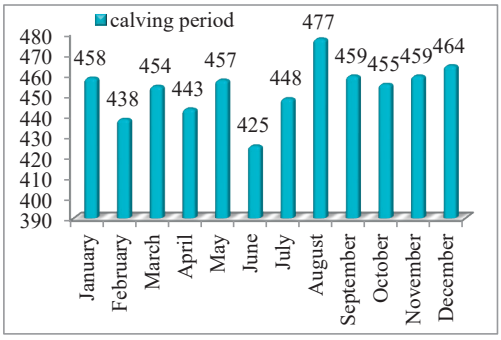

Figure 5. Length of the calving interval of the cows which calved in different months

The father does not affect the length of the calving interval. This probably stems from the inability to group the bulls due to their large number, the smaller number of daughters lactating in different farms, different years, seasons and so on. However, the difference in the length of the calving interval of the daughters of the individual bulls is considerable- from 248±104 to 805±144 days.

The shortest calving interval in Bulgaria was displayed by the daughters of IMPOSTEUR - FR 3993030061. In France IMPOSTEUR is ranked ISU - 102 with 95% milk yield reliability and 95% regarding linear traits. The daughters of UCIFE - FR 7401927569 had the longest calving interval of approximately 805±144 days. In France the UCIFE has ISU-84 but has a relatively low reliability with reference to the reproduction index- 66%.

CONCLUSIONS

During the first 8 years after the introduction of the Montbeliard cattle breed in Bulgaria the heifers calved at an approximate age of 31.6± 0.19 months, and the length of the calving interval is 453±20 days on average.

The farm, father, year and month of birth influence the age of first calving, and the farm, year and month of calving affect the calving interval length.

The cows born in Bulgaria have calved 2 months earlier and their calving interval is negligibly longer than that of the cows born in France, however, the difference observed in these two factors are unreliable.

The adaptation of the introduced animals is mainly affected by the breeding conditions in the farm. The Montbeliard adapts successfully and has good reproductive capacity in the farms which offer a good level of rearing and feeding technology.

After the initial adaptation period there is a steady downward trend in the first calving age and the length of calving interval of the Montbeliard cattle breed population in Bulgaria.

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STUDY OF PROTOCOLS FOR SYNCHRONIZATION OF ESTRUS IN HEIFERS WITH SEX-SORTED SEMEN

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Abstract

Two protocols for planned synchronization and resynchronization of estrus and ovulation of heifers (n-25), aged between 11 and 18 months, of the Aberdeen Angus breed, bred in Bulgaria, have been studied. An experiment was designed to evaluate pregnancy rates in beef heifers synchronized in two different protocols with consecutive synchronization and resynchronization. The first protocol is a combination of two standard and may in principle be divided into two consecutive stages. In the first stage, a group of 11 heifers are under Select Synch protocol extended period of observation and detection of estrus (6-20 days). In the second stage, after 32 days and ultrasound pregnancy detection, standard PRID-Synch protocol and timed artificial insemination, are applied for the resynchronization. In second protocol standard PRID-Synch protocol and timed artificial insemination are applied for synchronization and resynchronization of group of 14 heifers with sex-sorted semen. We found that the total pregnancy rate for the first protocol is 32.39 ± 10.54 % and for the second protocol is 23.8 ± 8.92 %.

Key words*: estrus synchronization; heifers; sex-sorted semen*

INTRODUCTION

Bulgarian beef cattle production is a subsector which has been paid more and more attention. Since the beginning of the century there has been a transition from dairy to meat cattle breading which is becoming even more intensive in the recent years. When compared to 2016, in 2017 the livestock farms in the country mark a general decline by 15.1% but the number of beef cows increases by 12.5% (MAFF, 2018) Beef cattle are monoproductive, and the major factor determining the production technology efficiency is the organization and management of the reproductive process. López-Paredes et al. (2018) have ascertained that the reduction of the first calving age leads to a cut in the heifers feeding expenses, decrease in the production costs and increase of the profits. According to Damiran et al. (2018), the heifers which calve earlier have higher productivity, remain in the herds longer, and throughout their productive live, they raise one calf more than the cows which are inseminated at a later stage.

The approaches related to the induction of earlier sexual development in heifers are concentrated around exogenous treatment with the hormone progesterone either individually or in combination with others such as oestradiol, GnRH or equine serum gonadotropin (eCG) which all bolster ovulation induction. These induction protocols are based on the presumption that the progesterone may reprogramme the immature reproduction system control in such a way that it may cause maturation and normal functioning. It is expected that during and after progesterone treatment, the secretion of hormones such as LH will increase and this will stimulate the follicles growth in the ovaries as well as the ovulation which occurs spontaneously or as a response of exogenous factors (Day and Nogueira, 2013).

The estrus synchronization has several major advantages- it can affect the length of the calving interval, it also reduces the necessity of constant monitoring for estrus displays in cows, facilitates the insemination campaign planning, equalized calves groups production, and so on. Application of artificial insemination in extensive beef cattle breeding may be eased through the use of effective protocols for synchronization of the estrus and the ovulation with a subsequent fixed time artificial insemination (FTAI) (Echternkamp and Thallman, 2011). According to Gabriel et al. (2016), the fixed-time artificial insemination is considered to be the most useful method for

increasing the number of inseminated cows in the herds.

The aim of our study is to examine the effectiveness of different protocols for synchronization and resynchronization of the estrus of Aberdeen Angus heifers bred in Bulgaria inseminated with a sex-sorted semen.

MATERIALS AND METHODS

The study was carried out in 2019. Protocols for planned synchronization and resynchronization of estrus and ovulation of heifers of the Aberdeen Angus Cattle breed (n-25), bred in „THRACE ANGUS FARM'' LLC, Montana region have been studied. The location of the farm is suitable for breeding of a specialized beef cattle breed such as the Aberdeen Angus. The manner of breeding in the farm is free in groups on deep litter bedding which is periodically cleaned. The buildings used are semi-opened, and the premises- zoned. Each cattle group and category has a yard for walks during the winter months. In the summer, the main categories, with the exception of the calves for fattening, are on the pastures. The farm is a single-sector and non-specialized one with a full reproduction cycle.

The heifers are aged between 11 and 18 months and are divided into two groups, depending on the synchronization protocol, following the method of analogues. The choice of heifers for the experiment in the groups is based on an ultrasound examination, attained minimum live weight of 350 kg, and an optimal physical development. The heifers included in the trial synchronization have a normally developed and healthy reproductive system but the presence of normal estrus cycle is not taken into consideration.

Two different protocols have been elaborated for the purpose of inducing estrus and ovulation synchronization and resynchronization.

The heifers were inseminated with a sex-sorted semen (for conceiving of female calves only). On day 32 after the insemination, the heifers were subjected to an ultrasound examination for the presence of pregnancy. A portable ultrasound machine ''WED3000V'' with a linear transducer with a frequency of 6.5 MHz was used for the performance of the ultrasound check-ups. The typical reference points for diagnosis of the pregnancy were the presence of an echogenic amniotic fluid in the uterine lumen; the ascertainment of an embryo differentiating as a hyperechoic structure; the presence of placentation and so on. During the manipulations the animals are fixed in a crush and all requirements and conditions for carrying out the procedure were met.

Two parameter groups were examined for the assessment of the two synchronization protocols. The first group includes financial assessment which is actually the valuation of a single synchronization and an average cost of a successful pregnancy. The financial value includes only the price of the veterinary and medical products, and the price of the semen and the work performed are not taken into account as they are largely fluctuating parameters. The average value of a successful pregnancy- Average pregnancy value (APV) is calculated with the following formula: APV= NS (number synchronization) * POS (price of one synchronization)/ NB (number of established pregnancies).

The second group of features analyzed- commercial parameters include the number of animal manipulations and protocol duration.

The pregnancy rate data are processed statistically via the specialized software SPSS21, IBM.

RESULTS AND DISCUSSIONS

Two protocols for synchronization and resynchronization of estrus and ovulation of heifers of the Aberdeen Angus Cattle breed with a sex-sorted semen were elaborated and tested for the purposes of the present study.

Figure 1 displays Protocol 1 (Pr1). The protocol is a combination of two standard synchronization schemes and can be generally divided into two consecutive stages. During the first stage a group of 11 heifers is subject to Select Synch protocol with an extended monitoring of estrus period (from 6th to 20th day). On day 0, each animal is intramuscularly injected 100 μg GnRH (gonadorelin) or 2ml OVARELIN®, Ceva Sante Animale France. On the sixth day 25 mg of PGF2α (ENZAPROST T, Ceva Sante Animale, France) one application of 5 ml of the product per animal was intramuscularly injected. From the sixth until

the twentieth day the animals were monitored for the ascertainment of heat and the heifers exhibiting typical signs of estrus are inseminated. On the twentieth day after the start of the protocol all animals which have not displayed estrus yet are injected PGF2α again and 72 hours later they are inseminated (FTAI). Thirty-second days after the artificial insemination (day 55), all heifers which are part of the protocol are examined with an ultrasound (US) and those which are not pregnant are subject to resynchronization (Second stage of the protocol). A standard PRID- Synch protocol and fixed-time artificial synchronization (FTAI) is applied during resynchronization. On day 55, each animal is injected 100 µg GnRH and progesterone-releasing intravaginal device (PRID delta, Ceva Animal Health) are inserted for heifers each containing 1.55g progesterone. The progesterone-releasing intravaginal devices are removed after they have remained in the vagina for seven days on day 62-nd, and at the same time the animals are intramuscularly injected with eCG 500 UI (Folligon, MSD Animal Health) and 25 mg of PGF2α (a single 5ml dose of the product per animal). The artificial insemination is performed at a fixed time on the 72 hour after the removal of the progesterone-releasing devices in combination with an intramuscular injection of 100 µg GnRH per animal.

VITAMIN AD$_3$E (Active substances: Vitamin A - 1,500,000 IU/100ml; Cholecalciferol (VitaminD$_3$) – 2,000,000 IU/100 ml; Alpha Tocopheryl Acetate (Vitamin E) - 1.0g/100 ml) is additionally injected on the 6th and the 20th day.

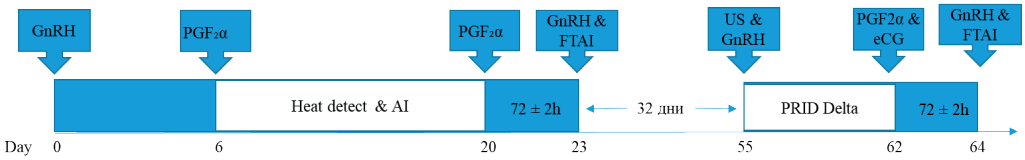

Figure 1. Protocol 1 for Synchronization and Resynchronization by Heat detect and AI and FTAI in beef heifers from the Aberdeen Angus Cattle breed

Figure 2 shows Protocol 2 (Pr 2) for synchronization and resynchronization performed through a two-time consecutive application of a standard protocol for synchronization of estrus with the help of intravaginal devices PRID delta. On day 0, each animal is injected 100 µg GnRH and inserted a PRID delta progesterone-releasing devices containing 1.55g progesterone each.

The vaginal progesterone-releasing devices are removed after they have remained in the vagina for seven days, and at the same time the animals are intramuscularly injected with eCG 500 IU (Folligon, MSD Animal Health) and PGF2α (ENZAPROST T, Dinoprost such as Trometamol, 5 mg/ml) -a single administration of 25 mg dinoprost or 5 ml dose of the product per animal. The artificial insemination is performed at a fixed time on the 72-nd hour after the removal of the PRID delta in combination with an intramuscular injection of 100 µg GnRH per animal.

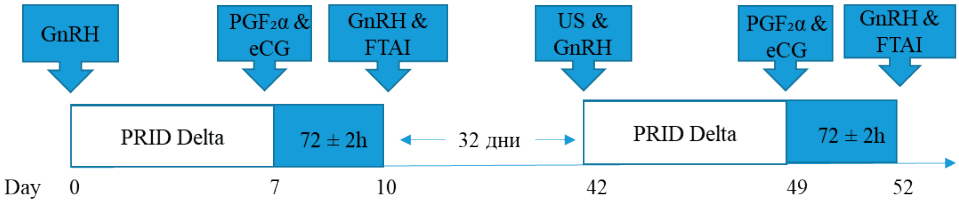

Figure 2. Protocol 2 for Synchronization and resynchronization of estrus and FTAI in beef heifers from the Aberdeen Angus Cattle breed

On the 32nd day after the artificial insemination, all heifers which are part of the protocol are examined with an ultrasound (US) for the presence of pregnancy. Those which are not diagnosed like pregnant undergo the same synchronization regimen again, i.e. resynchronization or second stage of protocol.

Table 1 displays the results of the protocol for synchronization of heifers application. According to Perry (2016), the estrus synchronization protocols which include progestin may induce estrus cycle in non-cycling heifers. However, we have ascertained that during the first stage of Pr 1, in which progesterone devices are not used, the pregnancy rate of heifers was almost two times higher- 27.27 % than it was during the second scheme (14.29 %) in which such devices were used in both stages of the synchronization. The difference during the second stage is negligible- 37.5 % and 33.3 % for Pr 1 and Pr 2, respectively. The total AI pregnancy rate indicated in our experiment is 31.58 % and 23.8 % in Pr 1 and Pr 2, respectively and have not been ascertained significant differences. On one hand, the low pregnancy rates might be explained by the inability the follicular waves in heifers to be fully synchronized. On the other hand, they might be due to the lower pregnancy rates which are expected upon sex-sorted semen insemination.

Usually, an average pregnancy rate expected in programs for synchronization of estrus is 40-60 % although the percentage of animals which exhibit estrus is bigger.

Table 1. Pregnancy rates upon testing of schemes for synchronization of estrus of heifers of the Aberdeen Angus Cattle breed

Stages	Protocol 1				Protocol 2			
	N	Pregnant, number	Pregnancies per artificial insemination (P/AI), %	±SE	N	Pregnant, number	Pregnancies per artificial insemination (P/AI), %	±SE
Stage 1	11	3	27.27	13.68	14	2	14.29	12.12
Stage 2	8	3	37.50	16.04	12	4	33.33	13.10
Total	19	6	32.39	10.54	26	6	23.81	8.923

Similarly to us, upon testing synchronization protocols for heifers, Butler et al. (2011) ascertain a pregnancy rate of about 30%. According to plenty of authors, the sex-sorted semen artificial insemination leads to a lower pregnancy rate in heifers which are subject to synchronization (Mallory et al., 2013; Thomas et al., 2014; Crites et al., 2018). According to Thomas et al. (2017), when using of sex-sorted semen, it is necessary maximum heat to be reached prior application of artificial insemination because the sex-sorted semen has a smaller number of spermatozoa per dose and a lower survival rate after thawing. In this respect, the explanation of the two times higher results of stage one in Pr 1 may be found not in the presence or the lack of progestins but in the insemination which is performed after heat detection.

In the process of studying some basic parameters related to the value, complexity and labour intensity of the protocols compared, we have ascertained that in Protocol 1 the number of the manipulations carried out and the time consumed for their realization are more than those in Protocol 2 which comes to prove that Protocol 1 is more complex and labour intensive (Table 2).

Table 2. Basic parameters in trial of schemes for synchronization of estrus

Parameters	Scheme	Scheme 1	Scheme 2
Financial parameters	Cost of veterinary and medical products , €	30	40
	Cost per pregnancy, €	95	173
Marketing parameters	Number of manipulations of animals	7	6
	Duration, days	67	53

The larger number of manipulations which are performed is connected with more labour and also leads to more stress for the animals themselves. On the other hand, the cost of the necessary materials per pregnancy is considerably lower and the success rate of Protocol 1 - higher.

CONCLUSIONS

In synchronization of the estrus of heifers of the Aberdeen Angus Cattle breed and subsequent sex-sorted semen insemination, higher results (pregnancy rate of 31.58%) are achieved in the protocol in which a scheme with an extended period of estrus detection without the use of progesterone-releasing intravaginal devices during the first stage is applied, while the pregnancy rate after the application of protocol for standard scheme synchronization and resynchronization with progesterone-releasing devices and FTAI is 23.8%.

In synchronization of the estrus of beef heifers inseminated with a sex-sorted semen, the protocol in which the insemination is after estrus detection is more suitable as it compensate the low sperm count and reduced viability, although it is more laborious and prolonged in time.

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SEASONAL DYNAMICS OF SOME TRAITS OF SEMEN PRODUCTION OF DANISH AND ENGLISH LANDRACE BOARS

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Abstract

The aim of the study was to analyse the seasonal dynamics of some traits of semen production of Danish and English Landrace boars. The concentration of spermatozoa was the highest in spring (412.72±7.72x10⁶/ml) and the lowest in summer (376.28±7.94x10⁶/ml). The volume of the semen (274.38±8.76 ml), the total concentration of sperms, (101.07±2.58x10⁹) and the number of insemination doses (16.89 ± 0.43) were the lowest in summer and the highest in winter (319.9±8.21 ml, 119.4±2.42x10⁹ and 19.94±0.43, resp.). The boars' race and the season of obtaining the ejaculates have a significant influence on the analysed semen traits. The Danish Landrace race surpass the English Landrace boars by all studied characteristics of semen production. The priority of the Danish Landrace in semen volume is between 3% and 19%, and in sperm concentration is between 1% and 14%. About the total concentration of sperms and the number of insemination doses, English Landrace boars give up with 29% during the different seasons.

Key words*: boars, ejaculate, Landrace, season, spermatozoa*

INTRODUCTION

Purebred animals on which an intensive selection of important reproductive, fattening, and slaughtering qualities is applied are a small part of pig-farming. Artificial insemination stations, as well as the nucleus herds of the hybrid pyramid where ejaculates of breeding animals are collected, have a significant influence on the economic efficiency of the branch. According to Kunowska-Slósarz & Makowska (2011), this can be achieved by insemination with ejaculates from boars with high-quality sperm and excellent reproductive potential. According to the same authors, pigs are a species which does have a season for its reproduction due to the fact that they are polycyclic.

Despite these species and physiological feature, the potential of the boars to produce quality sperm depends of a number of factors, like breed, frequency of collection of the ejaculates, age of the breeding animals, season of collection of the sperm, size of the testicles, and many others (Frangez et al., 2005; Ugwu et al., 2009; Huang et al., 2010; Marchev and Szostak, 2013; Knecht et al., 2014; Kowalewski et al., 2016; Malinova and Zapryanova, 2017).

In connection to this, we set our goal to study the seasonal dynamics of some traits of semen production of Danish and English Landrace boars.

MATERIALS AND METHODS

The study includes a total of 750 ejaculates, obtained from Landrace boars with Danish and English origin, in the period from September 2004 to July 2015, bred at the artificial insemination station at the Executive Agency for Selection and Reproduction, the town of Sliven. To achieve the goal, the semen production of boars of both breeds was studied at different seasons – spring (March, April, May), summer (June, July, August), autumn (September October, November,) and winter (December January, February).

The ejaculates were obtained with a manual method, collected in a graduated cup for semen collection, covered with a sterile gauze. Immediately after the acquisition and filtering, the material was evaluated by quantitative and qualitative semen traits, including:

- ejaculate volume (ml);
- sperm concentration ($x10^6$/ml), measured in sperm densitometer;
- total concentration of the sperms in the ejaculate ($x10^9$), calculated by multiplying the concentration of the sperms by the volume of the ejaculate;

When processing the data and establishment of the influence of some factors on the studied traits we used multifactor dispersion analysis as the linear model had the following statistical type:
Yijk=μ+Ai+Rj+eij (Model 1);
Yijk=μ+Ai+Rk+eik (Model 2);
Yijk=μ+Ai+Rj+CSij+eijk (Model 3); where: Yijkl – observation vector; μ- overall average constant; Ai, Rj are fixed effects corresponding to the season (i=4); the race of the sires (j=2); the individual (k = 8); CSij is random effect of interaction season*breed of the sires; eijk..- residual variance.

RESULTS AND DISCUSSIONS

Table 1 shows the average values (LS±SE) of the indicators of sperm production of boars in the different seasons. The lowest features of all the studies were reported in summer (274.38±8.76 ml for sperm volume, 376.28±7.94x10⁶/ml for sperm concentration, 101.07±2.58x10⁹ for total concentration, and 16.89±0.43 for number of doses).
Szostak et al., (2015) also discovered that during summer months, the collected ejaculates were with reliably lower values for volume, concentration, and total concentration than the other seasons.
In our study, the highest volume of the ejaculate (319.9±8.21 ml), the highest total concentration (119.4±2.42x10⁹), and the highest number of doses for insemination (19.94±0.43), were monitored during the winter period, except the highest sperm concentration (412.72± 7.72x10⁶/ml) which was registered in spring.

Table 1. Seminal characteristics of boars during different seasons (LS± SE) (N=750)

Season	Spring		Summer		Autumn		Winter	
Traits	LS	± SE	LS	± SE	LS	± SE	LS	± SE
Volume, ml	281.26	8.52	274.38	8.76	286.1	8.48	319.9	8.21
Concentration, 1x10⁶/ml	412.72	7.72	376.28	7.94	385.6	7.69	390.5	7.44
Total concentration, 1x10⁹	113.18	2.51	101.07	2.58	104.7	2.5	119.4	2.42
Number of insemination doses	18.90	0.42	16.89	0.43	17.44	0.42	19.94	0.43

The factors which are object of the analysis have a reliable effect on the studied indications of the sperm (Table 2). The individual appears to be a reliable source of variation in all the features of sperm production (p<0.001). The season of collecting the ejaculates has a reliable effect not only on the volume of the ejaculate and the sperm concentration (p<0.01) but also on the total concentration and the number of doses for insemination (p<0.001). The race of the breeding animals within the season has an analogical on the reviewed features as well.

Table 2. The effect of the season, race and the individual on the quality of sperm

		F-criterion and degree of reliability			
		Traits			
Model	Factor	Volume, ml	Concentration, 1x10⁶/ml	Total concentration, 1x10⁹	Number of insemination doses
1	Race	14.267***	9.789**	71.803***	74.248***
	Season	4.973**	3.285*	13.297***	13.446***
2	Individual	99.080***	35.462***	104.930***	105.202***
	Season	12.029***	6.596***	23.886***	24.056***
3	Season	5.881**	3.944**	11.065***	11.209***
	Race*Season	5.023**	3.938**	18.583***	19.129***

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

While analyzing the age dynamics of sperm production in our previous publications (Zapryanova and Malinova, 2019), we discovered that the breed of sires has a significant effect on the volume of the ejaculate, the total sperm concentration, the number of doses for insemination (p<0.001), as well as on the concentration of reproductive cells in the ejaculates (p<0.01).

There are a number of other authors who come to similar conclusions about the influence of the breed, the individual, and the season of collection of the ejaculate, and who express the importance of the volume of the ejaculate and the sperm concentration in it, for specifying the number of doses of insemination as well as for the reproductive potential of the sperm (Nacu et al., 2011; Savić et al., 2013; Savić and Petrović, 2015; Kamanová et al., 2017).

Season is an interaction of multiple dynamic factors of the environment such as temperature, humidity, duration of daylight, air movement, etc. (Hristev and Zapryanova, 2018). The data provided by the same authors shows that during the periods characterized with more significant deviations of the temperature and light factors, when the organism aims to reach homeostasis, some changes are observed which concern the quantitative and qualitative indications of sperm.

According to Gorski et al., (2017), domestic pigs, like their wild predecessors, show higher sexual activity during the season which is natural for their reproduction, as, at the same time, their reproductive potential decreases when the environmental factors are not favorable for reproduction in natural conditions. The highest volume of ejaculates in our current analysis in both monitored breeds is detected in winter, as the prevalence of Danish Landrace boars (347.33±13.44 ml) compared to those of English origin (292.46±9.42 ml) is 19% (Figure 1).

The difference between these two breeds is a percent less in autumn, and in spring this distance melts to 3% (278.00±14.72 ml and 270.75±9.50 ml, respectively).

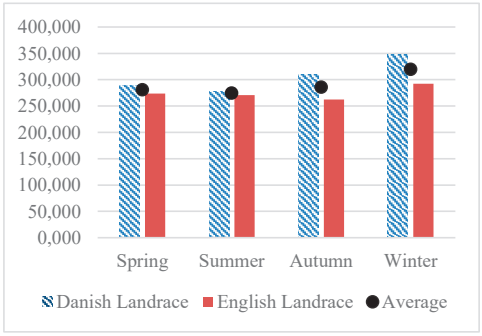

Figure 1. Dynamics of volume of the ejaculate (ml), depending on the season and breed

Kunowska-Slósarz and Makowska (2011) reach to similar results in their experiments to prove the seasonal effect on sperm production in different breeds and hybrid pigs. The authors find out reliably higher values of the volume of the ejaculate collected in winter and autumn, which is explained by the length of daylight, environment, and seasonal fluctuation in the sexual activity of male breeding animals. The lowest volume of ejaculates in the same study is in the period from March to June, which is explained with the reduced activity of boars. As for the seasonal variation of Danish Landrace, the highest volume is reported in winter (347.33±13.44 ml), which is 69.33 ml more than the lowest value of this indicator in summer.

The value of the quantitative feature of ejaculates of English boars is also highest in winter (292.46±9.42 ml), reaching its lowest levels in September, October, and November (262.23±7.87 ml).

Considering the seasonal dynamics of concentration of spermatozoa in the semen, we report prevalence of Danish Landrace sirs over the ones of English origin again (Figure 2).

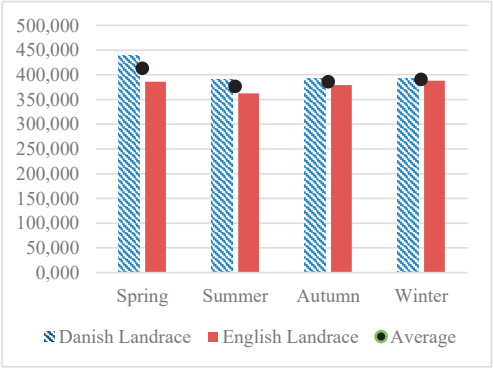

Figure 2. Dynamics of concentration of spermatozoa (x10⁶/ml), depending on the season and breed

The highest difference between these breeds is in spring – 21%, followed by the one collected in the months of summer (8%), and the difference is 1% in winter. In connection to the movement of the reviewed indicator of the breed, the highest detected values of concentration of spermatozoa in Danish breeding animals is in spring (439.45±12.84x10⁶/ml), while with the English ones, the highest concentration is in winter (388.19±8.54x10⁶/ml). In the other two seasons,

the direction of change in the studied indicator is equal, reaching its minimum in summer.

Our results are supported by those of Kunowska-Slósarz and Makowska (2011), who report the highest concentration of reproductive cells in the semen collected in the periods of winter-spring. In their studies, Gorski et al., (2017) find out that the longer the daylight, the lower the sperm concentration in ejaculates, and the shorter it is, the higher the sperm production becomes, which also leads to increased number of insemination doses.

The reported prevalence of the volume of ejaculates and concentration of spermatozoa of Danish Landrace boars, reasonably leads to the summary that the breeding animals from this breed have higher total concentration and collected number of insemination doses than the animals of English origin collected in the different seasons. The highest difference in the total concentration between the two breeds is in autumn – 29%, and the lowest one is in summer – 16%, in favor of the Danish genotype (Figure 3).

The values of the studied indicator in both monitored breeds are highest in winter and spring.

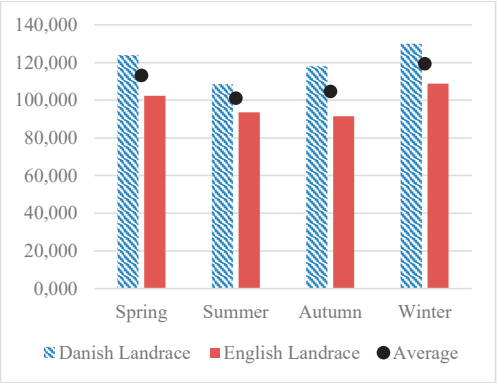

Figure 3. Dynamics of total concentration (x10⁹), depending on the season and breed

Danish Landrace boars reach the minimum of sperm production in summer (108.58±4.33x10⁹), while the breeding animals of the other race reach it in autumn (91.45±2.32x10⁹).

According to Kowalewski et al., (2016), the crucial factor of efficiency in the use of boars is the number of insemination doses. Malinova (2016) states that the total concentration is an important indicator which helps for the judgment of sperm production of breeding animals, and, more precisely, of the number of doses collected by one ejaculate.

In the conditions of the experiment we conducted, the values of this parameter in both studied breeds are the highest in winter (21.73±0.66 and 18.14±0.46, in Danish and English Landrace boars, respectively) (Figure 4).

The most significant difference between both genotypes is in autumn, when 29% more insemination doses were collected from the Danish boars than from the breeding animals of the English Landrace. The lowest contrast between the studied breeds is in summer (17%), but, again, in favor of the Danish genotype (18.18±0.72 against 15.59±0.47 numbers).

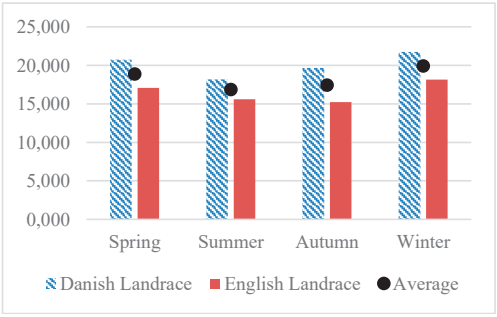

Figure 4. Dynamics of number of insemination doses, depending on the season and breed

CONCLUSIONS

The lowest features of all the studies were reported in summer (274.38±8.76 ml for volume, 376.28±7.94x10⁶/ml for sperm concentration, 101.07±2.58x10⁹ for total concentration, and 16.89±0.43 number of doses). In our study, the highest volume of the ejaculate (319.9±8.21 ml), the highest total concentration (119.4±2.42x10⁹), and the highest number of doses for insemination (19.94±0.43), were monitored during the winter period, except the highest sperm concentration (412.72± 7.72x10⁶/ml) which was registered in spring.

The boars' race and the season of obtaining the ejaculates have a significant influence on the analysed semen traits, as the Danish Landrace

boars surpass the English Landrace origin by all studied characteristics of semen production.

The highest volume of ejaculates in both monitored breeds is detected in winter, as the prevalence of Danish Landrace boars (347.33±13.44 ml) compared to those of English origin (292.46±9.42 ml) is 19%.

The highest detected values of concentration of spermatozoa in Danish boars is in spring (439.45±12.84x10^6/ml), while with the English ones, the highest concentration is in winter (388.19±8.54x10^6/ml).

And both studied breeds reach the maximum of their sperm production in winter and spring. Danish Landrace boars reach the minimum of sperm production in summer (108.58±4.33x10^9), while the breeding animals of the other breed reach it in autumn (91.45±2.32x10^9).

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CHARACTERISTICS OF SKIN THICKNESS AND GROWTH DYNAMICS OF HAIR-COAT COVERING OF MEAT CATTLES ACCORDING TO THE SEASON

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Abstract

Data on skin thickness and growth dynamics of hair cover of meat cattle of Aberdeen-Angus, Hereford and Limousine breeds, freely raised on the farm of the Research Institute of Mountain Stockbreeding and Agriculture, Troyan, are provided. Skin measurements in the neck, the elbow joint and the middle of the last rib were measured and analysed as well as the structure of different fiber categories of the hair cover. The measurements of skin thickness were performed with the aid of the skin-fold caliper in seasons (winter and summer). The hair weights in 1cm ^{2}, the length and percentage of the different fibers were determined. The animals surveyed show good adaptation plasticity in the process of generational change.

Key words: hair-coat covering, skin thickness, seasons, adaptation, meat cattle

INTRODUCTION

Skin and hair-coat covering of livestock play a particularly important role in the process of adaptation to the external environment conditions, which determines the amount of heat release to a significant degree (Kosylov and Zhaimysheva, 2009; Pozdnyakova et al., 2014; Pozdnyakova et al., 2015)

Many researchers have indicated seasonal, inter-breeding and age differences in skin thickness and hair structure and size (Dragnev, 1969, Balabanov 1972, Vlench, 1999, Pozdnyakova, 2001, Tsirendorjiev and Lumbunov, 2013).

Beef cattle breeds show good adaptive abilities to different climatic conditions due to their low and high temperatures because of their skin structure and construction and their well-differentiated, naturally cohesive hair-coat covering (Zelenkov, 1983; Eremenko, 2001; Baharev, 2011; Kayumov and colleagues, 2014).

Zimin (2006) found that in all the animals studied the individual characteristics of the hair-cover coating and skin were observed. An average skin thickness of 10113 mcm (10.01 mm), thin epidermis and relatively thick hairs were observed in cattle.

Morphological methods allow us to assess the resistance of cattle breeds to the elements of the seasons and their associated acclimatization abilities (Cherekaev, 2010)

The hereditary conditionality between the accumulation of subcutaneous fat and the increase in the thickness of hair-coat covering is a response of their organism to low temperatures, helping to adapt to the constantly changing environmental conditions.

The aim of the study was to investigate the subcutaneous fat accumulation and growth dynamics of the hair-coat covering in the adaptation to meat technology in the foothills of Bulgaria of Aberdeen-Angus, Hereford and Limousine cattle breeds.

MATERIALS AND METHODS

Subject of the study are beef cows of Aberdeen-Angus, Hereford and Limousine breeds, which have completed their development (after III lactation), raised on traditional, meat technology at the farm of the Research Institute of Mountain Stockbreeding and Agriculture. It involves raising cows in open air using a windproof canopy. The main activity of this technology is to inseminate the cows that have to give one calf at the end of the winter, to wean the calf in the end of the autumn in the same year. Thus, they could graze as much as possible and the additional nutrition is minimized. Technically, calves suck until late autumn and after weaning they are fattened.

Three groups of five cows were formed to study the adaptation processes in the animal body by the analogue method, taking into account the breed, age and live weight of each animal. In the first group (I) consisted of Aberdeen-Angus cows, in the second group (II) were Hereford cows, and in the third group (III) the cows of Limousine breed. The skin thickness and the degree of development of hair-coat covering in both groups were studied during the winter, summer and autumn seasons.

The following tests were carried out to measure skin thickness at the neck, at the tip of the hock and in the middle of the last rib in millimeters (mm) using skin-fold calliper.

Samples of the hair-coat covering were cut from the middle of the last rib during each season of the survey. We determined the weight of hair-coat covering in 1 cm² in milligrams (mg) using an analytical balance.

The length of hairs was determined in (mm) by a line. The structure of different hair categories were calculated in percent (%) per 100 examined hairs.

The data was processed by the methods of the variation statistic using the programs Microsoft Excel and Statistica 2000 and presented in tables.

The software products used for the purpose of presenting the correct visualization, as required, are from Microsoft office Word 365.

RESULTS AND DISCUSSIONS

Every modern meat technology is linked, dependent and coherent with natural-climatic factors. The town of Troyan is situated in the center of the Republic of Bulgaria at 400 m above sea level, with average annual air temperature of 14.4 ˚C. A humid continental, mountain climate is observed. Winter is long, with heavy snowfalls and low temperatures. The snow cover lasts for 40-60 days. The coolest month is February, with average temperatures of -3˚C. In the winter, frequent, strong northwest winds occur.

The analysis of the obtained data shows that the skin thickness in the different topographic regions shows differences both in breeds and in seasons.

Table 1. Skin thickness by seasons in mm (x ± Sx)

Indicator Measurements, mm	**Aberdeen-Angus** (I group) n=5	**Hereford** (II group) n=5	**Limousine** (III group) n=5
Winter (January)			
at the neck	10.2±1.10	9.4±0.89	9.6±1.34
at the top of the elbow joint	8.6±1.41	9.0±1.22	8.6±1.82
in the middle of the last rib	9.8±1.30	10.6±0.55	10.0±1.22
Summer (June)			
at the neck	9.2±0.29	9.0±1.41	9.40±0.1.34
at the top of the elbow joint	7.6±1.14	8.6±1.14	8.3±1.34
in the middle of the last rib	9.6±1.52	10.2±0.86	9.60±1.52

P<0.05

In winter, the greatest skin thickness in the middle of the last rib was found in Hereford cows, respectively, 10.6 mm, followed by Limousine cows with 10.0 mm, respectively, and the lowest skin thickness values was found at the last rib in Aberdeen-Angus cows, 9.8 mm and 9.6 mm, respectively. In percentage terms, this was 5.7% higher than Limousine breed, and 8.1% more than the Aberdeen-Angus cows (P<0.05).

During the winter season, the skin thickness of the examined body parts was greater compared to the summer period for all tested animals according to their inherited qualities. Similar peculiarities are also observed in the other animal body parts.

Breed differences in skin thickness are graphically depicted in Figure 1 and Figure 2.

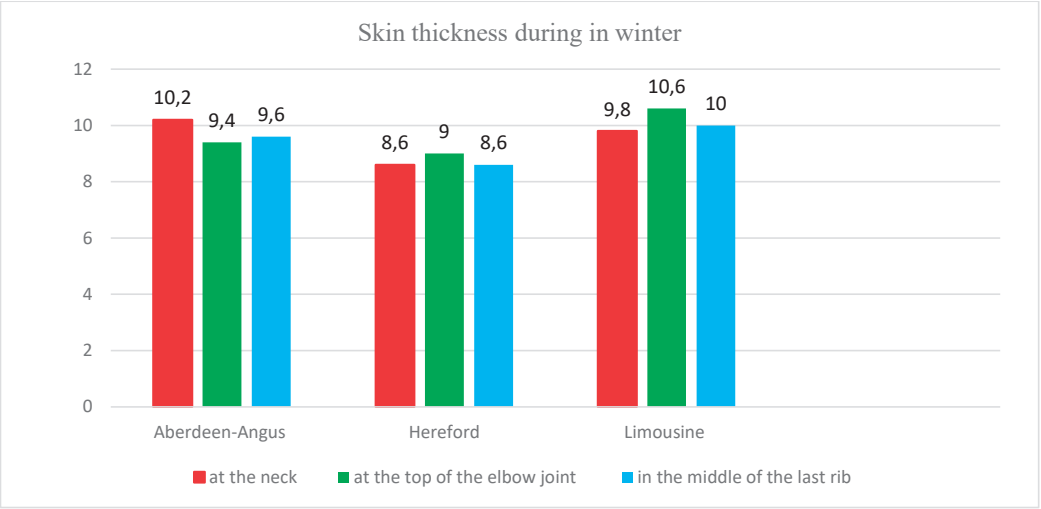

Figure 1. Skin thickness in winter

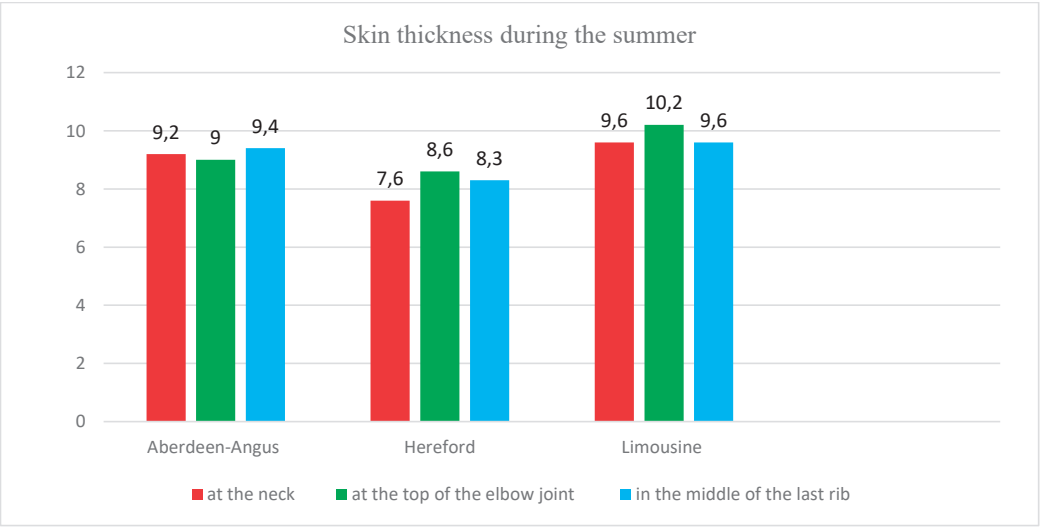

Figure 2. Skin thickness in summer

The results obtained by Zelenkov (1983) and Pözdnydkowa et al. (2015) are close to ours.

The hair-coat covering is a derivative of the skin and is related to its construction and its functions. In the adaptation of animals to the conditions of low temperatures, especially during the winter period, the hair-coat covering plays an important role in the process of regulating the heat exchange between the organism and the environment, and in rain and snow it protects it from wetness and cold (Pozdnyakova et al., 2015).

Table 2. Length, weight and percentage of hair-coat covering (x ± Sx)

Type of hair-coat covering	Breed		
	Aberdeen-Angus (I group) n=5	**Hereford** (II group) n=5	**Limousine** (III group) n=5
Winter (January)			
Length of fibers in mm including awny	35.20±0.13**	43.2±0.21**	41.6±0.41
Transitional	21.20±0.20**	28.4±0.22**	23.2±0.29*
Fluffy	12.0±0.17**	12.20±0.40	14.0±0.42
Weight of hair-coat covering for 1cm², mg	58.83±3.06	56.41±3.73	48.59±3.63
Percentage share of different fibers (%)	100	100	100
Awny	19.8	18.20	22.40
Transitional	18.8	40.20	39.60
Fluffy	61.4	41.60	38.00
Summer (June)			
Length of fibers in cm including Awny	31.04±0.35	38.39±1.36	36.16±0.22*
Transitional	18.51±0.29*	19.11±0.15***	17.34±0.38
Fluffy	9.97±0.14***	11.14±0.15	11.11± 0.26*
Weight of hair-coat covering per 1cm², mg	23.73±0.34*	23.42±0.43	22.92±0.27
Percentage share of different fibers (%)	100	100	100
Awny	22.4	25.1	26.4
Transitional	37.5	41.8	37.2
Fluffy	40.1	33.1	37.4

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

In the process of adaptation of animals to the conditions of low temperatures and snow cover during the winter, there were changes in the weight and structure of hair-coat covering.

The highest weight of 1 cm² hair-coat covering was found in Aberdeen-Angus cows, which surpassed Hereford breed by 1.35% and those of Limousine breed by 3.4%. In the summer, this weight decreased drastically and ranged from 22.92 mg in cows from Limousine to 23.73 mg in Aberdeen-Angus cows.

The differences in the length, weight and percentage ratio of the hair fibers are shown graphically by season in Figures 3, 4, 5 and 6.

Substantial changes are also observed in the structure of the hair-coat covering. The awn fibers were the longest in the winter cut from by Hereford Cows with 43.2 mm, followed by Limousine cows with 41.6 mm, and the smallest are those of the Aberdeen-Angus-35.3 mm (P<0.01).

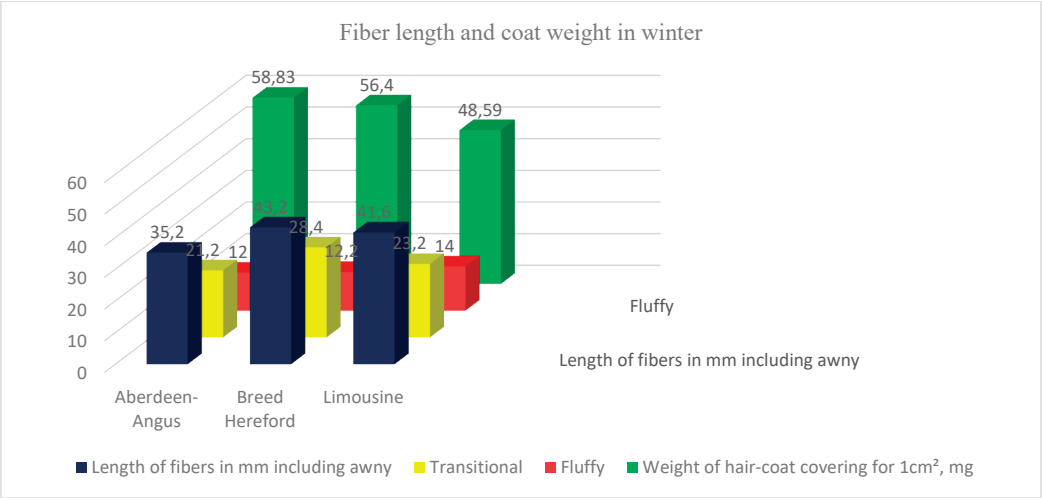

Figure 3. Fiber length and coat weight in winter

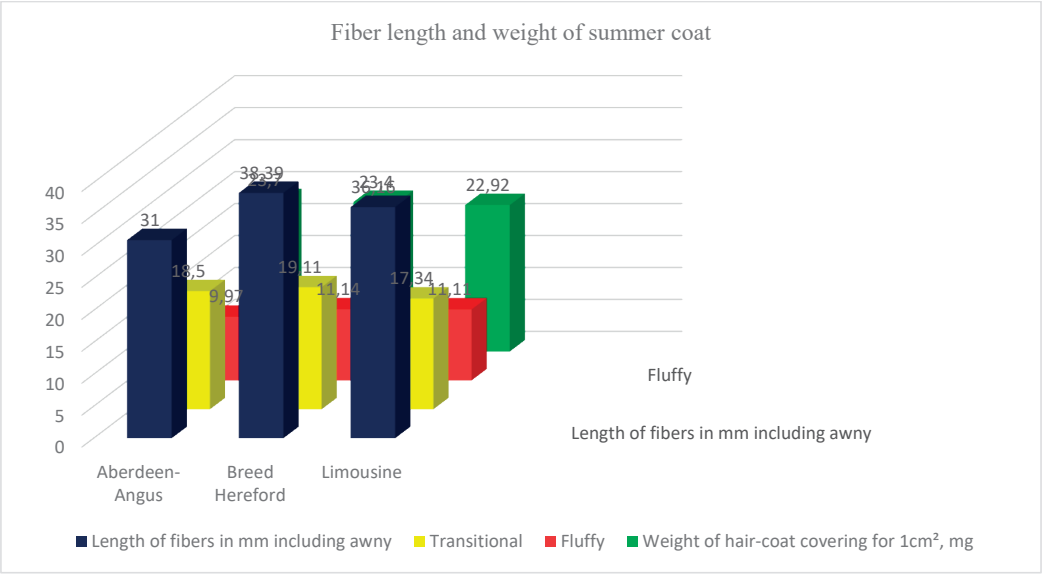

Figure 4. Fiber length and weight of summer coat

The length of the transitional and fluff fibers shows similarities to these differences. Fluff fibers of Aberdeen-Angus cows in winter surpassed by 17.4% Hereford breed, and Limousine cows by 6.7%.

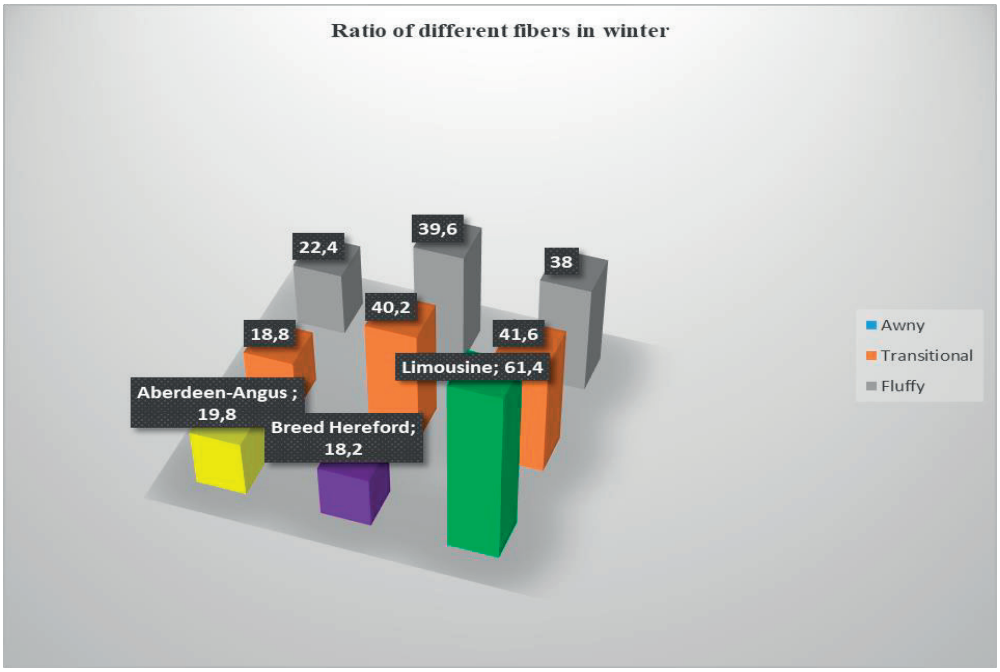

Figure 5. Ratio of different fibers in winter

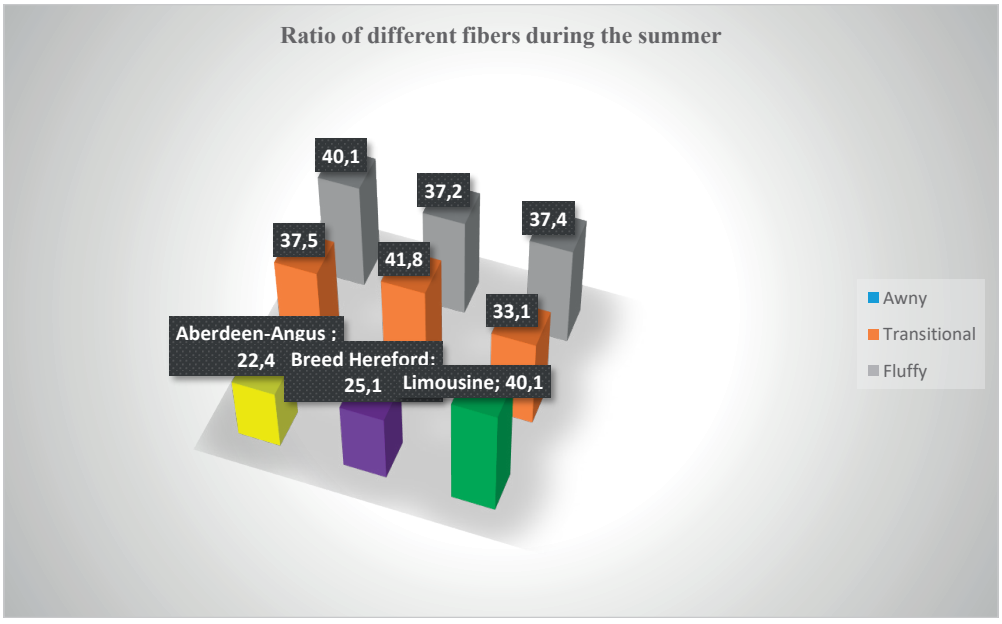

Figure 6. Ratio of different fibers during the summer

The highest values in transitional fibers were found in Hereford breed, followed by Limousine breed, and the lowest values were found in Aberdeen-Angus 18.8%. In summer the values were approximately the same for all three breeds tested - 37.2%, 37.4% and 41.8%. Similar results were obtained from Baharev (2011), Kayumov et all (2014) and Pozdnykova et al. (2015).

CONCLUSIONS

Breed and seasonal differences in skin thickness, hair length and density of hair-coat covering were found. In the winter period, during the adaptation of the animals to the technology, the three breeds increased the weight and length of their hair-coat covering, increasing the percentage of fluff in its structure. In the summer, there was a decrease in the number of fluff fibers and an increase in the number of transitional and any fibers. The studied breeds show good adaptive plasticity towards the humid continental, mountain climate of the town of Troyan, in the process of changing the generations.

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STUDY OF BIOCHEMICAL PROPERTIES OF BLOOD OF CALVES WITH A DIAGNOSIS OF KERATOCONJUNCTIVITIS

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Abstract

Eye diseases of young cattle are one of the reasons hindering the development of animal husbandry. Keratoconjunctivitis is a disease of the eye characterized by inflammation of the conjunctiva in the initial stage and subsequent transition process to the cornea. The disease is manifested in different forms and severity of inflammation. Keratoconjunctivitis causes serious economic losses due to high incidence and prevalence, both in our country and abroad. Losses from keratoconjunctivitis consist of slowing down the growth and development of young animals, loss of productivity, loss of live weight. On average, 25-30% of sick animals remain blind, the same amount of sight loss is 50%. In calves 5-6 months of age with obvious signs of keratoconjunctivitis contained in the conditions of the private livestock breeding complex, serious changes in ALT and AST activity were revealed during biochemical studies of blood plasma. Moreover, AST indicators during treatment exceeded the reference values characteristic of a given species and age of animals. Indicators of Albumin and Total protein fluctuated slightly, which indicates the activation of humoral and cellular immunity.

Key words: *keratoconjunctivitis, blood, plasma, calves.*

INTRODUCTION

Not a single doctor who stands guard over human health would begin treatment procedures, except in emergency cases, without a thorough analysis of the biochemical blood parameters results of his patient. Among veterinarians, there is also a kind of "tribute to fashion", when some of them regularly, although unsystematically, conduct blood sampling for biochemical studies. However, for the most part, all these studies, as well as their results, are not used in medical practice by veterinary specialists.

The results of biochemical studies could be of great service not only to veterinarians, but also to zootechnical specialists in terms of the formation of balanced diets for animals.

However, this method of work, when the results of biochemical blood tests reveal all the errors in feeding animals, does not always suit livestock personnel who are responsible for the quality of the same feeds. Any irrational approach of the application of this universal research method could point out two following ideas; the first is illiteracy and unwillingness to fulfill one's professional duty (for veterinary specialists); and the second, blood biochemical parameters are reliable facts that even specialists responsible for feeding animals cannot refute. The more veterinarians analyze biochemical parameters, the more detailed picture of an animal's health status they would receive.

It would not be new to say that a change in the physiological parameters of a large number of biochemical indicators reflects a certain list of pathological changes in the animal organism or the reasons that caused them.

Timely and competent use of these results would allow not only to preserve the health of animals, but also to receive high-quality products from them.

Livestock breeding is a complex and very costly branch of agricultural production, as it is associated with the need for daily care and attention to breeding animals, which, unlike their wild relatives, are fully supported by humans. In order to get maximum production from a small herd or large livestock complex, you need to constantly provide animals with high-quality feed and properly feed them (Pugh, 1986).

They should have unimpeded access to water and be housed in compliance with ethological principles in premises that meet the zoohygienic standards for their maintenance. It is important to establish daily care and medical care for them. The degree of exploitation of animals technological conditions should correspond to the biological capabilities of the body, should not weaken health and reduce the time of their production use. Failure to comply with at least one of these conditions reduces the profitability of livestock farming (Abinanti et al., 1961).

Reasonable results have been gained in certain biochemical indicators of blood plasma (total protein, albumin, ALT, AST and LDH) in the calves with keratoconjunctivitis and within the monitoring of dynamics of these indicators after the medical treatment with the use of the drug "Ligfol" in combination with various drugs.

The aim of this work is the identification and analysis of some biochemical blood indices of calves in the presence of clinical manifestations of keratoconjunctivitis, monitoring of the dynamics of biochemical parameters of blood plasma in the treatment of various preparations with local action and of the drug "Ligfol".

MATERIALS AND METHODS

As for any infectious disease, the kerato-conjunctivitis is also characterized by general emergence tendencies and spreads out with a certain coverage of the susceptible population, severity and duration of the course, outcome, etc.

Therefore, the study of all these epizootological problems is of great importance in the development of diagnostic methods, treatment and prevention. To find out the zonal features of the manifestation, we studied the epizootological features of this infectious disease in one of the agricultural enterprises of the Ulyanovsk region. According to our observations, in recent years the prevalence of keratoconjunctivitis infections has been increasing. First of all, calves are affected, especially from two days of age to a year. In adult animals, the disease is diagnosed less frequently and the inflammatory process in them proceeds more benignly. For several years, at “Megafarm – Oktyabrskay” in the Cherdaklinsky District of the Ulyanovsk Region (Russia), we were able to follow the development of enzootic of infectious keratoconjunctivitis in cattle. On this farm, in calves of the Holstein-Friesian breed, the disease progressed very quickly. A disease with eye damage of varying severity was recorded back in 2017.

The study was conducted in October 2018 on the basis of "Megafarm - Oktyabrskay" Cherdaklinsky district of the Ulyanovsk region. For this purpose there were formed 4 groups of calves. The total number of livestock in every group included 8 heads. All animals included in the group were diagnosed with clinical signs keratoconjunctivitis. Age of calves ranged from 5 to 6 months. All animals had a similar Constitution, were kept in the same microclimatic conditions, the diet was similar. For the study there were selected 4 treatment regimens, which we used for the appropriate groups. The main components of the regimen were: Daily eye treatment solution "Dioxidine" 0.5% and intramuscular injection of the drug "Ligfol" in a dose of 5 ml per head. The treatment was carried out for 14 days (Table 1). Blood samples for biochemical analysis were made at 1, 3, 7, 10 and 14 days of treatment. Blood was taken from jugular vein in vacutainer tubes with activator clot formation (SiO_2). Blood was taken in the morning, before feeding. The blood after collection was centrifuged to obtain plasma. Research plasma was produced in a clinical laboratory of the Department of surgery, obstetrics, pharmacology and therapy of the "Ulyanovsk state agricultural University". The researchers examined the biochemical indexes of blood plasma using the biochemical analyzer "Erba Mannheim XL-100». There was also determined a number of parameters: GPT, AST, LDH, Albumin and total protein. The data obtained were subjected to statistical processing in a computer program, "Statistika 12".

Table 1. Group treatment regimens

Group number	The total number of livestock in every group	Solution Dioksidini 0.5% + Ligfol 5 ml I/m + "additional drug"	Status
1	8	Tetracycline ointment 10 000 units	Background/control
2	8	Chloramphenicol 0,25%, 2-3 drops	Experience
3	8	Ciprofloxacin 0,3%, 2-3 drops	Experience
4	8	Gentamicin sulfate 3%, 2-3 drops	Experience

RESULTS AND DISCUSSIONS

Having examined the blood plasma, it was found that in calves the indicators of albumin in the course of treatment tended to decrease to the average 32.83 g/L. The average content of albumin in plasma at the time of the first study day (38.55 g/l) did not exceed norms for a given species (30-40 g/l).

The average value of the indicator "total protein" in the beginning of the study was 81.75 g/l, slightly higher than normal (60-80 g/l) for this species. After the treatment there was seen a tendency in decreasing of the indicator "total protein" value on day 14 was 70.18 g/l.

It was revealed a significant decrease of activity alanine aminotransferase (ALT), the average of which in beginning research made 100.91 u/l; normal 50 u/L. At the end of the treatment the average ALT activity fell to 31.15 u/l.

In the case of aspartate-aminotransferaza (AST), on day 14 of the treatment, it showed improved activity in comparison with the first day of the study. On the first day, the average AST in all groups was 36.26 u/l at norm for a given species 80 u/L. After the treatment, the increase in the average AST to 95.00 was observed (Figure 1).

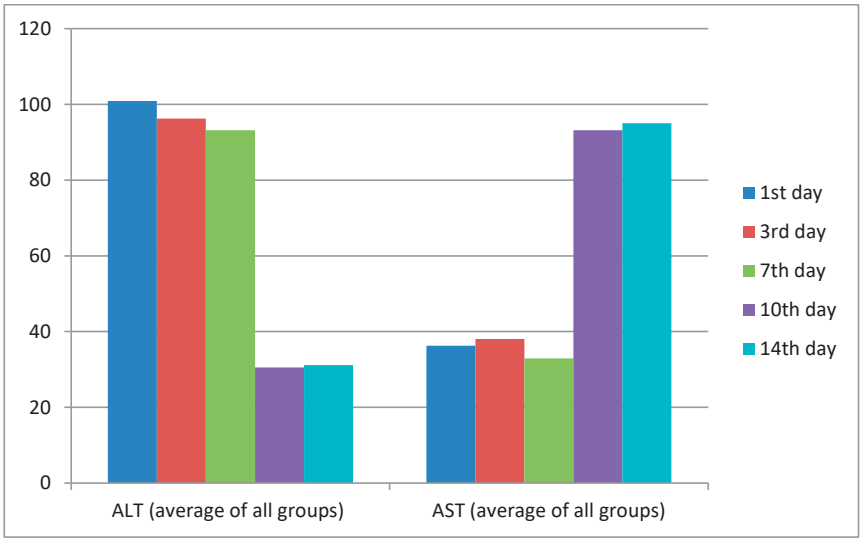

Figure 1. The ratio of the average ALT and AST activity during the treatment

The average activity of lactate dehydrogenase (LDH) at all stages of the study remained normal (1500 u/l). The average at the beginning of the study was 1451.46 u/l and at the end of the treatment he average indicator decreased to 1406.87 u/l (Table 2).

Table 2. Biochemical indicators of the blood plasma of calves during the treatment on 1,3,7,10 and 14 days

Group number	Day	Albumin g/l	Total protein g/l	ALT u/l	AST u/l	LDH u/l
Group 1	1	42.01±1.00	85.15±2.69	89,73±12,30	44.80±7.69	1,494.50±83.29
	3	32.05±1.70 ***	73.16±1.87 **	98.80±12.99	42.11±6.51	1,496.62 ±79.18
	7	32.76±1.52 ***	68.15±3.16 **	100.01±10.48	36.11±3.76	1,503.87±67.92
	10	31.12±0.97 ***	64.88±2.38 ***	33.82±1.84 ***	89.30±4.33 ***	1,473.75±57.09
	14	34.44±1.15 ***	65.08±2.77 ***	30.25±1.20 ***	97.47±9.40 ***	1,445.37±50.13
Group 2	1	35.70±1.30	76.48±2.52	113.82±18.51	35.00±3.90	1,437.50±64.63
	3	31.97±0.97 *	79.88±0.85	105.01±12.19	30.33±3.35	1,436.12±62.52
	7	32.46±1.18	74.21±2.34	97.15±9.37	33.13±1.20	1,432.62±58.09
	10	32.89±1.27	69.64±2.89	24.97±2.77 ***	87.38±4.01 ***	1,441.37±52.19
	14	32.40±0.97	65.64±3.98 *	32.70±2.06 ***	115.40±14.33 ***	1,428.75±69.32
Group 3	1	37.16±1.41	79.00±3.70	103.63±11.15	35.57±2.99	1,454.37±53.72
	3	35.89±0.84	81,.63±1.85	107.12±5.97	38.05±1.75	1,243.98±180.22
	7	33.49±1.46	70.19±2.62	91.35±8.59	31.61±2.62	1,385.12±46.10
	10	36.51±0.67	62.32±3.76 **	31.82±2.03 ***	102.11±9.76 ***	1,416.75±50.11
	14	30.30±0.63 ***	77.37±1.07	34.03±1.48 ***	92.16±4.06 ***	1,407.62±48.34
Group 4	1	39.97±0.60	86.36±1.88	96.46±7.72	46.30±14.63	1,419.50±58.38
	3	37.59±1.19	81.14±2.18 ***	74.22±9.65	41.81±6.75	1,397.50±60.30
	7	31.46±1.36 ***	67.71±2.80 ***	84.12±5.32	30.88±3.14	1,385.12±46.10
	10	29.74±2.11 ***	48.18±6.47 ***	31.51±1.21 ***	96.88±8.82 *	1,372.37±46.62
	14	32.83±1.14 ***	72.66±1.99 ***	29.86±1.10 ***	83.17±5.96 *	1,345.75±40.55

*Note: difference of values in comparison with the 1st day of the study: *** - p<0.001, * * p<0,01, *- p<0.05*

CONCLUSIONS

In calves aged 5-6 months with obvious signs of keratoconjunctivitis contained in the conditions of a livestock breeding complex "Megafarm - Oktyabrskay" in the course of biochemical studies of blood plasma revealed major changes in the activity of ALT and AST. The incidences of AST during treatment exceeded the reference values, characteristic of the species and age of animals. Indicators of Albumin and Total protein fluctuated slightly.

Most reliable abnormalities in comparison with the first day of treatment were detected in group nr.1 and group nr.4.

The indicators of activity of LDH throughout the treatment remained within the normal range.

A significant difference of biochemical indicators of the blood plasma, depending on the schema of treatment has not been established.

Eye diseases of young cattle are one of the reasons hindering the development of animal husbandry. Keratoconjunctivitis is a disease of the eye characterized by inflammation of the conjunctiva in the initial stage and subsequent transition process to the cornea.

Keratoconjunctivitis causes serious economic losses due to high occurrence frequency and prevalence, both in our country and abroad. Losses from keratoconjunctivitis consist of slowing down the growth and development of young animals, loss of productivity, loss of live weight. The study of biochemical parameters allows to identify the factors predisposing to the disease and identifying the causes of it under consideration.

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SYNERGISTIC EFFECT OF *THYMUS VULGARIS* AND VITAMIN E ON HEMATOLOGICAL PROFILE, SOME BLOOD BIOCHEMICAL INDICES AND LEUKOCYTE REACTION OF *OREOCHROMIS NILOTICUS* SPECIES

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Abstract

The aim of this research was to evaluate the influence of thyme and a mixture of thyme whit vitamin E on hematological profile, some biochemical indices and leukocyte reaction of Oreochromis niloticus species, reared in a semi-intensive aquaculture system. The experiment was made in replicate, during 31 days, and the experimental variants were: V1 – 1% thyme (Thymus vulgaris)/kg feed and V2 – 1% thyme + 500 mg vitamin E/kg feed. At the end of the experiment, in V2 variant, a significant increase ($p<0.05$) were registered in case of red blood cells count (RBCc), hemoglobin concentration (Hb), mean corpuscular hemoglobin concentration (MCHC), lysozyme activity, absolute number of leukocyte (lymphocytes, monocytes) and platelets. Also, in V2 variant, was obtained a significant decrease ($p<0.05$) in case of blood glucose concentration. In conclusion, we showed that the additivited diet of Nile tilapia, particularly, with a mixture of thyme and vitamin E (V2) had a synergistic influence on fish welfare status by improving the hematological profile, immune parameters (lysozyme activity, leukocyte reaction) and decrease of blood glucose concentration.

Key words*: hematological and biochemical indices, leukocyte reaction, lysozyme activity, Nile tilapia, Thymus vulgaris.*

INTRODUCTION

In recent years researchers have approached increasingly several studies in fish hematology because it plays an important role in assessing the health status. The study of the physiological and hematological characteristics of cultured fish species is an important tool in the development of aquaculture system, particularly in regard to the use in detection of healthy from diseased or stressed animal (Rainza-Paiva et al., 2000; O'Neal and Weirich, 2001).

However, the diet composition, metabolic adaptation and variation in fish activity are the main factors responsible for the change in hematological parameters of fish (Rehulka, 2003). Some authors reported changes in blood parameters indices of fish as result of feed (Kelly, 1979; Kilgour, 1987).

The fish welfare evaluation can be done by determining the hematological profile, some blood biochemical indices and leukocyte reaction. The main haematological indices are: erythrocytes number (RBCc), hemoglobin (Hb), hematocrit (PVC) and the erythrocyte constants: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). The analysis of some blood biochemical indices (total, protein, glucose, cortisol, lysozyme) and absolute and relative number of leukocytes aglucose from blood plasma is an important tool for determining the fish stress status and the immunity evaluation.

Ologhobo (1992) reported that the most common blood variables consistently influenced by diet are the RBCc, PVC, total protein and glucose levels.

Stress represents a major factor in the health of farmed fish, for this reason prevention is the ideal and it can be achieved through good management protocols, but also by development an optimal system design (Conte, 2004).
Tilapia (including all species) is the second most important group of farmed fish after carps, and the most widely grown of any farmed fish (FAO), because its ease of culture. *Oreochromis niloticus* are principally herbivorous, although occasionally they can be omnivorous. This is an efficient converter of waste foodstuff and appears to thrive well on artificial supplemental feed (Omoregie et al., 2009).
The mode of action of phytobiotics and their derivatives are attributed to the presence of many active components such as alkaloids, steroids, phenolics, tannins, terpenoids, saponins, glycosides, and flavonoids (Harikrishnan et al., 2011; Sivaram et al., 2004).
Herbs or spices have been reported to promote various functions among which hematological and biochemical status (Yılmaz and Ergun, 2012).
Among the various aromatic plants, thyme (*Thymus vulgaris* L.) is an herbaceous perennial plant belonging to the *Lamiaceae* family, and is a characteristic herb of the Tunisian and Mediterranean environment. It is recognized for its many beneficial properties: antiseptic, carminative, antimicrobial, and antioxidative properties (Baranauskiene et al., 2003). Also, thyme, has a strong antimicrobial and antioxidant activity due to its very high contents of thymol, p-cymene, carvacrol, eugenol, and 4-allylphenol (Gultepe et al., 2014). Thymol, a major component of thyme essential oils, has been widely studied for its antimicrobial properties (Dorman & Deans, 2000). Carvacrol, an isomer of thymol, is found in essential oils isolated from thyme.
Vitamins are micro-nutrients required for normal growth, reproduction, metabolism, and immune function (Safarpour et al., 2011).
Vitamin E is a fat-soluble vitamin that has two forms, tocopherols and tocotrienols. Aquatic animals cannot synthesize vitamin E, so it must be provided in their diet. Vitamin E is widely involved in anti-oxidant, cell signalling, reproductive development, immune regulation, and anti-stress processes (Galti et al., 2016; Hamre et al., 2011). It is a potent antioxidant that protects against oxidative damage in various fish tissues (Adham et al., 2000), enhances resistance of red blood cell (RBC) membranes (Kiron et al., 2004), and protects leukocyte functions (Sahoo et al., 2002).
It is an indispensable nutrient required to maintain normalphysiological functions infish (Hamre, 2011). Supplementation of dietary vitamin E has been shown to increase serum lysozyme activities in cobia (*Rachycentron canadum*) (Zhou et al., 2013) and serum complement activity in rainbow trout (*Oncorhynchus mykiss*) (Pearce et al., 2003).
Vitamin E also can regulate stress responses produced by micro cysts (Prieto et al., 2008), heavy metal pollution (Salehi et al., 2015), crowd stress (Liu et al., 2014), oxidized fish oil (Mourente et al., 2002), and high-fat diets (Lim et al., 2009). From this reason in this experiment we chose as phytobiotic the thyme and vitamin E for, well known, antioxidant effect.
The aim of this research was to investigate the influence of thyme and thyme with vitamin E combination on hematological profile and some biochemical indices of blood at *Oreochromis niloticus* species reared in a recirculating aquaculture system.

MATERIALS AND METHODS

Experimental design
This experiment was conducted in the research laboratory of the Department of Food Science, Food Engineering, Biotechnology and Aquaculture Aquaculture, from "Dunarea de Jos" University of Galati. The experimental period was 31 days. The recirculating aquaculture system includes four rearing units, with a volume of 0.5 m^3 each. For each growth units corresponded two external filters on the type Tetratec Ex 400 for water recirculation, while the water aeration was performed using a compressor and aeration stones.
In this research were used a total number of 100 Nile tilapia, with an initial average weight of 328.36 ± 37.68 g/fish, that were randomly distributed in 4 rearing units. Fish were fed with SOPROFISH pelleted feed, with 38% crude protein and 7% crude fat. The feed biochemical composition was related by Mogodan (Antache) et al. (2018). Fish were fed three times per day with a daily ration of 1%

from fish body weight. At the end of the experiment the individual average weight was 396.96 g/fish in V1 and 401.76 g/fish in V2.

The experimental variants were performed in duplicate and were organized as follows: V1 – 1% thyme (*Thymus vulgaris*)/kg feed, V2 – 1% thyme + 500 mg vitamin E/kg feed.

Blood sampling and analysis

At the beginning and at the end of the experimental period was sampling 3.5 ml of blood, from 10 fish of each growth unit, by caudal venous puncture using heparin as anticoagulant. A part of the blood was used for hematological study also, for determining glucose and total protein, and the other part was used for analysis of oxidative stress that is the subject of another study.

Blood analysis was performed by method used in fish hematology described by Blaxhall (1973). This analysis consisted in determination of erythrocytes number (RBCc x 106/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 using Vulpian as a diluting solution. The hematocrit was performed by duplicate using capillary tubes centrifuged for 4 minutes at 13000 rpm in 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) the erythrocyte constants were calculated: mean corpuscular volume (MCV, μm^3), mean corpuscular hemoglobin (MCH, pg), and mean corpuscular hemoglobin concentration (MCHC, g/dL).

In this experiment was analyzed four biochemical parameters of blood (glucose -mg/dL, cortisol - ng/mL, total protein - g/dL and lysozyme activity - 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 635nm 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 66mM 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 relative proportion of each type of white blood cells was obtained by microscopic examination of 200 leukocytes on blood smears (two per eacth fish), using Zeizz Axio Imager microscop and immersion objective (10 oc. X 100 ob.). The blood smears were 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.

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. Some researchers reported that 2-phenoxyethanol anesthesia had no effect on haematological profile (Velisek et al., 2007).

Statistical analysis

The hematological parameters of the experimental groups were statistically analyzed using descriptive statistics, t - Student test (for final variants) and ANOVA single factor test (for initially moment and final variants). Programs used were Microsoft Excell 2010 and SPSS Statistics 17.0. The results were presented as mean±standard deviation.

RESULTS AND DISCUSSIONS

Svobodova et al. (1991) reported that fish hematology would be useful in the assessment of suitability of diets and feed mixtures, evaluation of fish conditions, determination of toxic effect of substances, as well as diagnosis of disease.

The red blood cell indices (PVC, Hb and RBCc) can be an indicator of oxidative status, because erythrocytes are one of the major production sites of free radical and some of them can trigger peroxidation of sutured fatty acids in their membrane phospholipids, therefore altering their quality (integrity, size) and quantity (Pearce et al., 2003; Kiron et al., 2004; Ispir et al., 2011).

The values of hematological parameters obtained at the beginning (V0) and at the end of the experiment (V1 – 1% thyme/kg feed, respectively V2 – 1% thyme + 500 mg vitamin E/kg feed), are presented in the Figures 1 - 6.

The results obtained showed significant differences ($p<0.05$) in terms of the number of erythrocytes, hemoglobin concentration, mean corpuscular hemoglobin concentration and blood plasma glucose level both between initial and final moment, as well as between final experimental variants (V1 and V2). RBC count was significantly lower ($p<0.05$) at the initial moment compared to the values obtained at the end of the experiment. Although there were significant differences ($p<0.05$) on the number of erythrocytes, the values obtained are situated in the reference interval reported by Hrubec et al. (2000) and Bittencourt et al. (2003).

At the end of the experiment the best value of hemoglobin concentration was registered in V1 variant, in which was administered thyme. Because the high value of hemoglobin content denotes the occurrence of a stressor factor we can say that the additivation of fish feed with thyme has led to stress reduction. Hemoglobins are particularly important in fish adaptation as they constitute an interface between the organism and the environment (Landini et al., 2002).

Hematocrit values increased significantly ($p<0.05$) in the experimental variants compared to the values obtained at the beginning of the experiment, but these values were situated in the reference interval indicated by Bittencourt et al. (2003).

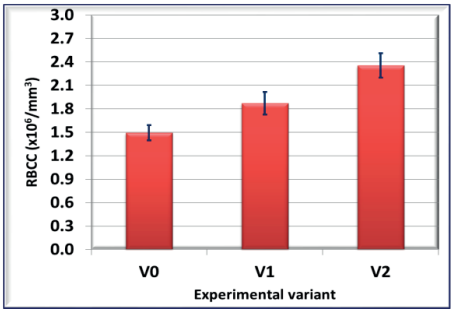

Figure 1. Changes in erythrocytes number (RBCc) of different experimental groups

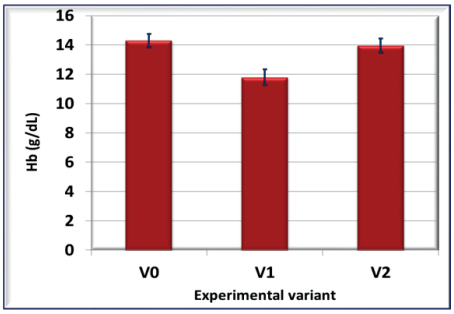

Figure 2. Changes in hemoglobine concentration (Hb) of different experimental groups

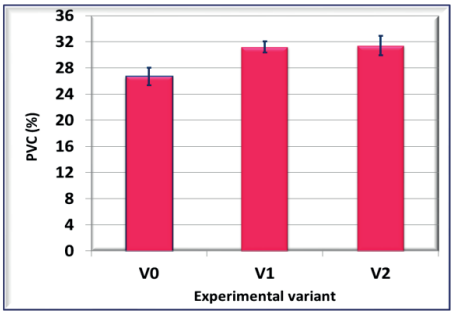

Figure 3. Changes in hematocrit (PVC) of different experimental variants during the experiment

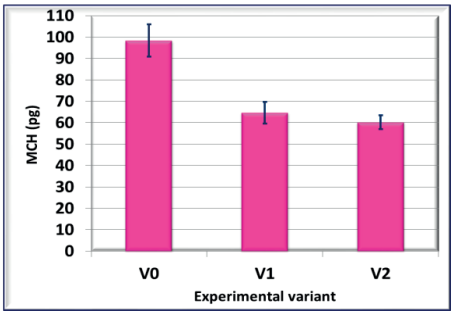

Figure 4. Changes in mean corpuscular hemoglobin (MCH) of different experimental variants during the experiment

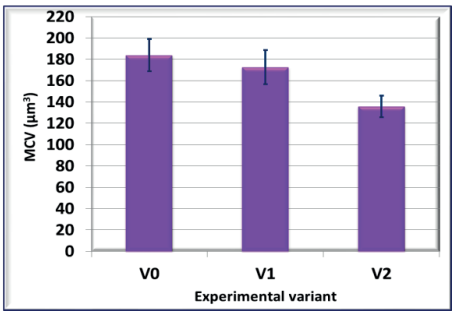

Figure 5. Changes in mean corpuscular volume (MCV) of different experimental variants during the experiment

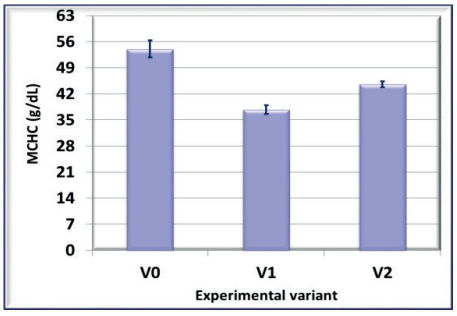

Figure 6. Changes in mean corpuscular hemoglobine concentration (MCHC) of different experimental variants during the experiment

Bai and Lee (1998) and Ispir et al. (2011) showed that the PVC of fish fed with basal diet was lower than that of fish fed with high level of vitamin E at *Sebastes schlegeli* species, respectively at *Oreochromis niloticus* species. This may explain the lower value obtained before starting the experiment. Because the increase of hematocrit was statistically significant between the initial and final moments but statistically not significant between the final variants, this shows that these hematological parameter was not relevant as an indicator of the stress response of the *Nile tilapia* induced by the introduction of thyme and thyme with vitamin E in feed.

Analyzing the results of the erythrocyte constants, it can be observed that the administration of thyme and thyme with vitamin E in feed has induced significant decrease in MCH ($p<0.05$) and MCHC ($p<0.05$) level that expresses a decrease in hemoglobin in red blood cells.

Regarding to the assessment of blood glucose levels, it is known that it is a good and fast indicator in assessing the level and intensity of stress on fish physiology. At the end of the experiment our results showed a significant decrease ($p<0.05$) in glucose level in V2 variant in which we administered thyme in combination with vitamin E in feed. We can say that the addition of vitamin E in feed, along with thyme, led to lower blood glucose level and consequently to stress reduction. However, it should be mentioned that the values obtained by us for blood glucose concentration are found in the reference interval reported by Bittencourt et al. (2003).

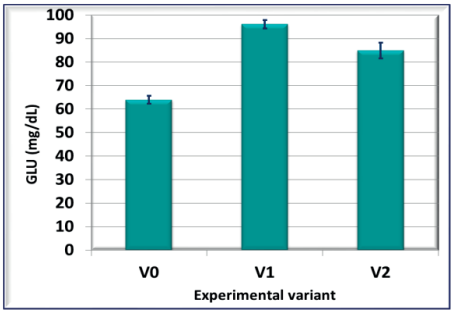

Figure 7. Changes in glucose concentration (GLU) of different experimental variants during the experiment

In case of cortisol concentration, at the end of the experiment no significant differences were obtained between the experimental variants ($p>0.05$) although, in case of glucose concentration were registered significant differences ($p<0.05$). The average value of cortisol concentration registered in variant V2 a slight increase with 9.11% compared to the value obtained in V1 variant.

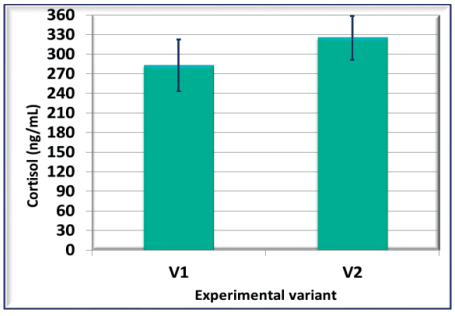

Figure 8. Changes in cortisol concentration of different experimental variants during the experiment

An increase of cortisol concentration, in the blood, represent the primary response to a stressor factor which affects the body and the

secondary response is given by blood glucose concentration (Begg and Pankhurst, 2004) we can say that the two diets administrated to *Nile tilapia* did not influence the occurrence of stress. So, either diet can improve the physiological state of the biological material.
From this reason, for a better assessment of the biomass culture in terms of occurrence of stress should not only determine the concentration of glucose in the blood because if an experiment glucose concentration may indicate certain differences between experimental variants.
Regarding the total protein from blood serum, the results showed insignificant differences both between the initial and final moment of the experiment ($p>0.05$) as well as between the final experimental variants, V1 and V2 ($p>0.05$). The values obtained are included in the reference interval indicated by Bittencourt et al. (2003). It should be noted that the measurement of total protein, from serum or plasma, is considered a diagnostic value in fish because it relates to general fish nutritional status, as well as to the integrity of the vascular system and liver functions (Abdel-Tawwab et al., 2008).

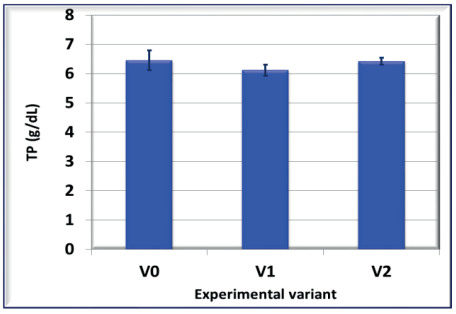

Figure 9. Changes in total protein concentration (TP) of different experimental variants during the experiment

Determination of lysozyme activity was performed in order to observe the resistance capacity of the fish biomass in case of disease. Moreover, lysozyme is a bacteriolytic enzyme that plays an important role in the defence of the body, because it has antibacterial and immuno-modulating properties (Takahashi and Itoh, 2011).
At the end of the experiment, the results of lysozyme activity showed a significant differences ($p<0.05$) between experimental variant. In variant V2, in which fed was additivated with thyme and vitamin E, was registered an intensification of the lysozyme activity.

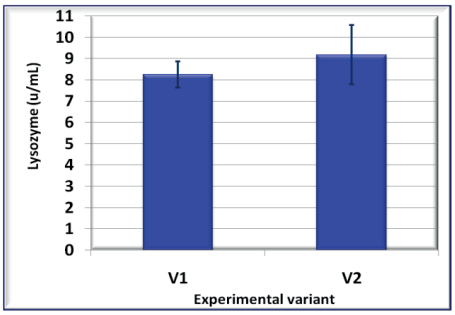

Figure 10. Changes in lysozyme activity (Lys) of different experimental variants during the experiment

For a better assessment of the health status of the biological material cultured under the conditions of the administration of different diets, the reactions of the leukocytar system was also analyzed. This analysis involved the determination of the relative and absolute number of leucocytes that can give us clear information about the possible changes that have occurred at the physiological level and the state of health in which the fish are.
After analysing the blood smears the leukogram of the studied specimens revealed the presence of agranulocytes, including small lymphocytes (Lm), large lymphocytes (LM), and the presence of neutrophilic granulocytes (N). Basophilic and eusinophilic granulocytes were absent. In figures 11, 12 and 13 are presented the leukograms (%) obtained from the blood smear analysis at the beginning and at the end of the experiment.
At the end of the experiment, the analysis of the relative number of leukocytes highlighted the aspects detailed below.
Lymphocytes (%). Regarding the small lymphocytes (Lm) between the experimental variants, there were no significant differences ($p>0.05$), but the highest value was recorded in variant V2 (97.35%). Although the initial value of the relative number of small lymphocytes was higher than the values obtained at the end of the experiment, no significant differences were obtained ($p>0.05$) (Figures 11, 12, 13). The relative number of large lymphocytes (LM) registered insignificant differences between the final experimental variants

(p>0.05) and neither between the initial mean value and the mean values obtained at the end of the experiment (p>0.05). However, compared to the initial value there was an increase of 82.61% in V1 and 86.96% in V2.

Monocytes (%). At the end of the experiment was found an increase of monocytes (M, %) in the experimental variants, compared to the average value obtained at the beginning of the experiment (0.33%), but insignificant (p>0.05) from statistical point of view Figures 11, 12, 13. In variant V2, in which thyme was administered in combination with vitamin E, was observed a decrease with 16.8% of the relative number of monocytes (%), compared to variant V1, but also insignificant (p>0.05).

Neutrophilic granulocytes (%). If at the level of the relative number of lymphocytes (%) and monocytes (%) there were not registered significant differences (p>0.05), regarding the relative number of neutrophils (%) a significant increase was obtained in V1 variant (1.99%), compared to V2 variant (p<0.05) and initial (V0) value (p<0.05).

The morphology of erythrocytes, leukocytes and thrombocytes, found on blood smears, can be seen in Figure 14.

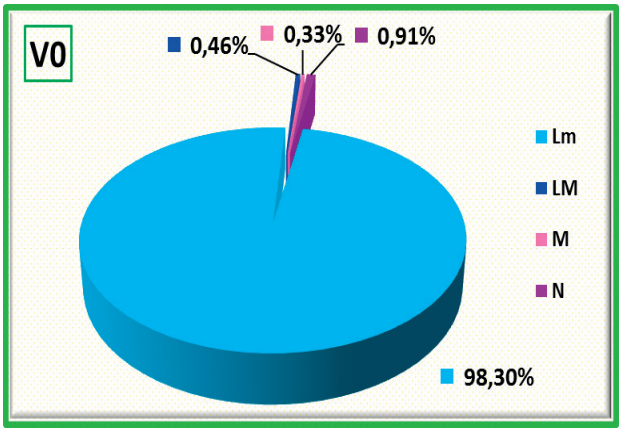

Figure 11. Nil tilapia leukogram in V0 variant at the beginning of the experiment

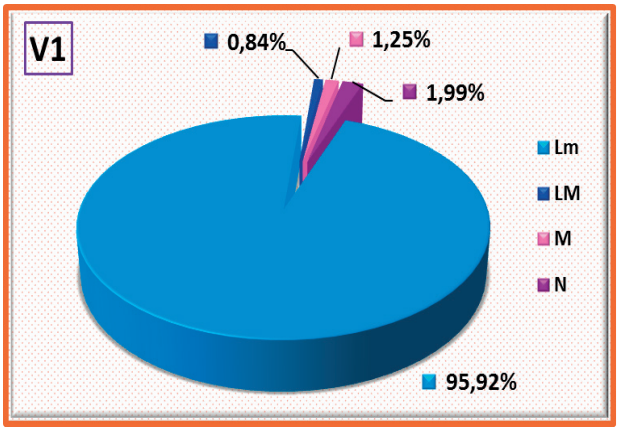

Figure 12. Nil tilapia leukogram in V1 variant at the end of the experiment

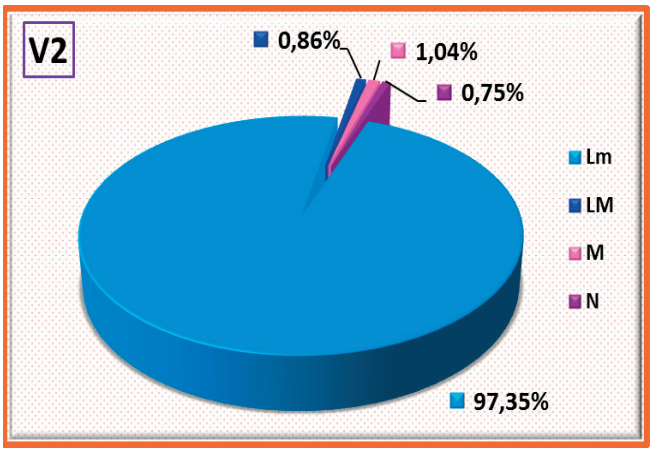

Figure 13. Nil tilapia leukogram in V2 variant at the end of the experiment

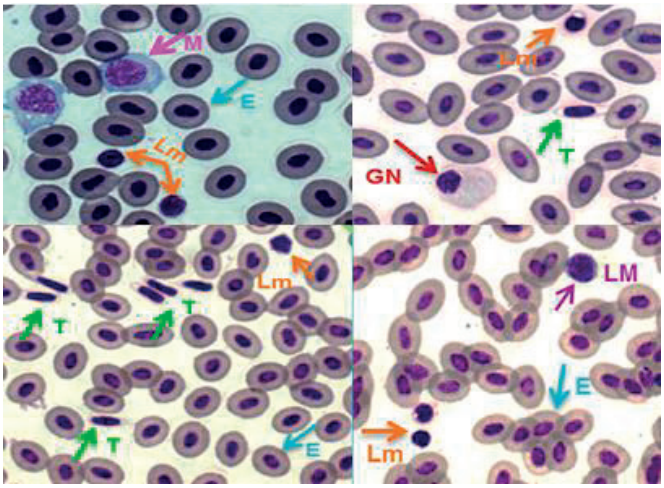

Figure 14. Morphology of cellular elements at Nile tilapia found on blood smears (photo original)
Note: E - erythrocytes; Lm - small lymphocyte; LM - large lymphocyte; M - monocytes; GN - neutrophilic granulocyte; T - thrombocyte

In order to obtain a clearer picture of the changes that take place in the leukocyte array, besides determining the relative number (%), the absolute number of white blood cells and the absolute number of platelets (number of cells/mm^3 blood) were calculated. The absolute modifications of the various types of cells that form the leukocytic complex are presented in Table 1.

Table 1. Variation in the absolute number of leukocytes and platelets at *Nile tilapia* during the experiment

Var. exp.	Absolut number (x1000 cel./mm^3)					
	Leukocytes	Lymphocytes		Monocytes	Neutrophilic granulocytes	Platelets
		small	large			
V0	56.83±62.68	55.86±6.54	0.26±0,06	0.18±0.11	0.53±0.11	4.10±1.41
V1	69.37±14.73	66.68±14.78	0.67±0,40	0.81±0.09	1.33±0.44	32.89±18.15
V2	114.91±39.45	112.18±39.07	1.12±0.72	1.07±0.52	0.78±0.27	45.47±8.57

Note: Exp. Var. – experimental variant. Results are expressed as mean±standard deviation.

Absolute number of leukocytes. At the end of the experiment, there was a significant increase ($p<0.05$) of the leukocytes number in the V2 variant compared to the initial average value, respectively with 102.20%. There were no significant differences between variants V1 and V2 ($p>0.05$), although the value obtained in V1 was with 39.63% lower compared to the mean value obtained in V2 (Table 1). Our values are registered within the reference range 21559-154690 leukocytes/mm^3 (Hrubec et al., 2000).

Absolute number of small lymphocytes. Because the small lymphocytes represent the largest proportion of the total leukocytes, also in their case were registered significant differences between the number obtained in variant V2 and the number of small lymphocytes obtained at the beginning of the experiment ($p<0.05$). It increased with 100.82% in V2, respectively with 19.37% in V1, compared to the initial absolute number of small lymphocytes. During the experiment the average values of the number of small lymphocytes were between 55.86 x 10^3 cel/mm^3 and 112.18 x 10^3 cell/mm^3. These are in the range presented in the literature for tilapia (6.78-136.39 x 10^3 cel/mm^3) (Hrubec et al., 2000).

The ***absolute number of large lymphocytes*** shows the same growth tendency at the end of the experiment as the number of small leukocytes and lymphocytes, but they were not registered significant differences ($p>0.05$). The number of large lymphocytes increased with 157.69% in V1, respectively with 330.77% in V2, compared to the initial value (0.26 x10^3 cel/mm^3) (Table 1). The number of large lymphocytes recorded was lower than that recorded by Hrubec et al. (2000) (2.85-30.83 x10^3 cel/mm^3).

Regarding to the ***absolute number of monocytes***, were registered significant differences between the values obtained at the end of the experiment compared to the initial (V0) value ($p<0.05$). The highest number of monocytes was recorded in the variant in which thyme was administered in combination with vitamin E (V2) - 1.07 x 10^3 cel/mm^3, and the lowest in the variant in which thyme was administered (V1) - 0.81 x 10^3 cel/mm^3 (Table 1). At the end of the experiment were not recorded significant differences between the experimental variants ($p>0.05$). If the absolute number of leukocytes obtained at the end of the experiment falls within the reference range; 0.40-4.29x10^3 cel/mm^3 (Hrubec et al., 2000); the number of monocytes obtained at the beginning of the experiment does not fall within the range, being smaller - 0.18x10^3 cel/mm^3.

The ***absolute number of neutrophilic granulocytes*** registered a significant increase in case of V1 variant was administered, compared to the variant V2 ($p<0.05$) and compared to the absolute number determined at the beginning of the experiment ($p<0.05$). The number of neutrophils increased from the initial moment with 150.94% in the V1 variant, respectively with 47.17% in the V2 variant (Table 1). The mean values of the absolute number of neutrophil granulocytes ranged between 0.53±0.11 x 10^3cel/mm^3 and 1.33±0.44 x 10^3 cel/mm^3 (Table 1). The results obtained are found in the optimum range for tilapia; 0.56-9.87 x 10^3 cel/mm^3 (Hrubec et al., 2000).

Neutrophilic granulocytes represent the first white blood cells who arrive in the place where the tissue is lysed due to a particular pathogen (in case of infections) (Toazza et al., 2013). These have a very important role in ensuring native immunity because they have the ability to recognize, phagocyte and destroy the pathogen without stimulating other cells that play a role in the defense of the body (Tavares-Dias and Moraes, 2007). Although, the number of neutrophils increased significantly at the end of the experiment, it can not be about the presence of an infection because the obtained results fall within the optimal limits for tilapia.

The ***absolute number of platelets*** registered a significant difference ($p<0.05$) between the average value obtained at the beginning of the experiment (4.10±1.41 x 10^3 cel/mm^3) and the average values obtained at the end of the experiment in V1 variant (32.89±18.15 x 10^3 cel/mm^3) and V2 variant (45.4±8.57 x 10^3 cel/mm^3). In V2 variant, the absolute number of platelets increased with 38.25% compared to V1 variant, but this was insignificant ($p>0.05$). In tilapia, the optimal range for absolute platelet count ranges from 25.06 x 10^3 cel/mm^3 to 85.24 x 10^3 cel/mm^3 (Hrubec et al., 2000). Thus, the results showed that the number of platelets from the beginning of the experiment

is below than lower limit described by Hrubec et al. (2000), but according to Tavares-Dias and Oliveira (2009), who claim that in fish the number of platelets is between 2.00-78.90 x10^3 cel/mm^3, the results obtained being less than inferior limit.

The low number of platelets from the beginning of the experiment showed that the fish have a deficient immune system, being more sensitive to the attack of different pathogens (Tavares-Dias and Oliveira, 2009). This is also certified by the small number of leukocytes, more specifically lymphocytes, recorded at the beginning of the experiment. Ndong and Fall (2011) showed that the leukocytes have grown in the variant in which they administered garlic, to a tilapia hybrid, in 0.5% concentration, fish immunity was also improved.

In fish, increasing the leukocyte numbers in circulating blood may be associated with increasing of leukocyte production in the hematopoietic tissue of the kidney, respectively in the spleen (Manolescu, 1999). It has been shown that the main function of lymphocytes is to produce antibodies and other chemicals that serve to protect the body against infection (Ramesh & Saravanan, 2008). Thus, it is confirmed that the administration of thyme in combination with vitamin E has led to a significant improvement of immunity due to increase in number of lymphocytes. The administration of thyme (V1) has contributed to the improvement of the immune system in a smaller proportion.

CONCLUSIONS

In the past few years for ensuring the fish welfare status will be introduced phytobiotics in fish feed against antibiotics and other medicinal products administration. The main disadvantages of using different types of medicines are represented by high costs and environmental pollution.

This experiment reveals that the thyme in combination with vitamin E shows a synergistic effect leading to a decrease of blood glucose level, but, due to the decrease of RBCc, Hb and MCHC in V1, we can say that the introduction of thyme in Nile tilapia diet not constitute a stressor factor from the physiological point of view.

Also, the increase in leukocyte numbers, especially in lymphocytes and monocytes, respectively in the number of platelets, showed an improvement of the fish immunity in the variant in which fisf fed was additivated with thyme and vitamin E (V2).

At the same time, the blood biochemical analysis reflects a better condition in the variant in which thyme was administered in combination with vitamin E, due to the results obtained in case of cortisol, total proteins and lysozyme activity.

In conclusion, in our case, the additivited diet of Nile tilapia, particularly, with a mixture of thyme and vitamin E (V2) had a synergistic influence on the fish welfare status by improving the hematological profile and immune parameters.

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SPERMOGRAM IN BREEDING RAMS - BREED OF TSIGAIA WOOL-MEAT-MILK

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Abstract

The assessment of the quality of the semen taken from the breeding rams of the breed of Tsigaia Wool-meat-milk has been the subject of our research. As a result of the research it was found that the volume of sperm was 1.38 ± 0.40 ml, the sperm mobility of 81.17%, the sperm concentration of 1.47 ± 0.10 mlrd/ml and the number of sperm moving in a straight line of 53.5%. The spermogram indices of the semen obtained from the breed of Tsigaia wool-meat-milk correspond to the standard of the Tsigaia breed and allow its use in artificial sowing of sheep. In the subsequent follow-up, research was conducted on the kinetics of freshly collected raw sperm.

Key words*: ram, semen, insemination, spermogram.*

INTRODUCTION

In breeding sheep male reproducers play a particularly important role. From this point of view the breeders require maintenance and qualitative nutrition in order to ensure a high spermatogenesis, mainly during the breeding period. During this period the breeding ram must produce a larger amount of sperm in order to be able to mount more sheep. At the same time, the sperm produced must also be qualitative, that is, it corresponds to qualitative indices that would ensure a resultant amount. Several methods of ejaculate sampling (in the artificial vagina, electro-ejaculation, etc.) are used in the process of harvesting the ram's sperm, and each method influences in its own way the sperm indices analyzed later (Darie, 2016).

The determination of sperm quality includes the analysis of a series of indices and parameters, which characterize the aptitudes of the sperm (Nauk, 1991). A high quality of sperm will lead to a longer preservation of sperm viability and eventually increase their fertilizing capacity (Ostasco, 1978). In the last years, with the development of the informational software and the improvement of the research techniques and methods, the methods of analysis of the spermatic indices have been improved (Zahan, 2017). For sperm harvesting for refrigeration or cryopreservation purposes, previously tested, healthy and vigorous race rams are selected. In the main mountain season it is recommended to harvest sperm daily, 1-2 ejaculates from each ram, and in the secondary season once every 2-3 days (Darie et al., 1992). Before harvesting the rams are washed and sanitized to avoid sperm contamination in the process of sampling with various bacteria and impurities.

MATERIALS AND METHODS

As research material, the rams - breed of the Tsigaia wool-meat-milk and fresh sperm from them served. The breeding sheep were kept at the sheep farm of the Technological - Experimental Station "Maximovca", of the Scientific-Practical Institute of Biotechnologies in Zootechnics and Veterinary Medicine R. Moldova. Maintenance and nutrition conditions corresponded to the zoo-hygienic norms according to the requirements of the breeding sheep for breeding sheep. The semen was taken using the artificial vagina. Immediately after

sampling, the sperm was subjected to a macroscopic analysis after which the volume of sperm, consistency, color, odor and some visible unknown impurities were analyzed. Subsequently or performed and microscopic analysis - mobility, concentration and rate of progression of spermatozoa, assessed with the help of a specific computerized program that allowed a detailed analysis of the spermogram to be performed on all the ejaculates taken from the rams included in the research. Thus, in our researches the appreciation of the quantitative and qualitative parameters of the sperm taken was performed with the help of the computer software of the "CEROS" Program. The nominated program performs the analysis of sperm samples in a very short time and allows the determination of an entire block of quantitative and qualitative indices. As a result, we expressly obtain the spermogram of the analyzed sperm of each ram and even of each ejaculate with the determination of the average level of the indices.

The analysis for the assessment of the sperm concentration in the ejaculate was performed under the microscope using the Goriaev chamber after dilution of the sperm by 1:1000.

REZULTS AND DISCUSSIONS

Appreciation of the qualitative indices of the semen of the sheep breed Tsigaia of wool-meat-milk, with the elaboration of the individual spermograms was performed both under the conditions of the main breeding season and in the secondary season. This condition was respected because of the need for appreciation of the behavior of the breeding rams in both seasons, the existence and manifestations of the libido and as a result finding changes in sperm quality depending on the season. During the period of the researches to monitor the spermatic indices from the breeding rams, 46 ejaculated were taken per season. The organoleptic analysis of the sperm found that the color of the semen was normal, white to yellow in all the ejaculates analyzed, the specific odor of proteins, of fresh egg which is characteristic of the smell of natural sperm, freshly taken. Following the data from the figures shown below, we find that the values of the characteristics of the raw sperm fall within the specific limits of the Tsigaia breed.

The volume of ejaculate raw ram sperm is shown in Figure 1.

The average volume of ejaculate (Figure 1) is 1.38 ml in the main breeding season and 1.23 ml in the secondary season, with individual limits of variation from 0.9 ml to 1.52 ml. The volume of the ejaculate with minimum values it was mainly recorded in the secondary breeding season of sheep. In appreciating the quality of the ram's sperm, a large seed has the sperm concentration in the ejaculate.

Figure 2 shows the data on sperm concentration in 1 ml of sperm.

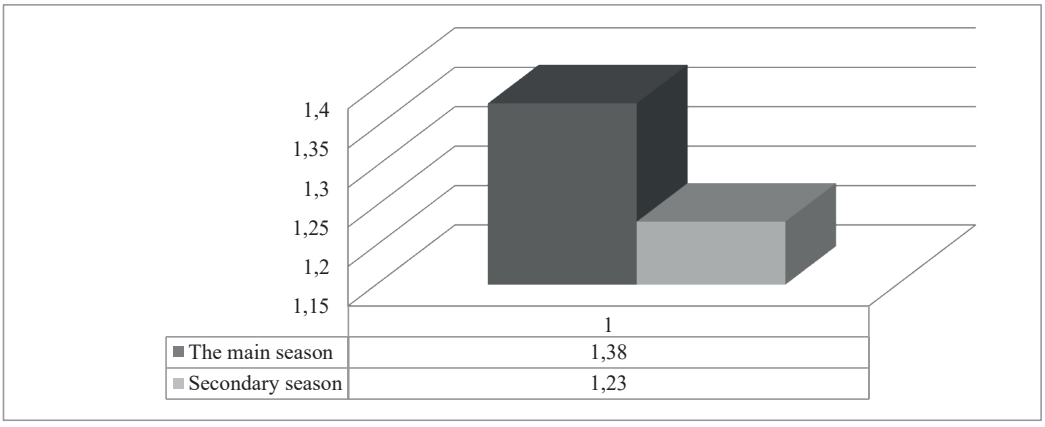

Figure 1. The volume of the ejaculate

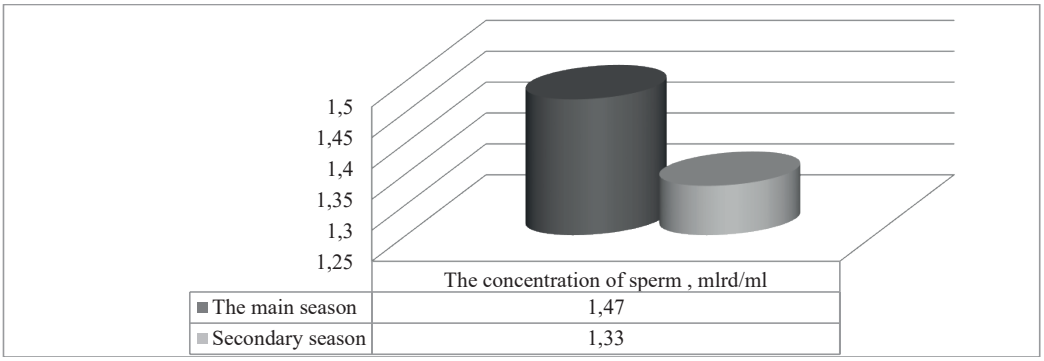

Figure 2. Sperm concentration in the ejaculate

The concentration of sperm (Figure 2) in the main season is 1.47 billion/ml and 1.33 billion/ml in the secondary season, with oscillations between 0.683 billion/ml and 2.313 billion/ml. The wide limits of the sperm concentration oscillation are expressed by the fact that ejaculates were taken from breeding rams of different ages.

The quantity (amount) of sperm in the ram's sperm ejaculates is shown in Figure 3.

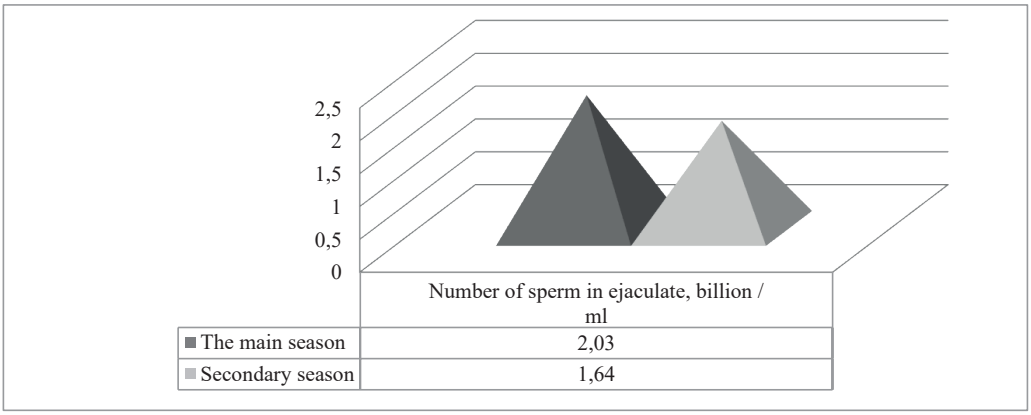

Figure 3. The number of sperm in the ram's ejaculate

As we can see, in the ejaculates taken from the breeding rams in the main mountain season the number of sperm constitutes 2.03 billion/ml, and in the secondary season 1.64 billion/ml, the difference in favor of the main season being quite essential. A large role in assessing the quality of the sperm has the mobility of the sperm, shown in Figure 4. The viability of the sperm depends largely on the values of this index.

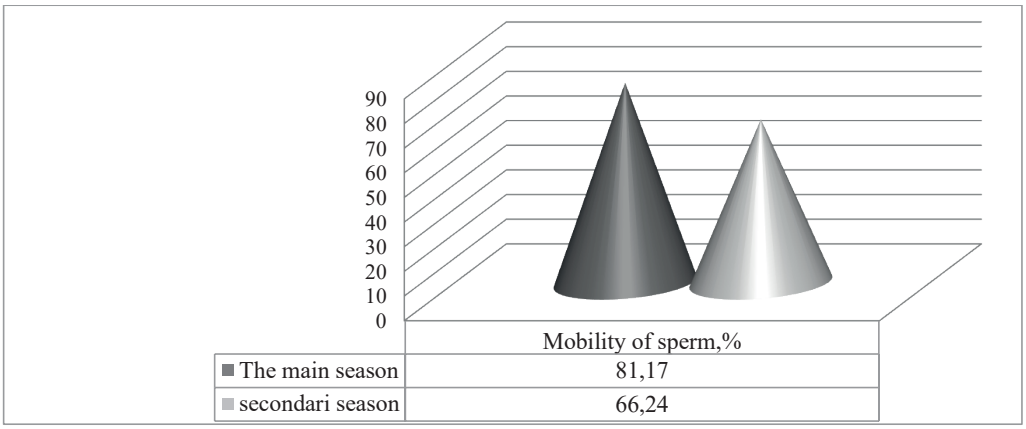

Figure 4. Ram sperm mobility, %

The mobility of sperm is quite high, even sometimes very high, with average rates of 81.17% in the main breeding season, and the limits of variation are relatively low. The minimum grade for sperm mobility of 0.8 allows the use of ejaculates for further processing, even for those that are qualitatively lower ranked. In this regard it is important to note that the minimum requirement for sperm mobility was 0.7 points. As we can see from Figure 4, the mobility of sperm in the extra-season is much lower than in the main breeding season and constitutes 66.24% compared to 81.17% in the main season. A major ability of sperm is their rate of advancement. The evaluation of the rate of sperm advancement was performed with the help of computer software "CEROS" by finding 3 main indices; - average forward speed - VAP, - straight forward speed - VSL, and - curvilinear speed - VCL. Sperm movement rate indices (VAP, VSL, VCL), both in the breeding season and in the extra-season, are shown in Tables 5, 6, 7. Figure 5 shows the average sperm advancement rate.

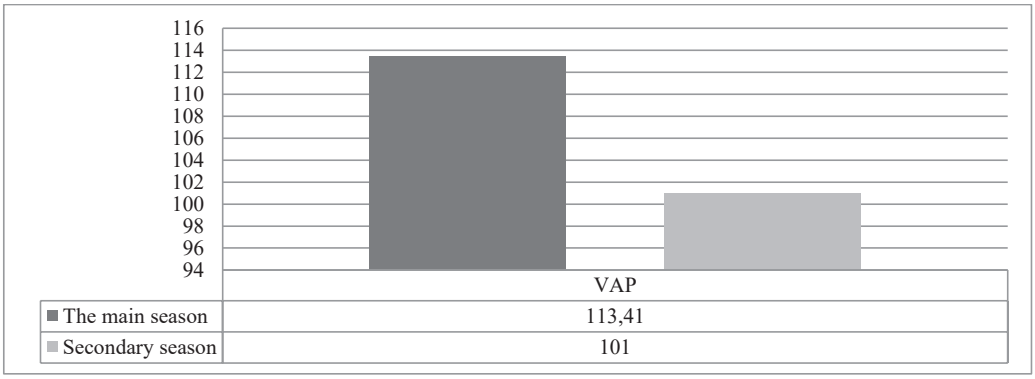

Figure 5. The advance speed (VAP) of ram sperm, µm/s

The data in Figure 5 show that the average advancement rate (VAP) of rams' sperm in the main breeding season is 113.41 µm/s and in the secondary season it is 101.0 µm/s. Figure 6 shows the advancement speed (VSL) of ram sperm.

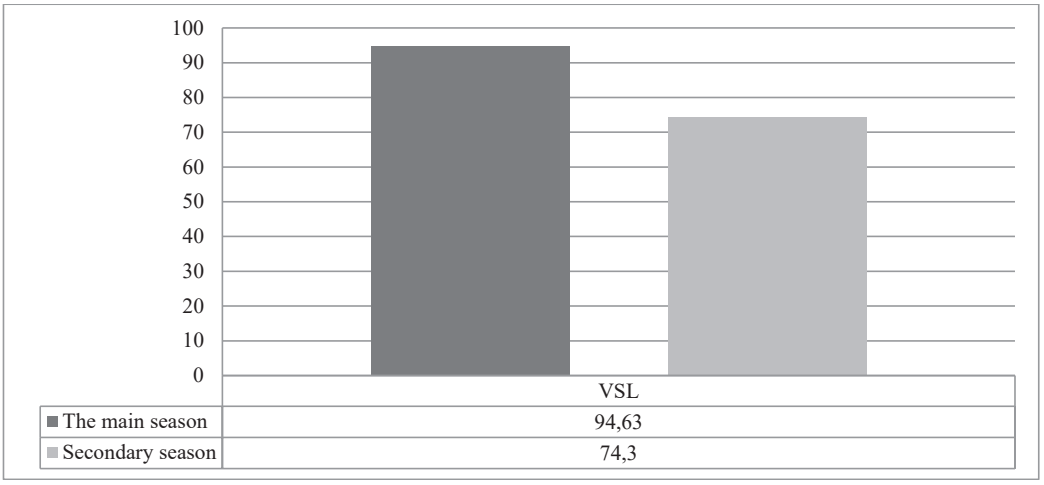

Figure 6. The advancement speed (**VSL**) of ram sperm, μm/s

From Figure 6 it can be seen that the velocity of movement in the straight line (VSL) of the sperm in the main breeding season is 94.63 μm/s, and in the secondary season the speed is only 74.3 μm/s. The data in Figure 7 show that the curvilinear movement speed (VCL) of the sperm in the main breeding season is 202.57 μm/s, and in the secondary season it is much lower and constitutes 164.8 μm/s.

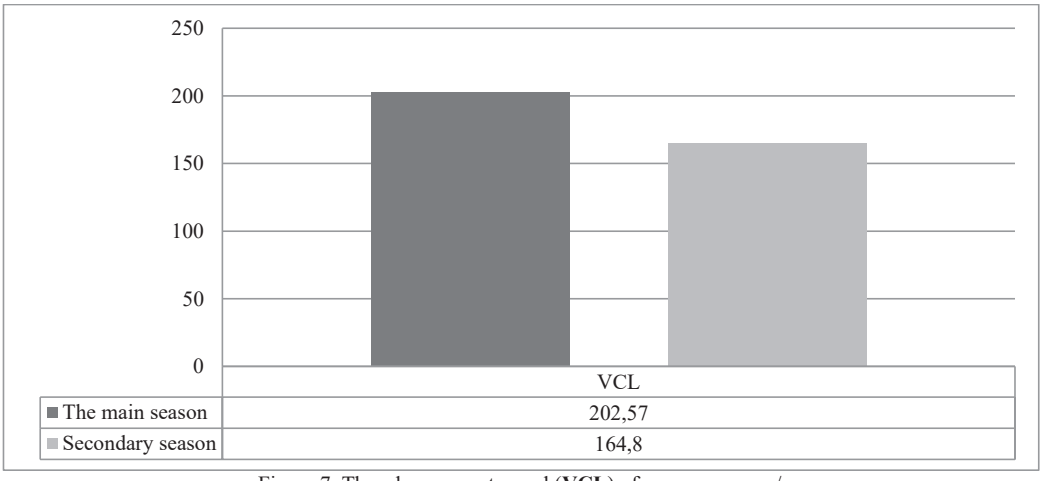

Figure 7. The advancement speed (**VCL**) of ram sperm, μm/s

The decrease of the qualitative and quantitative indices of the ram's sperm in the extra-season compared to the period of natural reproduction are dictated by the character and the particularities of the species. For these reasons, there are differences in the value of the spermogram indices in the species of animals with seasonal reproduction, when the activity of the breeders and the libido in the extra-season are relatively minimized. The average rate of advancement of rams 'spermatozoa according to some scientists' data is 77 μm/s (V.K. Milovanov, 1962). Sperm with an average advancement rate of 113.41 μm/s are 25.01% of the total sperm volume, and with a rectilinear movement speed of 94.63 μm/s - 31.46%. Thus, we can see the fact of obtaining a qualitative sperm of the Tsigaia breed of wool-meat-milk, in the main breeding season. In the secondary season the average rate of advancement of the native sperm of the domestic breed Wool-meat-milk shingles is

relatively low and constitutes 101.0 μm/s, the speed of movement in the straight line is 74.3 μm/s, and those with curvilinear motion they have a speed of 164.8 μm/s. As we see the rate of sperm progression is relatively lower in the secondary season than in the main breeding season. Thus we find the manifestation of a stable tendency to reduce the movement rate of the sperm in the secondary sheep breeding season. The percentage of sperm with forward movement in the straight line prevails in ejaculates taken in both seasons, but in the main breeding season it is significantly higher than the secondary season (31.46 V% compared to 21.42 V%).

CONCLUSIONS

1. The values of the characteristics of the raw sperm of the rams of the domestic breed Tsigaia of wool-meat-milk fall within the specific limits of this breed.
2. The volume of the ejaculate and the qualitative indices of the sperm change during the year depending on the breeding season of the sheep.
3. The mobility of sperm in the main breeding season is quite high, with average indices of 81.17 ± 0.01%, with relatively low variation limits.
4. In the main season, the number of sperm with a rectilinear feed rate of 94.63 μm/s constitutes 31.46% of the total ejaculate volume.
5. In the secondary breeding season the mobility of the sperm is lower than in the main breeding season (66.24% against 81.17%).

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CRYOGENIC CHANGES OF PROTEINS DURING CRYOPRESERVATION OF BULL AND ROOSTER SPERM

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Abstract

In the field of cryobiology, there are a large number of publications devoted to the study of the most labile lipid composition of gametes and their membranes. However, less attention is paid to the state of the protein component of cells. Therefore, the purpose of the presented paper was to study the protein spectrum and its changes in the process of cryopreservation of bull and rooster sperm. The presence of albumins, alpha, beta and gamma globulins was determined by the spectrophotometric method. Most of the proteins are contained in the seminal plasma, in gametes the total protein content is slightly lower. Of the seminal plasma proteins, most contain albumins, and of the gamete proteins, most contain gamma globulins (in the sperm of both animal species). With the passage of technological stages, the number of some proteins increases, while of others it decreases. This indicates about the destruction of protein structures, violation of the permeability of biological membranes and changes in the chromatographic mobility of proteins. From the data obtained, it was found that the protein spectrum of bull and rooster sperm is very different. If alpha globulins (18.2±1.84 mg/billion) prevail in the plasma of the bull`s sperm, and gamma globulins (16.8±3.27 mg/billion) prevail in the gametes, then in rooster sperm, both in plasma and gametes, most of all is albumin (6.0±2.45 and 11.8±0.47 mg/billion, respectively).

Key words*: proteins, cryopreservation, gametes, seminal plasma.*

INTRODUCTION

Living organisms are characterized by a wide variety of proteins, which form the basis of the structure of the body and provide many of its functions. It is believed that in nature there are approximately 10^{10}–10^{12} different proteins, which explains the great variety of living organisms. In unicellular organisms, there are about 3 thousand different proteins, and in the human body - about 5 million (Artemuk et al., 2010).

Protein molecules consist of one or more polypeptide chains organized in a characteristic three-dimensional structure. Individual proteins have a certain chemical composition. Their molecular weights span from 6,000 to over a million. The metabolism, structures, and function of each cell are decisively determined by proteins. Chemical reactions in a cell that would proceed extremely slowly in vitro are accelerated by special catalytic proteins, enzymes, hundreds of thousands of times. It goes without saying that this does not affect the equilibrium state of the reaction, but the speed of its onset. Other proteins perform external or internal protective functions (Iakubche et al., 1985).

MATERIALS AND METHODS

The following methods of protein research were used in the investigations: the method for determining the total protein, the determination of protein fractions by the express method and the solubilization of proteins. Quantitative determination of the protein was carried out by Lowry et al., which is based on the ability of the protein in combination with copper to restore the Folin solution with the subsequent formation of colored reaction products. The protein content was determined spectrophotometrically at a wavelength of 750 nm.

The determination of protein fractions by the express method was determined by McCord in the modification of Karpiuk described by Golban et al. (Golban et al., 1988). The principle of the method is based on the property of

phosphate solutions of different concentrations to precipitate proteins. The optical density of the solutions was determined on an SF-46 spectrophotometer at a wavelength of 750 nm. The content of a single fraction was determined in gram percent. Taking the amount of total protein for 100 and knowing the amount of each fraction, the percentage of protein fractions is calculated.

Gamet protein solubilization was carried out by the method described by (Meddi, 1979). In this case, a fraction of hydrophilic proteins was obtained after shaking the gametes in double-distilled water for 20 minutes. The fraction of alkali-soluble protein was obtained by solubilization of them in a 0.1 molar solution of sodium hydroxide, and acid-soluble proteins by solubilization in a 0.1 M solution of hydrochloric acid under analogical conditions.

RESULTS AND DISCUSSIONS

Albumin is the main blood protein of mammals, where its concentration is 500-700 μM. The albumin molecule is not coated with a carbohydrate shell and can bind a variety of molecules and atoms: water and metal cations (Ca^{2+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, Cd^{2+}, Co^{2+}, Pt^{2+}, Au^{+}), free fatty acids and fat-soluble hormones, unconjugated bilirubin, and bile salts, transferrin, nitric oxide, aspirin and other compounds (Fasano et al., 2005). The first publications devoted to albumin appeared at the end of the 19th century, and the three-dimensional structure of human serum albumin (HSA) was studied only at the end of the 20th century. A similar structure of bovine serum albumin (BSA) was obtained in 2012, and the three-dimensional structure of rat serum albumin (RSA) has not yet been obtained. The serum albumin molecule is formed by a single polypeptide chain consisting of 585, 584 and 583 amino acid residues for human, rat, and bovine albumin, respectively (Consortium T.U., 2015; Majorek et al., 2012; Strauss et al., 1977). The structure of albumin is conservative for all mammals: the molecule consists of three homologous domains, each of which consists of ten helices and can be divided into two subdomains, A and B, containing six and four helices, respectively; these two subdomains are connected by a long loop (Bujacz, 2012). The human and bovine albumin molecule has 17 disulfide bonds and one cysteine residue with a free SH- group (Bujacz, 2012; Ghuman et al., 2005). In the primary sequence of rat albumin, there are as many cysteine residues as there are in the human and bull sequences, and they are located at the same positions (Strausberg et al., 2002), so there is every reason to assume that RSA also has 17 disulfide bonds and one single cysteine in its three-dimensional structure. Alignment of the primary sequences of amino acid residues of the HSA, RSA, and BSA molecules using Clustal Omega (Sievers et al., 2011) showed that the HSA and BSA molecules have the highest identity – 75.6%. However, the percentage of primary structure identity in the molecules of HSA and RSA is 73.0%, while in BSA and RSA it is 69.9%. If we compare directly the amino acid residues of the Sadlow I site (the Sadlow II site is conservative in all three types of albumin), then here the HSA and RCA molecules have the highest identity - 75%, while the BSA shows only 57% identity with both the RCA and the HSA. In addition, BSA is dominated by substitutions of arginine for lysine residues at the Sadlow I site (Lys195 → Arg195, Lys199 → Arg199), which probably affects its configuration, since the side chains of arginine are more branched. The three-dimensional structure of albumin is quite labile, so that when interacting with the albumin molecule of different substances, there are such effects as cooperativity and allosteric modulation, usually inherent in 6 multimeric proteins (Ascenzi et al., 2010). However, due to the above differences in primary structures, according to the results of a study of the functional characteristics of albumin of one animal species, it cannot be a priori asserted that albumin in another species will have the same characteristics. For example, HSA when interacting with fatty acids in solution has high plasticity and flexibility, while the characteristics of BSA in solution with respect to fatty acids are not very different from the calculated data obtained for its crystal structure (Reichenwallner et al., 2013).

From the variety of physico-chemical processes occurring in the cell and consequently in the plasma membrane during freezing and thawing (Belous et al., 1982; Gulevschii et al., 1988; Moiseev, 1984), it is shown that decisive of these is the occurrence of transmembrane

defects in the plasma membrane, the development of which depends on the degree of modification of the protein skeleton and changes in the physico-chemical properties of lipids and proteins in the membrane at different stages prior to freezing. A specific feature of the biological effect of cold is that primary damage in gametes is localized only in extremely small volumes of molecular size. If injuries of gametes refers to the unique structures of the cell (chromosomes, nucleus) and affect the composition of the nuclear or cell membrane, these injuries may secondarily cause large irreversible functional impairments (Nauc, 1991; Novikov et al., 1992). Damage of membranes can significantly disrupt the stationary state in gametes by changing intermolecular interactions, their diffusion parameters, the course of enzymatic reactions and other vital processes (Ortman et al., 1994; Zamfirescu et al., 2010; Katkov, 1985). It follows that the efforts of researchers should be aimed at stabilizing primary, reversible changes, while irreversible ones are not subject to regulation. Damage of biological objects are among to the fundamental problems of modern cryobiology. Interest in this problem increases for a number of reasons, and first of all – it is stimulated by practical tasks. At present, there are more and more questions about the possibility of life for people, animals and plants in conditions unusual for normal functioning – at high and low temperatures and pressure, increased radiation, high accelerations, with a lack of water, high salt content, etc.

Cryo-damage occurs at all stages of sperm processing. However, there are critical zones on the temperature scale (Malinovskaia, 1983). Thus, studies of temperature-induced structural transitions on the water-lipid surface of membranes (Timbal et al., 1995) revealed the rearrangement of water-protein interactions at temperatures of 30, 25, 17 and 7ºC. Moreover, these interactions occur both on the outer surface of the proteins accessible to the free solvent, and in the cavities of biomacromolecules due to the tertiary structure, as well as the contacts of subunits in oligomeric ensembles of proteins. Similar temperature zones were determined (Jukovschii et al., 1987) when studying the mechanisms of stabilization of the native conformation of water-soluble proteins.

In our laboratory was studied the content of plasma and gametes proteins in the native and thawed sperm of bull, ram and boar (Table 1).

Table 1. Protein content in native and thawed sperm of farm animals

Indicators	Animal species		
	Bull (n=8)	Ram (n=4)	Boar (n=3)
Native sperm			
Proteins of plasma, mg/ml	52.1±4.28	20.5±1.26	14.1±1.98
Water-soluble protein of gametes, mg/billion	5.6±0.87	4.6±1.22	3.6±0.43
Alkali-soluble protein of gametes, mg/billion	2.2±0.22	3.1±0.62	2.9±0.46
Acid-soluble proteins of gametes, mg/billion	3.8±0.38	5.0±1.20	7.2±1.23
Total gamete protein, mg/billion	11.6±1.02	12.7±2.93	13.8±0.49
Thawed sperm			
Proteins of plasma, mg/ml	48.4±5.11	18.9±1.47	13.6±2.27
Water-soluble protein of gametes, mg/billion	5.0±0.65	4.5±0.38	2.8±0.43
Alkali-soluble protein of gametes, mg/billion	2.5±0.40	2.5±0.46	2.1±0.03
Acid-soluble proteins of gametes, mg/billion	3.8±0.23	5.0±0.83	9.6±1.31
Total gamete protein, mg/billion	11.3±0.74	12.2±1.83	14.5±1.40

As can be seen from the table, the most protein is contained in the plasma of bull semen (52.2±4.28 mg/ml) and the least in boar semen (14.1±1.98 mg/ml). An analogous trend is also observed in the study of the content of water-soluble gamete proteins. If alkali-soluble proteins are contained at the same level, the content of acid-soluble proteins is specific for each species. The latter are most detected in boar gametes (7.2±1.23 mg/billion) and least in bull gametes (3.8±0.38 mg/billion). The total protein content in gametes is almost at the same level.

Technological processing of the sperm does not have a significant effect on the content of the

studied proteins in the bull and ram semen, and only in the boar there is a tendency to decrease alkali-soluble proteins.

Thus, the total protein content of animal spermatozoa during cryopreservation does not undergo significant changes. The data are presented in Table 2.

Table 2. Protein spectrum of native and thawed bull sperm

Name of proteins	Native sperm	%	Thawed sperm	%
Plasma, mg/ml				
Albumin	3.6±0.26	13.5	13.7±2.84*	35.8
Alpha globulins	18.6±1.84	68.7	17.2±0.84	44.9
Beta globulins	2.0±0.40	7.6	4.0±1.48	10.5
Gamma globulins	2.7±0.18	10.2	3.4±1.48	8.8
Total	26.5±1.91	100	38.3±3.63	100
Gametes, mg/billion				
Albumin	5.1±1.48	14.4	12.8±7.92	49.7
Alpha globulins	7.6±4.0	21.6	4.0±1.13	15.6
Beta globulins	5.8±1.27	16.4	2.5±1.15	9.7
Gamma globulins	16.8±3.27	47.6	6.5±1.73*	25.0
Total	35.7±5.52	100	25.8±8.27	100
Total, plasma + gametes	61.8±5.84		64.1±9.03	

*Statistically authentic differences compared with native sperm

It is possible that although the total protein content during freezing and thawing does not significantly change, there may be conformational changes in individual protein fractions (Tables 2 and 3).

Table 3. Protein spectrum of native and thawed rooster sperm

Name of proteins	Native sperm	%	Thawed sperm	%
Plasma, mg/ml				
Albumin	6.0±2.45	47.8	4.8± 1.90	46.3
Alpha globulins	2.8±0.88	22.1	1.5±0.21	14.5
Beta globulins	3.8±1.08	30.1	2.6±0.50	25.0
Gamma globulins	0	0	1.5±0.23	14.2
Total	12.6±2.82	100	10.4±1.99	100
Gametes, mg/billion				
Albumin	11.8±0.47	54.3	2.1±0.91*	9.6
Alpha globulins	2.7±1.53	12.4	1.2±0.12	5.5
Beta globulins	1.9±1.85	9.0	0.6±0.08	2.8
Gamma globulins	5.3±0.70	24.3	17.9±1.08*	82.1
Total	21.7±1.72	100	21.8±1.42	100
Total, plasma + gametes	34.3 ±3.30		32.2±2.44	

*Statistically authentic differences compared with native sperm

From the data of these tables it follows that the protein spectrum of the bull and rooster sperm is very different. If alpha globulins (18.6±1.84 mg/billion) prevail in the plasma of the bull's sperm, and gamma globulins (16.8±3.27 mg/billion) prevail in the gametes, then the rooster's sperm has the most albumins in both plasma and gametes (6.0±2.45 and, respectively 11.8±0.47 mg/billion).

A distinctive feature of the protein spectrum is that the rooster gametes contain much fewer globulins than those of a bull (the total content of alpha, beta and gamma globulins in a rooster was 9.9±1.89 mg/billion, and in a bull – 30.2±5.32 mg/billion).

Changes in the protein spectrum during cryopreservation are specific for each species. And only the amount of alpha and beta globulins in bull and rooster gametes during thawing has the same character - decreases.

Thus, although the total protein content of the animal sperm during cryopreservation does not change (see Table 1), its protein spectrum underwent quite pronounced modifications. In the process of cryopreservation of a sperm in its plasma, changes in the content of alpha, beta and gamma globulins are specific for each animal species, while in gametes, the dynamics of alpha, beta and gamma globulins are specific.

CONCLUSIONS

The total protein content of animal sperm does not change during cryopreservation, while the protein spectrum undergoes quite pronounced modifications.

In the process of cryopreservation of a sperm in its plasma, changes in the content of alpha, beta and gamma globulins are specific for each animal species, while in gametes the dynamics of alpha and beta globulins is non-specific.

The process of cryopreservation of rooster sperm, unlike that of a bull, causes a decrease in the amount of albumins. This can be explained by the denaturation of proteins, namely albumins.

The appearance of gamma globulin fraction in thawed rooster seed plasma, a sharp decrease in albumin content in thawed rooster seed plasma, and a sharp decrease in albumin content in gametes allows us to note that low temperatures

can initiate translocation, aggregation, and disaggregation of proteins.

A specific manifestation of these disorders may be a decrease in the viscosity of the phospholipid bilayer, which leads to a violation of the barrier properties of plasma membranes in the cryopreservation cycle.

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PROTEIN-LIPID AND CHOLESTEROL-PHOSPHOLIPID RATIO AS AN INDICATOR OF CRYORESISTANCE OF GAMETES OF FARM ANIMALS

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Abstract

In our previous studies, it was shown that the sperm of farm animals differs in both species and individual composition, so the purpose of the research, the results of which are presented in this paper was to identify the resistance of gametes of various animal species to the action of cryopreservation factors. The content of proteins and lipids, cholesterol and phospholipids was determined by biochemical methods, and their quantitative ratio was also calculated. It was found that the resistance of diluted sperm to freezing-thawing is directly proportional to the protein-lipid ratio, while the relationship between cholesterol-phospholipid ratio and cryostability of the sperm is inversely proportional. The results of the conducted studies convincingly show that phospholipids undergo the greatest changes and that the molar ratio of cholesterol phospholipids in gametes changes towards one, especially after thawing of sperm.

Key words*: gametes, cryopreservation, protein-lipid ratio, cholesterol-phospholipid ratio.*

INTRODUCTION

A cell is a highly organized structure containing many functional units, characterized by the presence of plasma and internal membranes. The plasma membrane is the outer membrane of the cell, and the inner membranes are membranes of cell organelles. Biomembranes are characterized by extreme diversity and are able not only to separate the cell contents from the external environment and to ensure the separation of the internal cell volume into compartments, but also participate in the regulation of many processes (Ghennis, 1997). For example, plasma membranes provide a diffusion barrier, active transport, electrical excitability, intercellular communication, hormonal and immune responses etc. On the membranes of the endoplasmic reticulum are synthesized proteins, fats and carbohydrates. Membranes of nerve cells are able to transmit impulses in the form of changes in electric potential etc. The uniqueness of the functions of each membrane is largely determined by the properties of the membrane proteins that make up its composition. Proteins - various enzymes, transport proteins, receptors, pores, channels etc. - make a significant contribution to the formation of the structure of cell membranes (Ghennis, 1997). The average protein content in the membranes is approximately 60% (by weight of dry matter), while the composition of biomembranes also includes lipids - 30% and carbohydrates - 10%. Naturally, the ratio between these components can vary significantly depending on the nature of the membranes. Thus, the protein content in membranes can vary from 20% in myelin to 80% in mitochondria (Ghennis, 1997). Lipids-phospholipids, glycolipids, cholesterol - make up the backbone of the membrane and are responsible for the integrity of the membrane structure. Carbohydrates are found in the composition of membrane proteins (glycoproteins and proteoglycans) or lipids (glycolipids) (Ghennis, 1997). In addition, the membranes contain a relatively large amount (~30%) of bound non-freezing water. Despite the diversity of biomembranes, the basic principles of structural organization of all membranes of animal, plant and bacterial origin are the same. According to the widely accepted "liquid mosaic" model, originally proposed in 1972 by Singer and Nicholson (Singer et al., 1972), the biomembrane is represented as a

fluid phospholipid bilayer in which proteins are immersed. Subsequently, however, it became apparent that the molecular organization of the membranes is much more complex than it follows from the liquid mosaic model. In particular, it was shown that not all membrane proteins diffuse freely in a liquid lipid bilayer (Ghennis, 1997). Some parts of the membranes differ in structure from the classical lipid bilayer due to lipid polymorphism. Within the same membrane, regions with different lipid composition and functions can adjoin (Epand, 1998). At present, it is believed that the complex dynamic structure of biomembranes, which is characterized by curvatures, phase transitions, thickness variations and the formation of non-layer structures is determined by the specific interactions of membrane proteins with lipids (Epand, 1998). Such interactions in many respects ensure the effective fulfillment by membranes of various cellular functions arising during metabolism. The dynamic properties of biomembranes can be illustrated by the example of a "metamorphic" model, which includes the basic membrane processes.

MATERIALS AND METHODS

To separate the plasma membranes, a two-phase polymer system was used, consisting of dextran with a molecular weight of 500000 and polyethylene glycol-6000 D produced by Fluka A G Busch (Switzerland). The components of the polymer system were dissolved in 0.1 M phosphate buffer with a pH of 6.5. The resulting solution was transferred to a long funnel and kept for 12 hours, after which the upper and lower phases were separated, which were stored until use. All operations to isolate plasma membranes were performed at 4°C.

In contrast to the method (Ivanov et al., 1981), the membranes were separated using a dividing funnel, where the phases are not concentrated at the separation boundary. In addition, in connection with the detection of a color change in the samples and the construction of a calibration curve, an albumin working solution was prepared using Tris HCl (or EDTA at the determination of protein in gamete homogenate). The determination of the strength of gamete plasma membranes was performed by using the method of electrochemical surf of cell membranes due to the diffusion potential difference based on the registration of changes in the pH of the non-buffer environment. When cells are placed in a non-buffer isotonic medium, chlorine ions exit through the plasma membrane, which leads to the appearance of a diffuse potential on the membrane. This can be used to record changes in the transmembrane potential by determining the pH of the incubation medium. At a transmembrane potential exceeding the critical value, acidification is replaced by reverse alkalization, which is regarded as a consequence of the release of potassium ions from the cell as a result of electrical breakdown of the membranes.

Lipid extraction was carried out by the method of (Bligh et al., 1959). The method is based on the destruction of protein-lipid bonds by polar solvents, followed by extraction of lipids by a non-polar solvent combined into a single mixture containing water in a ratio of 1:2:0.8.

The content of total lipids was studied using the Brandon method described by Skorohod et al. (1983). The principle of the method is based on the ability of potassium dichromate to oxidize lipids. Spectrometry of the resulting chromic acid was carried out at a wavelength of 590 nm.

RESULTS AND DISCUSSIONS

The study of the role of membrane organization of proteins directly in a living organism is difficult due to the complex organization of living matter and the simultaneous occurrence of many interrelated processes. However, the possibility of carrying out of gene mutation that provides selective changes in the structure of expressed proteins, for example, the expression of only soluble forms of proteins, allows in some cases to show the importance of the functioning of membrane-bound proteins (Esther et al., 1997; Huang et al., 1992). Consider this using the example of a KIT-ligand, one of the membrane-bound growth factors of mammals.

Note that the importance of binding to the biomembrane is revealed not only for integral proteins, but also demonstrated in some cases for peripheral proteins, for example, pyruvate oxidase - a peripheral enzyme that catalyzes the

oxidation of pyruvate to acetic acid and the restoration of ubiquinone. The specified enzyme circulates in the body and binds to the plasma membrane only in the presence of a substrate and cofactor; in this case, a C - terminal lipid-binding domain is formed in the protein molecule.

Thus, the biological role of various membrane enzymes can be largely determined by their ability to bind to the membrane. Firstly, binding to a biomembrane provides localization (concentration) of enzymes in a certain part of the cell and/or in that region of the membrane where the substrate is concentrated. For example, acetylcholinesterase is fixed in the postsynaptic membrane, where the concentration of acetylcholine is high. Secondly, the adsorption of enzymes on the membrane makes it possible to interface the processes of catalysis and transmembrane transfer. Thus, during the functioning of membrane-bound enzymes involved in the hydrolysis of starch and proteins, a locally high concentration of soluble product molecules is created near the cell membrane, which contributes to their effective absorption by the cell. Thirdly, for many enzymes, at binding to the membrane ensures the availability of water-insoluble substrates. These can be integral enzymes involved in the processing of membrane proteins (for example, secretase of membrane proteins, see above), as well as peripheral enzymes: phospholipases (Burack et al., 1994), protein kinase C (Newton et al., 1998), pyruvate oxidase etc. Finally, optimal microenvironment is formed upon binding providing native conformation and catalytic activity of membrane enzymes.

Proteins of biological membranes make up a significant part in most living organisms - about a quarter of the genome of prokaryotes and eukaryotes encode these proteins (Fagerberg et al., 2010; Liu et al., 2001). Membrane proteins play a vital role in the implementation of many cellular functions, such as signal transmission, energy production and conversion, recognition and transport of substances through the membrane. According to the biophysical classification, membrane proteins are subdivided into integral ones, which penetrate the membrane through them, often several times, “sit” rigidly in it and can be extracted from it using, for example, non-polar organic solvents or detergents, and peripheral ones that are weakly bound to the membrane or associated with its surface due to non-covalent bonds and can be separated from it by an aqueous buffer solution without destroying the integrity of the entire biological membrane. There are also anchored membrane proteins that hold onto the membrane by immersed in the hydrophobic region of fatty acid or prenyl residues attached to the polypeptide chain as a result of posttranslational modification. Currently available information about the structure and mechanisms of action of membrane proteins is rather sparse - among all protein structures in Protein Data Bank, data on membrane proteins make up only 1-2% of the total number. This is due to the difficulty of conducting structural and functional studies of membrane proteins.

The lipids of a bilayer of biological membranes are amphipathic molecules consisting of a hydrophilic polar head and a hydrophobic tail. The classification of lipids is based on their chemical structure - depending on the structure of the polar head, they are charged (anionic) and uncharged (neutral and zwitterionic). Lipids also differ in the structure of the hydrophobic tail (the number of chains, the length of the hydrocarbon chain, with the presence or absence of double bonds, cycles etc.). The main molecules of the membrane matrix are glycerophospholipids. In addition to them, the membrane contains many other types of lipids, of which sterols (for example, cholesterol), sphingolipids, glycolipids, and others are worth mentioning separately (Sezgin et al., 2017). Due to the chemical structure and the uniqueness of the physicochemical properties of each type of lipid, the structure of associates into which they are able to spontaneously organize in aqueous mediums under different external conditions is also diverse. In 1925, Gorter and Gredel suggested (Cherezov et al., 2007) that lipids are able to form bilayer structures - in the aqueous medium, when the hydrophobic tails are densely packed to each other, while the hydrophilic head parts look outward, directly contacting with the water phase. Subsequently, this idea took shape in a two-dimensional fluid model with lateral diffusion of membrane

lipids, which was called the mosaic model of biological membranes (Nicolson, 2014).

The presence of cholesterol, which is an essential component of mammalian cell membranes, increases the packing density of lipids in the membrane, which leads to changes in the viscosity or permeability of the membrane and is involved in the formation of rafts (Burger et al., 2000; Sezgin et al., 2017). The lipid composition of cells of different organisms differs from each other, which determines both the structure of the bilayer formed by them and the variety of functions that lipids perform in membranes (Sezgin et al., 2017). For example, bacterial membranes mainly consist of phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and cardiolipin (CL) (Burack et al., 1994). Eukaryotic membranes are composed primarily of glycerophospholipids, sphingolipids and sterols. The main anionic lipid in them is phosphatidylserine (PS).

Our laboratory staff noticed that the biochemical composition of plasma membranes is of great interest in elucidating the causes for the different resistance of gametes to low temperatures and investigated the cryogenic changes in the ratio of protein/lipid plasma membranes of gametes of different animal species (Table 1).

As can be seen from the table, the highest ratio of protein/lipid in plasma membranes is observed in carp gametes and is somewhat lower, but still higher than in other animals - in the bull, whose gametes tolerate freezing well.

The results of the researches showed that the ratio of cholesterol/phospholipids was the same in the diluted gametes of bull and ram (Table 2). The lowest ratio of these components was found in the membranes of boar gametes, which are characterized by high sensitivity to cooling and freezing. The value of this indicator in the membranes of the rooster and ram gametes occupies an intermediate position.

Table 1. Cryogenic changes in the ratio of protein/lipid plasma membranes of animal gametes

Animal species	Protein/lipid ratio of membranes			Number of repetitions
	Native gametes	Refrigerated gametes	Thawed gametes	
Bull	0.43±0.014	0.3±0.11	0.25±0.019*	6
Ram	0.36±0.031	0.38±0.13	0.52±0.032*	6
Boar	0.17±0.009	0.17±0.09	0.20±0.004*	5
Rooster	0.40±0.042	0.4±0.08	0.52±0.041*	5
Carp	0.65±0.07	0.54±0.15	0.42±0.08*	3

*Statistically authentic cryogenic changes

Apparently, a decrease in the protein/lipid ratio during thawing is more advantageous for preserving functional usefulness than its increase, because the semen of bull and carp in the gamete membranes of which the indicated decreases is better preserved during cryopreservation than the semen of other animal species.

In the following studies, the goal was to study the dynamics of changes in the molar ratio of cholesterol/phospholipids in the process of gamete cryopreservation.

During cooling, freezing and thawing of the semen, there is a tendency to shift the molar ratio in the direction of increase. In bull gametes, this phenomenon is most pronounced. The observed changes are of certain interest, since the phase transition of lipids disrupts the plasticity of membranes, leading to the formation of a rigid membrane structure, and a decrease in the thermal stability of cells.

Table 2. Indicators of the impact of technological processing of semen on the ratio of cholesterol/phospholipids (C/Pl) in the gametes of bulls (n=7), rams (n=6) and boars (n=8).

Lipids	Dilution		Refrigeration		Thawing	
	Mol %	C/Pl	Mol %	C/Pl	Mol %	C/Pl
Bull						
Cholesterol	1.07±0.04	0.282	0.99±0.04	0.326	0.89±0.04*	0.382
Phospholipids	3.79±0.06		3.04±0.06		2.33±0.10*	
Ram						
Cholesterol	1.11±0.03	0.281	1.05±0.04	0.302	0.93±0.05*	0.293
Phospholipids	3.95±0.06		3.48±0.03		3.18±0.03*	
Boar						
Cholesterol	1.25±0.05	0.349	1.18±0.03	0.375	1.11±0.03*	0.381
Phospholipids	3.58±0.09		3.15±0.05		2.84±0.04	

*Statistically authentic cryogenic changes

The results of these studies convincingly show that phospholipids undergo the greatest changes and that the molar ratio of cholesterol/phospholipids in gametes changes in the direction of one, especially after thawing the semen, that is, in the direction of the ratio that eliminates the phase transition of lipids.
Consequently, changes in the ratio of cholesterol/phospholipids, as well as loosely bound cholesterol in gametes during cryopreservation, have a non-specific character, that is, the nature of their modification is the same for the gametes of all studied animal species.

CONCLUSIONS

Changes in the ratio of cholesterol/ phospholipids and the content of loosely bound cholesterol in gametes during cryopreservation are non-specific, that is, their modifications are the same for the studied animal species.
The observed changes are of particular interest, since the phase transition of lipids disrupts the plasticity of membranes, leading to the formation of a rigid membrane structure, and a decrease in the thermal stability of cells.
During cooling, freezing and thawing of the semen, there is a tendency to shift the molar ratio in the direction of increase. In bull gametes, this phenomenon is most pronounced.
It is proved that phospholipids undergo the greatest changes and that the molar ratio of cholesterol/phospholipids in gametes changes in the direction of one, especially after thawing of the seed, that is, in the direction of the ratio that eliminates the phase transition of lipids.

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EXPERIMENTAL STUDY OF CARDIOVASCULAR RECEPTORS BEHAVIOR IN RAT HEART AND AORTA. THE IMPACT OF SERUM FACTORS FROM HYPERTENSIVE SUBJECTS

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Abstract

Hypertension or elevated arterial blood pressure is the most common risk factor for cardiovascular disease and death and by the year 2025 the number of people with arterial hypertension will reach 1.5 billion. Objectives are to get some insights concerning the mechanisms of induction of arterial hypertension (AHT). We used freshly collected fragments of rat heart and aorta incubated with serum from hypertensive (HT) subjects as well as with different Na_2HPO_4 concentrations in order to follow up the changes in $^{45}CaCl_2$ fluxes, in ^3H Serotonine, ^3H Noradrenaline (NA) as well as in ^3H Cortisol uptake in rat aorta and heart. Our data have pointed out significant changes in $^{45}CaCl_2$ uptake in rat aorta and heart incubated with serum from (HT) subjects which can be pathologically correlated with the mechanism of (AHT) induction. A significant decrease in ^3H Serotonine and ^3H NA has been recorded both in rat aorta and heart incubated with (HT) serum which can be accounted for a suppressing of normal inhibitory mechanism .As far as ^3H Cortisol uptake is concerned, no change was noticed in the case of incubation with sera from (HT) subjects both in heart and aorta. When different Na_2HPO_4 have been used, ^3H NA and ^3H Serotonine uptake in rat aorta have pointed out an increase in ^3H NA uptake at phosphate concentrations close to the physiology ones, while in the case of ^3H Serotonine, there is a proportional increase in its uptake at higher Na_2HPO_4 concentrations. It seems that $^{45}CaCl_2$ admission depends very much on organic Na_2HPO_4 concentration.

Key words*: rat heart muscle, rat aorta, cell receptors, ^3H serotonine,^3H noradrenaline, hypertension*

INTRODUCTION

Hypertension or elevated arterial blood pressure is the most common risk factor for cardiovascular disease and death and by the year 2025 the number of people with arterial hypertension will reach 1.5 billion (Wenzel et al., 2016).

This disorder is a major risk factor for many common causes of morbidity and mortality including stroke, myocardial infarction, congestive heart failure, and end-stage renal disease (Lifton, 2001).

Stiffening of large elastic arteries and aorta is a hallmark of vascular aging and one of the most important determinants of the age-related increase in blood pressure and cardiovascular disease events (Cecelja, 2016).

Increased intraarterial pressure causes more tension in the arterial wall, both in smooth muscle cells and endothelial cells and increased production of growth factors (Lagerlof, 1989). During ageing, the distension of the arterial wall decreases progressively (Lithel, 1988), stiffness is also due to glycation process of cellular matrix (Fleckenstein, 1987).

Abnormal vascular smooth muscle cell proliferation is thought to contribute to the pathogenesis of vascular occlusion including atherosclerosis.

Atherosclerosis is multifactorial process extremely complex, a disease with slow progression, its onset is related with childhood, but became obviously in the middle or advanced age, when clinical symptoms indicate organic lesions (Aviram, 1993). Atherosclerosis, or hardening of the arterial blood vessel wall, is a chronic progressive inflammatory disorder. The disorder presents with coronary artery disease, carotid artery disease, peripheral artery disease, or combined, cardiovascular disease (CVD) Aberrant endothelial cells (EC) - vascular muscle cells (VSMC) interaction could promote atherogenesis (Mana Li, 2018).

Atherogenesis is a process characterized by the formation of a neo intima lesion that progressively occludes arterial lumen and neo intima thickening is due to accumulation of cellular and extra cellular substances in the space between intima and the underlying medial vascular smooth muscle (Nilsson, 1986).
Endothelial dysfunction is characterized by lipid accumulation and increase adherence of monocyte/macrophage and T lymphocytes which then migrate through endothelium and localize sub endothelium (Gimbrone, 2016).
In patients with essential arterial hypertension (Linder (1987) pointed out the effects of a circulating factor on intracellular Ca in normal platelets.
Objective: In order to get some insights related to the induction mechanisms of hypertension, we studied the modifications in $^{45}CaCl_2$ transport as well as the uptake of ^{3}H Serotonine, ^{3}H Noradrenaline and ^{3}H Cortisol in rat aorta and heart incubated with serum from hypertensive subjects as well as in the presence of different phosphate concentrations

MATERIALS AND METHODS

The study was done on 10 white Wistar rats aged 8 months old, with a weight between 180-200 grams, fed on standard chew.
Following anesthesia, the animals were killed by cervical dislocation, and thoracic aorta and heart were quickly removed by surgical opening of thorax cadge and were placed on ice bath in physiological saline solution for being processed for radioisotope uptake experiments within one hour.
10 ml of peripherial blood were collected on heparin test tubes by vein punction from hypertensive subjects and then serum has been separated from plasma by 3000 rpm centrifugation.
The heart fragments and aorta rings (50 mg each) were placed in test tubes and incubated with:
a) 20 µl of serum from hypertensive patients and then with $^{45}CaCl_2$, ^{3}H Cortisol, ^{3}H Serotonin and ^{3}H NA;
b) The incubation medium of rat aorta/heart fragments included the following chemicals:
- Na_2HPO_4 0.6 mM, 1.2 mM, 2.5 mM
- Na_2HPO_4 1.2 Beta glycerol phosphate 2.5 mM
- Na_2HPO_4 1.2 mM beta glycerol phosphate 5 mM solutions and then radioactivated by adding the following radioisotopes in the following concentrations:
1) $^{45}CaCl_2$ 0.15 uCi/ml
2) ^{3}H Serotonine 0.05 uCi/ml
3) ^{3}H Cortisol 0.05 uCi/ml
4) ^{3}H Noradrenaline 0.025 uCi/ml
The tissue samples were incubated over the night at 37°C. The extraction was done on next day by introducing biological tissue fragments in HCl 1N after being previously rinsed 3 successive times in bidistilled water.
The next day, these have been processed for radioactive uptake: the tissue fragments have been removed from extraction medium and 0.2 ml of incubation and 0.2 ml of extraction media have been introduced in 5 ml vials containing scintillation liquid. Then, the samples from incubation and extraction media were subjected to estimation of radioactivity uptake assay has been done in fluid phase in Beta Berthold Scintillation Counter for beta radiations.

RESULTS AND DISCUSSIONS

The data have been statistically processed and their percentage values were referring to control values. The significance of the difference between the two groups of samples incubated with serum from hypertensive subjects, under study divided by standard deviation of the difference was then calculated:

$$t=d/Sd \qquad d=Xm-Xp$$

$$Sd=\frac{d2-(d2)/h}{n(n-1)}$$

P values (counter probability) was calculated in reference to the t value and the degree of experimental freedom. The difference between samples under study was considered more marked (Table 1).

Table 1. Representation of values statistically calculated (t and p) obtained after calculation of difference significance between the two groups of biological samples incubated with serum from HT subjects

Radioisotope	$^{45}CaCl_2$		^{3}H Serotonine		^{3}H Cortisol		^{3}H NA	
Biological material	t	p	t	p	t	p	t	p
Heart	2.3	5-2%	2.1	5%	0.11	60%	3.17	1%
Aorta	2.4	5-2%	2.8	2-1%	1.31	40-20%	1.94	5%

The normal behavior of the vascular and cardiac cell membrane permeability in terms of their ability to uptake $^{45}CaCl_2$ changes in contact with sera from hypertensive patients (an enhanced Ca uptake is to be noticed in the case of both aorta and heart tissue, p ranges from 5-2%) (Linder, 1987).

In the case of samples incubated with serum from hypertensive subjects, the uptake of ^{3}H Serotonine falls below the confidence levels. The drop is more marked in aorta p=2%-1% than in heart p=5%. The role of Serotonine in neurotransmission is hypothetical in some forms of experimental arterial hypertension.

Concerning ^{3}H Cortisol uptake, there are no changes in rat heart or aorta when these are incubated with serum from hypertensive subjects. When incubated with serum from HT subjects in the presence of ^{3}H NA the uptake is much lower in rat heart (p=1%), and close to the significance ranges in rat aortic tissue (p=5%).The depressed uptake of ^{3}H NA is relatively difficult to interpret; this phenomenon might mean a suppressing of a normal inhibitory mechanism (Table 2, fig.1).

Table 2. Mean values of $^{45}CaCl_2$, ^{3}H Serotonine, ^{3}H Cortisol and ^{3}H NA in rat aorta and myocardium incubated in Hanks physiological solution with serum from control patients (cpm/g tissue)

Biological material	$^{45}CaCl_2$	^{3}H Serotonine	^{3}H Cortisol	^{3}H NA
Heart	154.68	81.40	101.72	88.18
Aorta	151.17	74.10	121.49	86.83

There is almost constant values of radioactive uptake in rat aorta and heart in the presence of serum from control patients free of HTA pathology (fig. 2).

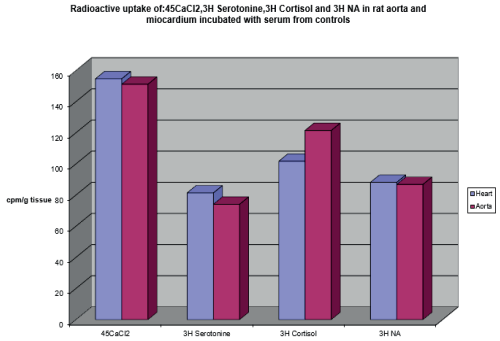

Figure 1. Histogram of of $^{45}CaCl_2$, ^{3}H Serotonin, ^{3}H Cortisol and ^{3}H NA in rat aorta and myocardium incubated in Hanks physiological solution with serum from Control patients (cpm/g tissue)

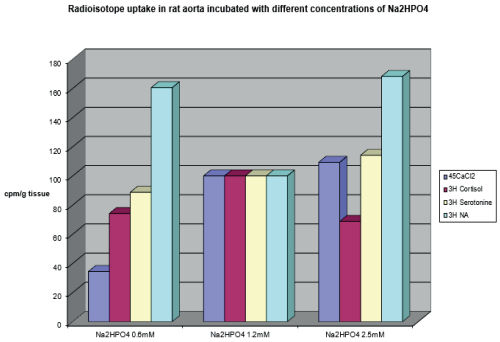

Figure 2. Radioisotope uptake in rat aorta in presence of different concentrations of Na_2HPO_4

Table 3. Radioisotope uptake in rat aorta incubated with different concentrations of organic and inorganic phosphate + mixture of 1.2 mM Na_2HPO_4 and 5 mM Beta Glycerol phosphate (B.G.P.)

Phosphate	Na_2HPO_4			Na_2HPO_4 1.2 mM	
Concentrations	0.6mM	1.2mM	2.5mM	B.G.P. 2.5mM	B.G.P. 5mM
Radioisotope $^{45}CaCl_2$	34.4	100	109.3	101.6	99.1
^{3}H Cortisol	74.2	100	68.8	69.1	93.4
^{3}H Serotonine	88.7	100	114.1	96.04	175.14
^{3}H N.A.	160.5	100	168.2	161.1	98.2

1.2 mM Na_2HPO_4 was considered physiological normal (fig. 3).

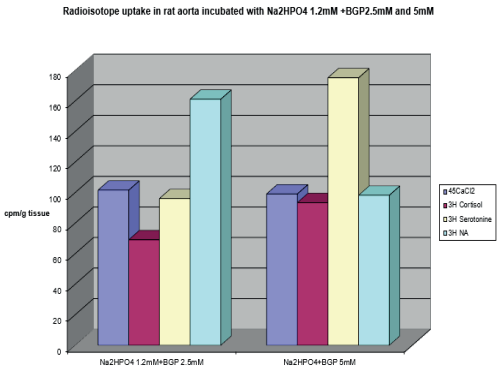

Figure 3. Radioisotope uptake in rat aorta incubated with Na_2HPO_4 1.2mM+B.G.P. 2.2mM and 5mM

From our data, it seems that $CaCl_2$ admission depends very much on organic phosphate concentration.

It is obvious that at lower concentrations to those physiological ones, Ca^{+2} transport is very much slowed down (only 34.4%) versus control (fig. 1) while at higher concentrations as well as different concentrations of organic phosphate, this process is not significantly modified.

Concerning ^3H Cortisol uptake, at both below and above normal phosphate concentrations there is a decrease in ^3H Cortisol uptake and the phosphate in higher concentration determines an uptake of ^3H Cortisol in rat aorta close to that found in controls (Tabel 3).

There are no significant differences between the radioisotope admission at 1.2 mM Na_2HPO_4 in comparison with the mixture of 1.2 mM Na_2HPO_4 and 5 mM Beta Glycerol phosphate (B.G.P.).

From our radioactive uptake of ^3H Serotonin in the presence of different phosphate concentrations, it seems that this process is not influenced by concentrations close to normal ones, but it is very much increased in presence of phosphate in excess.

The behaviour of ^3H NA uptake is very different. This uptake of ^3H NA is very much increased at different organic phosphate concentrations than physiological normal concentration and close to normal in the presence of phosphate in excess.

CONCLUSIONS

The data on $^{45}CaCl_2$, ^3H Serotonine, ^3H NA uptake by rat heart and aorta in the presence of serum from HT subjects pointed out a change in normal behavior of cell membrane receptors and in the ionic flow and neural mediators uptake.

In the presence of different Na_2HPO_4 concentrations, there are modifications of $^{45}CaCl_2$ incorporation in rat aorta, which can be pathologically correlated with the mechanism of arterial hypertension induction.

The decrease in ^3H Serotonine and ^3H NA uptake is relatively difficult to interpret, these phenomena can be accounted for a suppressing of a normal inhibitory mechanism.

Our studies seem to point out that in the case of arterial hypertension there are not any modifications in ^3H Cortisol uptake.

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REPRODUCTIVE RESPONSE AT ILE DE FRANCE EWES AFTER 5 DAYS PROGESTAGEN TREATMENT PLUS OR WITHOUT PREGNANT MARE SERUM GONADOTROPIN (PMSG)

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Abstract

The aim of the present study was to study the reproductive response at Ile de France ewes after 5 days progestagen treatment plus or without pregnant mare serum gonadotropin (PMSG). The experiment was carrired out with 26 multiparous ewes. Three experimental groups were formed depending on whether a PMSG was used or not, as well as the time of treatment with a synthetic analogue of PGF 2α (before or after the removal of sponges). Group 1 (PGF /Pg/PMSG) (n=8) - at the time of the placement of the sponges, the ewes were treated with a synthetic analogue of PGF 2α. At the time of the sponge removal, 300 UI PMSG was put i.m. Group 2 (PGF /Pg) (n=9) - at the time of the placement of the sponges, the ewes were treated with a synthetic analogue of PGF 2α. Group 3 – (Pg/ PGF) (n=9) - the ewes were treated with a synthetic analogue of PGF 2α at the time of the sponge removal. The protocol, that consisted PMSG, successfully synchronized estrus (100%) with high levels of fertility (87.5%) and fecundity (171.4%). Protocols, which were without PMSG, were not suitable for ES, because of unsatisfactory results, that were obtained.

Key words: *estrus, ewes, PMSG, progestagens, synchronization.*

INTRODUCTION

The synchronization of fertilities and births of ewes are main elements of the reproductive management in sheep breeding. Synchronization of estrus allows control and shortening of lambing and kidding, with synchronization of weaning and uniform batching of animals to slaughter; it also allows more efficient use of labor and animal facilities (Abecia et al., 2011). In Europe, the most common hormonal method for estrus synchronization (ES) of small ruminants is with intravaginal sponges, impregnated with progestagen (flurogestone acetate FGA or medroxyprogesterone acetate MGA) (Danko, 2003; Menchaca and Rubianes, 2004; Abecia et al., 2011). In the traditional protocols for synchronization of oestrus with intravaginal sponges, they stay in the vagina for 12 to 14 days (so-called long term treatments), followed by a PMSG (eCG) injection during the removal of sponges and after about 48 hours an oestrus occurs and the sheep can be inseminated (Abecia et al., 2011). The long-term progesterone treatments efficiently synchronize estrus, but with variable fertility (Menchaca and Rubianes, 2004) and also related to the development of vaginitis and problems with lack of sponge retention (Suarez et al., 2006; Martins et al., 2009), which are not consistent with what is desired from an animal welfare and health perspective (Maritnez-Ros et al., 2018).

According to the new concepts of follicular growth (the wave model, and that each follicular wave appears every 5-7 days), were developed various alternative, short-term progestagen treatments in sheep and goats, consisting of the induction of 5-7 days progesterone background followed by the injection of PMSG (Menchaca and Rubianes, 2004). Short progestagen treatments are effective both during the non-breeding season (Ungerfeld and Rubianes, 1999; Ataman et al., 2006; Martinez-Ros et al., 2018[1]; Maritnez-Ros et al, 2019[1]) and during the breeding season (Viňoles et al., 2001; Ataman et al., 2006; Ustuner et al., 2007; Karaca et al., 2009; Metodiev and Raicheva, 2011; Cox et al., 2012; Metodiev et al., 2014; Maritnez-Ros et al., 2019[1], Maritnez-Ros et al., 2018[2]; Metodiev, 2019; Maritnez-Ros et al, 2019[2]; Maritnez-Ros et al, 2019[3]; Maritnez-Ros and Gonzalez-Bulnes, 2019). Short-term protocols are more and more frequently used for sheep artificial

insemination under field conditions, but even with the advantages with use of this protocol there is still less use of this progestogen treatment regimen than that of the classical long-term treatments (Maritnez-Ros et al., 2018[1]).

In fact, all protocols for ES that used progestagens ends with PMSG treatment. Currently, there is a highly active movement in European countries against the use of eCG and it is necessary to look for alternative protocols for the induction and ovulation which would not include eCG (Maritnez-Ros et al., 2019[1]).

The aim of the present study was to study the reproductive response at Ile-de-France ewes after 5 days progestagen treatment plus or without PMSG.

MATERIALS AND METHODS

The experiment was carrired out with 26 ewes of the Ile-de-France breed, raised in the Experimental farm of the IAS - Kostinbrod during May, 2019. Before and after the experiment, the animals were raised outdoors, grazed on pasture with supplement of 300 g. of sheep concentrate mix (12% crude protein, produced in a forage factory) per ewe per day. During the time when the ewes were with sponges, they were raised indoors, fed with meadow hay ad libitum with supplement of 300 g. of sheep concentrate mix (12% crude protein) per ewe per day. All experimental ewes were multiparous, aged 2-4 years, live weight (70-75 kg), body condition (BCS= 3.0-3.5), clinically healthy and with normal deliveries in the previous lambing. The intravaginal sponges (30 mg FGA Synchropart®, Ceva Sante Animale, France) were put on day 15 after the begining of controlled matings to all experimental animals for 5 days. Control matings (or mating campaign) means that every day at ewes were introduced teasers (1 teasers to 50 ewes) and ewes in heat were inseminated naturally with rams according the breeding plan. Usually mating campaigns with all flocks in Experimental farm of the IAS - Kostinbrod lasts 40-45 days. For those 15 days, 23 from 110 multiparous ewes came in estrus. The three experimental groups were formed with ewes depending on whether a PMSG was used or not, as well as the time of treatment with a synthetic analogue of PGF 2α (before or after the removal of sponges). The ewes in the experimental groups did not come in estrus up to this moment.

Group 1 (PGF /Pg/PMSG) (n=8) - at the time of the placement of the sponges, the ewes were treated with a synthetic analogue of PGF 2α - 1.0 ml Alfabedyl (Ceva Sante Animale, France, contains Alfaprostol 2 mg/ml). At the time of the sponge removal, 300 UI PMSG (Folligon®, MSD Animal Health) was applied.

Group 2 (PGF /Pg) (n=9) - at the time of the placement of the sponges, the ewes were treated with 1.0 ml Alfabedyl. At the time of the sponge removal, no treatment.

Group 3 – (Pg/ PGF (n=9) - at the time of the placement of the sponges, the ewes were not treated with a synthetic analogue of PGF 2α. At the time of the sponge removal, ewes were treated with 1.0 ml Alfabedyl.

The ultrasound screenings of the ovaries were done on next days: the day of the placement of the sponges (Day 1), the day of the removal of the sponges (Day 5) and 47 hours after sponge removal (Day 7). The equipment included a digital portable ultrasound system ALOKA ProSound 2 (Aloka Co., Ltd.) supplied with electronic linear transducer UST 5820, with frequency of 7.5 MHz. The total number and mean diameters of ovulatory sized follicles (diameters ≥ 5.00 mm) were counted for every ewe. Also presence or not of corpus luteum was observed. If there was of corpus luteum, the number of it was counted.

At 48 h after the sponge removal, the ewes were tested for presence of a heat with an estrous detector (Draminski Ltd). All ewes that had electrical resistance ≤ 350 units were considered to be in estrus (according to users' manual and our field observations). Ewes with boundary values for estrus (360-440 units) were also recorded. At 49 h after sponge removal ewes in heat according estrus detector and those with bound values were put to individual mating. At 56 h after sponge removal, the ewes that were not mated at 49 hour were tested again for presence of a heat with an estrous detector (Draminski Ltd) and the same procedure, like 48 hours, was done. Those in heat and with bound values were put to mating on 57 hour. For every mating only

one service was done. Six clinical healthy rams were used. Every ram did maximum 4 services per day (2 at 49 h and 2 at 57 hours).

Statistical analysis

The data for follicles and corpora lutea was presented in number means ± SEM. The mean diameters of follicles were compared by one way - ANOVA, Data analysis, Excel, Microsoft Office). All ovarian data was presented in number, means ± SEM.

The following reproductive parameters were studied:

- Estrus synchronization rate (ESR) – all ewes in estrus on Day 2 after sponge removal (detected by to estrus detector and by ram mating) - ewes in a heat/ all ewes x 100.
- Fertility - lambed ewes/ inseminated ewes x 100.
- Fecundity - the number of born lambs (included all born lambs - live or dead) / lambed ewes x 100.

Fertility and fecundity were calculated after lambing. All results were presented in percentage. The significance of the differences between groups about the synchronization effect and fertility, were established by the Fisher's exact test (http://graphpad.com/quickcalcs/contingency1/).

RESULTS AND DISCUSSIONS

During the treatment, ewes from the experimental groups had similar number of ovulatory follicles (Table 1.) At Day 7 (47 hours after sponge removal) ewes from Group1 had the biggest number of ovulatory sized follicles – 21, whereas the Group 2 had the smalest – 16, but with the biggest mean size – 6.41 mm. The mean sizes follicles were not significantly different at all studied days. At the day of placement of sponges (Day 1) all ewes from Group 1 had corpora lutea, whereas 1 from Group 2 and 2 from Group 3 didn't (Table 1).

Table 1. Descriptive values for the follicles and corpora lutea

	Day 1				Day 5				Day 7		
Group	Total number of follicles	Mean diameter of follicles	Number of ewes with corpora lutea	Mean number of corpora lutea per ewe	Total number of follicles	Mean diameter of follicles	Number of ewes with corpora lutea	Mean number of corpora lutea per ewe	Total number of follicles	Mean diameter of follicles	Number of ewes with corpora lutea
First (n=8)	14	6.17±0.20	8	1.87	20	5.88±0.15	0		21	6.35±0.17	0
Second (n=9)	10	5.8±0.17	8	1.87	18	5.67±0.13	0		16	6.41±0.13	0
Third (n=9)	14	5.8±0.12	7	1.71	17	5.59±0.11	8	1.62	18	6.17±0.15	0

At the day of sponge removal there weren't ewes from Group 1 and Group 2 with corpora lutea, whereas only 1 ewe from Group 3 did not have coprus luteum. After 47 hours, all ewes from Group 3 didn't have corpora lutea.

The ESR, according to estrus detector, was similar at Group 1 and Group 2 - 7 ewes, whereas the results for Group 3 were worse - 5 ewes. The ESR, according to ram service, was highest at Group 1 - 100% and its value was significantly different from values of Group 2 and Group 3 (Table 2).

Fertility for Group 1 was 87.50% and its values was significantly different from values of Group 3 - 0% (neither of three ewes in estrus was pregnant after ram service) (Table 3). Fertlity of Group 2 was 66.67 % but with low fecundity - 1 lamb from 1 ewe. Fecundity for Group 1 was 171.4%.

Table 2. Estrus synchronization rate (ESR) of the three experimental groups according to estrus detector and ram service

Group	Total number of ewes in estrus, detected by ED	Total number of ewes, that were serviced by ram	ESR according to estrus detector, %	ESR according to ram service, %
First (n=8)	7	8	87.5	100.0*
Second (n=9)	7	3	77.77	33.33
Third (n=9)	5	3	55.55	33.33

Note. * Significant differences at P< 0,01 between Group I and Group II, Group I and Group III

Table 3. Fertility and Fecundity of the three experimental groups

Group	Fertility, %	Fecundity, %
First (n=8)	87.50*	171.4
Second (n=3)	66.67	100
Third (n=3)	0	

Note. * Significant differences at P< 0,001 between Group I and Group III

DISCUSSIONS

The aim of the present study was to study the reproductive response at Ile de France ewes after 5 days progestagen treatment plus or without PMSG. All of our experiments so far with short progestogen treatments were at the beginning of the breeding campaign. Over the last few years (2016, 2017, 2018, personal observations) the breeding campaign with the sheep from the flock have been stretched over time. Therefore, we chose to postpone the experiment two weeks after the onset of the breeding campaign to accelerate the campaign and also to see whether cyclic activity has started in sheep that have not exhibited so far estrus.

The presence of corpora lutea in 23 of all 26 sheep proved that sheep started to cycle (Table 1). The treatment of the first and second groups with a synthetic analogue of PGF 2α proved effective luteolysis. In the third group, which did not receive a synthetic analogue of PGF 2α, demonstrated that a 5-day treatment with synthetic progestogen was insufficient to regress the corpus luteum. This finding was in accordance with conclusion of Menchaca and Rubianes (2004), that in order to obtain good results in estrus induction after short treatment during the estrus season, it is necessary to provide regression of the corpus luteum. If luteolysis is induced at the beginning of short treatments, all females will maintain similar and adequate serum levels of exogenous progesterone during treatment. In our previous experiment (Metodiev and Raicheva, 2011) we evaluated the effect of 6 day progestagen treatments plus PMSG prior ram introduction on the estrus synchronization and the fertility of Ile de France ewes in the beginning of breding campaign we had two experimental groups – Group with Alfabedyl treatment and Group without Alfabedyl. Both groups had 91.66 ES, but with different fertility - 63.64% vs. 45.45%. On the basis of present and previous studies, we can conclude that treatment with a synthetic analogue of PGF 2α is obligatory at sponge placement, when scheme with short-term progestagen treatment are used.

In all three ultrasound observations, the state of ovaries in terms of the number and average size of the ovulatory follicles were the same. During the treatment, ewes from the experimental groups had similar number of ovulatory follicles and mean sizes follicles were not significantly different at all studied days (Table 1). These observations confirmed the conclusions of Menchaca and Rubianes (2004) that short-term progestagen treatments were adequate in the duration of a follicle wave.

The present experiment was the first, in which we used two different methods to detect estrus synchronization - estrus detector and rams. In our previous experiments we used only one of them. But in all previous experiments we used PMSG after sponge removal. The ESR according to the estrus detector was similar at Group 1 and Group 2 - 7 ewes, whereas the results for Group 3 were worse - 5 ewes. ESR according to ram service was highest at Group 1 – 100% and its value was significantly different from values of Group 2 and Group 3. The ESR in Group 1 was consistent with the results obtained in our previous experiments and also to these, reported by other authors – 80.0 and 100% until 144 hours after sponge removal (Ungerfeld and Rubianes, 1999; Viñoleset al., 2001; Aköz et al., 2006; Ustuner et al., 2007; Karaca et al., 2009; Martemucci and D'Alessandro, 2011; Metodiev and Raicheva, 2011; Metodiev and Raicheva, 2014; Metodiev et al., 2018, Martinez-Ros et al., 2018[1]; Martinez-Ros et al., 2019[3]; Martinez-Ros and Gonzalez-Bulnes, 2019)

The results for Groups 2 and 3 are more puzzling. Judging by the results of the estrus detector, those of Group 2 were similar to those of Group 1, while those of Group 3 were slightly lower in value. It was interesting why in sheep with ovulatory follicles and electrical resistance of the vagina responding to animals

with oestrus, these animals were not allowed to be covered. We suppose, this was due to the lack of sufficiently strong stimuli for long-term secretion of GnRH from the hypothalamus. Caraty et al. (2002) found that GnRH is involved in the control of receptivity in a ruminant species and suggested that in the cycling ewe the sustained preovulatory GnRH plays a physiological role in extending the duration of estrus.

We could conclude that if schemes without PMSG are used, stimuli of axis GnRH/LH should be done, for example external GnRH or new products, like the kisspeptin-10 analog C6. Martinez-Ros and Gonzalez-Bulnes (2019) concluded, that 5 days progestagen protocols with one dose GnRH, put on 56 hour after sponge removal, or two dose GnRH (put on sponge placement and second put on 56 hour after sponge removal) offer similar yields to eCG 5-days protocols. Decourt et al. (2019) examined the effect of the kisspeptin-10 analog C6 (palm-γ-Glutamyl-Tyr-Asn-Trp-Asn-Ser-GlyC[Tz]Leu-Arg (Me)-Tyr-NH2) as alternative of PMSG and the obtained results were promising.

Fertility for Group 1 was 87.50% and its values was significantly different from values of Group 3-0% (neither of three heat ewes was pregnant after ram service). Fertlity of Group 2 was 66.67% but with low fecundity - 1 lamb from 1 ewe. Fecundity for Group 1 was 171.4%. High fertility in Group 1 was in conformity with the suggestion of Menchaca and Rubianes (2004) that high short-term progestagen treatments follow PPSG treatment may better control follicular dynamics and improve fertility in small ruminants than long-term programs. In general, the results of fertility for Group 1 and Group 2 were in correspondence with the results to other authors, obtained after short terms progestagen treatment, natural mating and multiparous ewes (Ungerfeld and Rubianes, 1999 - 66.7%, Viñoles et al., 2001 - 87.0%; Karaca et al., 2009 - 71.6%, Martemucci and D'Alessandro, 2011 - 80%, Martinez-Ros and Gonzalez-Bulnes, 2019 - 76.5%, Martinez-Ros et al., 2019[1] - 80-90% (in breeding season), Martinez-Ros et al., 2019[3] - 60-80%).

Low values of fecundity (100%) of Group 2, compared to fecundity of Group 1 (171.4%), as well as zero fertility of Group 3 (Table 3), demonstrated that treatment without PMSG was unreliable for usage.

CONCLUSIONS

The protocol that consisted 5-day progestagen treatment plus synthetic analogue of PGF 2α put at the time of the placement of the sponges and PMSG at the time of the sponge removal successfully synchronized estrus with high levels of fertility and fecundity. Protocols, that consisted 5-day progestagen treatment plus synthetic analogue of PGF 2α put at the time of the placement of the sponges or removal of the sponges without PMSG are not suitable for ES, because of unsatisfactory results, that were obtained.

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RESEARCHES CONCERNING THE USE OF FEED INGREDIENTS TO REDUCE GREENHOUSE GAS EMISSIONS IN DAIRY COWS FARMS

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Abstract

From the multitude of solutions proposed to reduce greenhouse gas emissions, the strategies based on feed solutions are the most effective in the ruminant breeding sector. In most cases, the benefit is twofold, namely limiting the greenhouse effect and improving animal production. In practice, a reduction in gas emissions in the future is possible provided that farmers are aware of the need to invest in order to ensure this food adaptation to the new recommendations of researchers in the field. One way to reduce methane emissions is to increase the proportion of concentrated feed in the ration, which has been found to reduce CH_4 emissions per unit of ingested dry matter and liter of milk, as production remains the same or increases. Experiments performed on lactating cows indicated linear decreases in CH_4 emissions with an increase in the proportion of concentrated feed in the ration. Concentrated feeds also generally provide more digestible nutrients compared to bulky feeds, which could increase animal productivity. As a result, the decrease in fiber content and the faster passage of leguminous through the digestive tract of cows decrease the production of CH_4.

Key words*:dairy cows, feed, methane gas.*

INTRODUCTION

Gas emissions have increased by about 20% in recent years, which has exacerbated the greenhouse effect. This situation is also due to the emissions of polluting gases that ruminants produce.

The amount of greenhouse gas (GHG) that human activities release into the atmosphere each year is the equivalent of 49 billion tons of CO_2, and from this value the livestock sector, namely the amount of methane gas eliminated by ruminants, is equal to 13.5%, lying behindthe energy sector (25.9%), industrial (19.4%), forestry (17.4%), but ahead the transport sector (13.1%) (Lesschen et al. , 2011). The situation of gaseous emissions from the agricultural sector thus becomes one of the targets of the fight against global warming. CO_2 emissions exceeded the carbon-absorbing capacity of terrestrial and ocean vegetation almost a century ago.

Greenhouse gas (GHG) emissions associated with animal production contribute with an equivalent of 7.1 gigatonnes of carbon dioxide per year (14.5% of the total emissions from human activities), according to the Food and Agriculture Organization of the United Nations (FAO, 2014).

Globally, ruminants are estimated to produce 5.7 gigatonnes of CO_2 equivalent per year, accounting for about 80% of emissions in the livestock sector. In the case of dairy cows, emissions account for 35%,respectively 30% of emissions in the animal sector (equivalent to 4.6 gigatonnes of CO_2).

The average emission intensity corresponding to the products obtained from ruminants was estimated at 2.8, 3.4 and 6.5 kg CO_2 equivalent/kg milk corrected according to fat and protein corrected milk (FPCM) for milk from cattle (FAO, 2014).

It is estimated that a cow can emit more than 100 kg of methane each year during rumination. Methane is an important factor in the greenhouse effect, whose heating potential is twenty times higher than that of carbon dioxide (Euractiv, 2019).

The main sources of emissions are: production, processing and transport of feed (45% of the total); digestion of ruminants (39%); manure decomposition (10%). The rest is attributed to

the processing and transport of animal products (post-slaughter transport, refrigeration and packaging of animal products) (Dunca et al., 2011).
By carefully analyzing how these emissions occur, the significant reduction in emissions is within the reach of animal producers. Adopting current best practices and technologies for feeding, raising and ensuring animal health, manure management, and greater use of technologies for biogas generators and energy-saving devices would help the global livestock sector reduce greenhouse gases by almost 30%.
In this paper, the objectives are to analyze methods to reduce greenhouse gas (GHG) emissions in dairy farms.

MATERIALS AND METHODS

In animal production systems there is a strong link between resource efficiency and the intensity of GHG emissions.
Many of the actions recommended by FAO (2014) to improve efficiency and reduce greenhouse gas emissions also improve the production process, resulting in a quantitative and qualitative increase in food and higher incomes, with benefits for food security. and poverty reduction.
For animal production systems, emissions of nitrogen oxide, methane gas and carbon dioxide are losses of nitrogen, energy and organic matter which reduce their efficiency and productivity. Possible interventions to reduce emissions are therefore largely based on technologies and practices that improve production efficiency at the animal and livestock level.
Although the ways to reduce greenhouse gas emissions need to be adapted to specific local targets and conditions, currently available limitation options include:
- the use of feed and feeding techniques that take into account the digestibility, quality and composition of the feed ration, which can reduce the methane generated during digestion, as well as the amount of nitrogen and methane released by the decomposition of manure;
- improving the genetics and health of animals which contributes to a better conversion of feed into animal products, further reducing the intensity of emissions of animals;
- improving breeding strategies (replacement rate, age at first calving), reducing the number of non-productive animals in the herd and, therefore, emissions per unit of product generated in the herd;
- the modality of crop management on land surfaces, respectively the extension of pastures, is an important factor of emissions management, improving productivity;
- the application of current manure management techniques influences the release of methane and nitrogen oxide, ensuring the recovery and recycling of nutrients and energy, as well as the use of energy saving devices.

RESULTS AND DISCUSSIONS

For ruminants, the processes preceding the absorption of nutrients take place in a completely different way from that of animal species with a single-cavity stomach.
The digestive peculiarities of ruminants are determined by the existence of a gastric complex consisting of 4 compartments, the most important being the fact that it has a microflora and microfauna that allows the feeding of ruminants to be mostly fibrous, even if a single feed is used.
While the ruminant consumes fodder, certain microorganisms in the prestomacal cavity extract hydrogen. Hydrogen is used by other methanogenic bacteria to reduce carbon dioxide (created after digestion) into methane (Constantin, 2006). This process consumes between 2 and 15% of the energy provided by food. The synthesis of methane in the rumen environment is facilitated by the activity of methanoforming bacteria, such as *Methanobacterium ruminantium*. It is very sensitive to environmental conditions (Ellis et al., 2008). When these conditions are not favorable, methane production is reduced, which can lead to the switching of metabolic pathways of catabolism of pyruvic acid in the direction of propionic acid formation. The main conditions that determine the inhibition of the activity of methanoforming bacteria are: a high level of ingestion; finely processed fodder; their high starch content. Under these conditions, the rate of methane production is reduced, decreasing the production of acetic

acid and increasing the one of propionic acid (Morgavi et al., 2010).

Rumen fermentation is also influenced by the order of administration of feed assortments; it is more appropriate to administer the hay beforehand, followed by succulents and finally concentrates.

The administration of fibrous 90 minutes before the concentrates achieves a better digestion of cellulose in the rumen, maintains a wider ratio between acetic acid and propionic acid, increases the rate of bacterial protein formation, factors that contribute to maintaining normal limits within 24 hours for the rumen physiological value. Thus, there is a direct relationship between acetic acid production and methane production: the increase in the amount of pyruvic acid directed through the synthesis of acetic acid determines the increase in methane production (Ellis et al., 2008).

There is also a relationship between methane and propionic acid production: as the pathways of pyruvate metabolism are directed through propionic acid synthesis, methane production decreases (Miresan et al., 2003).

One way for farmers to reduce greenhouse gases in dairy farms is to choose the type and quality of feed that make up the rations of ruminants.

An important characteristic of feed that can have an impact on the production of enteric CH_4 is the ***quality of the feed***, especially its digestibility. Increased intake of poor quality feed with lower digestibility may have an insignificant effect on CH_4 production (Garg et al., 2018; de Vries et al., 2019). On the contrary, the increase in the ration intake of fodder with a higher digestibility causes a decrease in the amount of CH_4/kg of fodder consumed.

A number of factors, such as plant species, variety, crop maturity and preservation, can affect feed quality and digestibility. As the plant matures, the content of structural carbohydrates increases and that of fermentable carbohydrates decreases. Feeding at the right time is important to increase the amount and digestibility of nutrients in feed (Hristov et al., 2013b). Also, the different processes used for preserving feed (hay, silage, etc.) can negatively influence the nutritional value, if not done correctly.

Drehmel (2017) observed that the decrease in fiber content and the faster passage of legumes through the digestive tract of cows decrease the production of CH_4.

A substantial effort has also been made to ***develop varieties of fodder rich in important nutrients*** (proteins, lipids, carbohydrates) to ensure a mitigation of greenhouse gas emissions.

Hristov et al. (2013b) propose several options for reducing the amount of methane removed by ruminants by improving feed quality, including the correct management of pastures. As a result of these measures, which will ensure the quality green fodder with a high nutrient digestibility for dairy cows, increases in the efficiency of milk production will be obtained, which will probably lead to a decrease in CH_4 emissions.

Pasture management practices to reduce gaseous emissions from ruminants include shortening the grazing time, moving the animals from the pasture to time intervals to prevent the emission of N_2O or CH_4 into the soil. These measures will improve production efficiency and reduce GHG emissions per unit of production.

Keady et al. (2012) analyzed the effects of ***forage silage*** on the productive performance of animals and showed that a 10 g/kg increase in the concentration of digestible organic matter in the dry matter of the grass silo could increase the daily yield of milk obtained from cows with 0.37 kg. They also highlighted the unfavorable effect of silage on some plants that exceeded the optimal harvesting period. At the grass silo they found that the delay in harvesting the plants reduced digestibility by 3 to 3.5%. The same team of researchers pointed out that the use of bacterial inoculants in silage, with the addition of formic acid, especially in difficult silage conditions, can increase the performance of animals, which will reduce CH_4.

The inclusion of corn and alfalfa pickled fodder in lactating cow rations can also improve animal production (Groff and Wu, 2005) and the efficiency of nitrogen metabolism (Wattiaux and Karg, 2004), which could lead to a decrease in nitrogen in the urine and N_2O emissions from the application of manure.

The ***use of probiotics*** (lactic acid bacteria, Bacillus, yeasts) as a way to reduce ruminal methane gas can be used in ruminants to improve ruminal fermentation, dry matter intake and milk yield (Beauchemin et al., 2008). Due to the low prices and their wide use in feeding ruminants, the acceptance of probiotics in order to reduce CH_4 has a high probability.

Weinberg et al. (2003) and Huyen et al. (2020) indicated that probiotics based on lactic acid bacteria added to silage can positively influence fermentation by buffering rumen pH and oxygen consumption, improve nitrogen utilization and increase microbial protein synthesis in rumen. Another way to influence the composition of the ruminal microflora was to use as a probiotic the bacterium *Lactobacillus plantarum* in silage alfalfa (Mohammed et al., 2012) or *Enterococcus faecium* which increased the concentration of volatile fatty acids but decreased the concentration of methane (Mamuad et al., 2019).

There are also studies that have not shown any difference in methane levels in the case of consumption of pickled fodder with *Lactobacillus fermentum* or *Enterococcus faecium* (Jalč et al., 2004).

To reduce ruminant emissions, researchers at the University of Davis in California have experimented with ***alternative ways of feeding***, namely a combination of cow feed made from hay mixed with 1% natural red algae, *Asparagopsis armata*. To improve the palatability of the ration and mask the salty taste and specific smell, molasses was added (Searby, 2019).The conclusion was that the animals that consumed the ration containing algae had a significant reduction in methane gas. During experiments lasting two or three weeks, in which the concentration of algae was different, it was observed that the cows that consumed the highest content of algae eliminated half of the initial amount of gas.At the beginning of the experiment, it was assumed that algae could influence the taste and aroma of milk, but the sensory analysis of milk obtained from 25 people showed that the taste is normal. The only disadvantage was the decrease in milk production. An impediment to the use of algae, however, is the taste of the algae and the difficulties in obtaining large amounts of algae.

Another way to reduce methane emissions is to ***increase the proportion of concentrated feed in the ration***, which has been found to reduce CH_4 emissions per unit of ingested dry matter and animal product, as production remains the same or increases (Ferris et al. 1999; Sauvant and Giger-Reverdin, 2009; Aguerre et al., 2011).

Use of feed additives

Nitrates and sulfates have been studied as CH_4 emission mitigation agents (Yáñez-Ruiz et al., 2017; Brown et al., 2011). The potential problems with these compounds stem from the fact that the adaptation of the rumen microflora of the ruminant is not sufficiently known.

Plant extracts can be a natural and effective solution in limiting, as much as possible, green-house gases from ruminant breeding. Herbal extracts refer to phytonutrients or phytobiotics, such as phenolic compounds, saponins or terpenoids present in plant essential oils.

Munteanu et al. (2016) highlighted the importance of garlic polyphenols, and Gligor et al. (2017) identified and characterized these compounds in order to investigate their effect in the ration of dairy cows on productive performance, health, and ruminal fermentation.

Busquet et al. (2005), Benchaar and Greathead (2011) have observed that garlic essential oil reduced the proportion of acetic acid and volatile fatty acids with branched chain, and increased the proportion of propionic and butyric acid, as well as the concentration of nitrogen. These changes are consistent with the inhibition of methane in ruminal fermentations and bring beneficial changes in the rumen microbial activity.

Garlic inhibits methanogenesis and increases the acetate/propionate ratio (Blanch et al., 2016), as well as the concentration of butyric acid in ruminal fluid (Klevenhusen et al., 2011). Foskolos et al. (2015), Patra and Yu (2015) showed that dairy cow rations, supplemented with garlic phytonutrients, had antimicrobial action on a broad spectrum of bacteria, reduced nitrogen and increased propionic and butyric acid levels, highlighting in vivo the favorable effects of the use of active garlic compounds introduced in the feed of dairy cows.

Kolling et al. (2018) have observed that the use of essential oils extracted from oregano and green tea administered to feed lactating cows during 28-87 days of lactation reduced methane gas emissions during the first third of lactation and have the potential to be used as additives for cows.

Other studies have been conducted by researchers at Aarhus University in Denmark, who found that oregano essential oils do not have the ability to reduce gas emissions from digestion in ruminants (Olijhoek et al., 2019).

Other plant compounds with potential for greenhouse gas emissions produced by ruminants are tannins that are active both as inhibitors of methane and as modulators of NH_3 emissions from excretions (Woodward et al., 2001).

Methane gas emissions can be reduced by using tannin and saponin extracts in vitro, accompanied by a reduction in dry matter digestibility, organic matter digestibility and ammonia (Yogianto et al., 2014).

The addition of fat to the ration of ruminants has been used to reduce CH_4 emissions. The addition of fats reduces the fermentation of organic matter, the direct inhibition of methanogens in the rumen by the hydrogenation of unsaturated fatty acids. The greatest reduction is determined by unsaturated fatty acids, which act on the hydrogen in the rumen by dehydrogenation (Boadi et al., 2004).

Of the medium-chain fatty acids, caproic (C6), caprylic (C8) and myristic (C14) acids in coconut or palm oil are the most effective in attenuating CH_4 emissions. Moreover, fats are not metabolized in the rumen and therefore do not contribute to methanogenesis.

According to the studies conducted by researchers at the National Institute for Agricultural Research in Clermond-Ferrand, the use of flaxseed oil in the diet of dairy cows has led to a reduction in methane emissions, respectively cows that received flaxseed oil, 6% of them have reduced their methane emissions by 27 to 37% (Md Najmul, 2018).

CONCLUSIONS

From the multitude of solutions proposed to reduce greenhouse gas emissions, the strategies based on feed solutions are the most effective in the ruminant breeding sector.

In most cases, the benefit is twofold, namely limiting the greenhouse effect and improving animal production.

In practice, a reduction in gas emissions in the future is possible provided that farmers are aware of the need to invest in order to ensure this food adaptation to the new recommendations of researchers in the field.

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EXPERIMENTS ON A HOLSTEIN-FRIESIAN LINE ON THE EFFECT OF SELECTION FOR ROBUSTNESS ON FEEDING BEHAVIOUR

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Abstract

The consumption behavior of fodder in kg dry substance (SU) per day was followed for the duration of the fodder consumption in minutes per day, and the duration of the rumination in minutes per day, at 6 first-calf heifers in each group for 3 consecutive days. During the stall period, the group 1 first-calf heifers had higher fodder consumption than the group 2 first-calf heifers. The SU consumption was 19.5 kg/day in the group 1 and 17.2 kg/day in the first group 2. The duration of fodder consumption was higher in group 1 (robust first-calf heifers) compared to the duration of fodder consumption in group 2 (256 vs. 236 minutes per day). The average time to consume one kg of SU was on average 13.12 minutes for group 1 of animals and 13.70 minutes for group 2 of animals. The ideal cow for pasturing systems is the cow that consumes large quantities of green mass and efficiently transforms it into high milk production per kg live weight. The first-calf heifers in group 2 are slightly more efficient in pasturing than the robust first-calf heifers. Grass consumption was, on average, 16.4 kg Dry Substance in group 2 of animals compared to group 1 of animals 14.2 kg SU. The difference between the two consumptions 22.2 kg SU is statistically significant ($p<0.05$).

Key words*: robustness, body conformation, productive longevity, heifer.*

INTRODUCTION

The exploitation of dairy cows is based on the following types of behaviour: ingestion, dietary, metabolic or trophic behaviour; excretion or feeding behaviour; social behaviour; comfort, rest and sleep behaviour; exploratory and orientation behaviour in the environment (Cola and Cola, 2019).

Ingestion behaviour (food) is the innate and learned action of the animal to nourish and drink water, an action based on nervous and humoral mechanisms. This behaviour comprises 3 phases: the search for food, the procurement and contact with the food and the consumption of food and manifests itself differently in the pasture and shelter (Georgescu et al., 2007).

Excretion behaviour involves two physiological processes, resulting from metabolism-defecation and urination.

Social behaviour represents the group manifestations through which the relationships between animals are established. In the extensive growth the social behaviour approaches the natural one, and in the semi-intensive and intensive one there are constraints in their manifestation.

Rest and sleep behaviour is the interruption of activities to restore the body's power. This behaviour is different in pasture and shelter.

Exploratory and orientation behaviour represent the actions of investigating the environment in order to know and orientate the cows in the environment in which they live.

The daily time of the behavioural activities was measured by establishing a "behavioural routine" (Table 1) that serves as a basis for the evaluation of the productive performance and the economic losses due to a faulty management. The time allocated to the behavioural activities in 24 hours represents the net response of each cow to its growth environment.

Table 1 Daily time allocated to behavioural activities in lactating cows *

Activity	Time allocated to the activity per day
Fodder consumption	3-5 hours (9-14 meals a day)
Rest behaviour	12 – 14 hours
Social behaviour	2-3 hours
Rumination	7-10 hours
Water consumption,	30 minutes
Exploratory behaviour (including milking)	2.5-3.5 hours
Other activities	30 minutes

* Adaptation after Grant and Albright (2000)

Albright (1993) measured the daily behaviour of the Beecher Arlinda Ellen cow during lactation in which she set the world milk production record by recording the following: 6.3 hours of fodder, 13.9 hours of rest and 8 hours of rumination, of which 7,5 hours lying down and 30 minutes standing.

Dairy cows need to perform daily behavioural activities and should not interfere with the management routine.

Satisfying basic behavioural needs lasts almost 21 hours daily. Given this absolutely necessary time, it is easy to see how managerial practices can disrupt the behavioural time span. A deprivation of 3.5 hours from the rest area, fodder or water will force the cows to give up other activities or shorten the time allocated to the activities. Often the rest time and the fodder time are reduced with negative consequences on the productivity of dairy cows.

Starting from these aspects, several experiments were conducted at SCDA Şimnic Craiova regarding the behaviour of the primipara resulted in the first generation from the basic herd subject to improving their robustness.

MATERIALS AND METHODS

From the total herd of dairy cows from SCDA Şimnic was selected a genealogical line of Holstein Friesian cows with common genes from the famous STARBUCK bull (Canada). The database includes information from cows born in 2015, 2016 and 2017 and from the pedigrees of 10 breeding bulls. New phenotypic values are recorded periodically. It was expected that by 2018 the base herd (50 cows) will produce 105 F_1 products, of which 52 females and 53 males. Each group of features is composed of indicators that in turn are complex phenotypes formed of physiological phenotypes. These experiments took place in 2018 at SCDA Şimnic Craiova Dolj.

The experiments lasted 68 days, of which 34 days during the maintenance period at the stable (May 1-June 3) and 34 days during the grazing period (June 4-July 8).

Two primipara groups were brought to the experiments, of which group 1 of robust primipara (PR) and group II control group primipara (PM), primipara contemporaries with PR but from mothers not included in this study within the research Biobase of dairy cows in Şimnic. Each group included 11 animals.

During the stall period, the primipara were housed in two separate boxes, free on straw bedding and fed with a ration comprising 60% concentrates and 40% volume fodder. The composition of the concentrated fodder provided consisted of maize 472 kg, sunflower seed 276 kg, wheat 175 kg, minerals 40 kg and vitamins 37 kg (all in kg of dry substance per ton of concentrated mixture). Volume fodders included alfalfa hay and maize silage. The ration adjustment was done with brewers' grains. An accommodation period of 20 days and a measurement period of 14 days were allocated. The milking of the animals was done twice daily: 5:30 - 6:30 in the morning and, respectively, 15:30 - 16:30 in the evening, in a milking room 2 x 5 De Laval type. Animal feeding was done twice daily. During the 14 days of measurements the fodder consumption per primipara group was calculated as the difference between the quantities of fodder ingredients administered the day before and the quantities of fodder ingredients that were not consumed. The quantity consumed multiplied by 1.1 represents the quantity offered to the cows the next day.

During the grazing period the animals grazed a surface of Lolium perenne. An amount of approximately 18 kg of Dry Substance of green mass calculated at a cutting height of 4 cm was allocated daily. This allocation was made daily by measuring 4 quarts of 0.25 m^2 grass surface cut to a height of 4 cm from the ground with the sickle and chosen randomly.

The amount of grass in each quarter was weighed and samples were taken to determine the dry substance content (S.U.).

The height of the grass before grazing and after grazing was determined daily. The crude protein, acid detergent fibre, detergent fibre, raw energy and starch content and water soluble carbohydrate content were analyzed in the laboratory in the main fodders offered in the two experimental periods.

The consumption behaviour of the fodder in kg of Dry Substance per day was followed for the duration of fodder consumption during the day, and the duration of the rumination in minutes per day, at 6 primipara in each group for 3 consecutive days.

The data from the experiments was statistically private with MC Excel 2010.

RESULTS AND DISCUSSIONS

The content of Dry Substance of crude protein (PB), neutral detergent fibber (NDF), acid detergent fibre (ADF), starch, water soluble carbohydrates and raw energy of the fodders offered during an experimental period is presented in Table 2. The concentrates had an average content of Dry Substance of 854 g/kg, 236 g/kg of Dry Substance crude protein and 16.2 MJ/kg Dry Substance raw energy during the stall period and 864 g Dry Substance/kg, 220 kg/Dry Substance crude protein and 16.4 kg MJ/kg of raw energy respectively during the grazing period. The maze silage offered had a Dry Substance content of 334 g/kg and a starch content of 260 g/kg of Dry Substance.

The green mass (*Lolium perenne*) offered had a Dry Substance content of 182 g/Kg, crude protein of 195 g/kg of Dry Substance and raw energy of 11 MJ/kg of Dry Substance.

Before grazing, the height of the grass was on average 9.7 cm in the plot offered to the animals in group 1 and 10.4 cm in the plot offered to the animals in group 2.

During the stall period, the group 1 primipara had a higher fodder consumption than the group 2 primipara (Table 2.)

The consumption of Dry Substance was 19.5 kg/day in group 1 and 17.2 kg/day in group 2. It is worth mentioning that the difference in live weight between the two groups was 40 kg in favour of group 1. The duration of fodder consumption was higher in group 1 (robust primipara) compared to the duration of fodder consumption in group 2 (256 vs. 236 minutes per day).

The average time to consume one kg of Dry Substance was on average 13.12 minutes for group 1 animals and 13.70 minutes for group 2 animals.

During grazing, grass consumption was higher in group 2 animals compared to group 1 animals (16.4 kg Dry Substance vs. 14.7 kg Dry Substance). The grazing time was higher in group 2 compared to group 1 (582 vs. 530 minutes/day), and the duration of rumination was on average 366 minutes in group 2 animals and 350 minutes in group 1 animals.

Table 2 Chemical composition of the main fodders offered during the experiments

Specification	Period of stall				Grazing period			
	Concentrates		Silo maze		Concentrates		Green mass	
	$\dot{X}$	±ds*	$\dot{X}$	±ds*	$\dot{X}$	± ds*	$\dot{X}$	± ds*
SU g/Kg	854	20.2	334	10.8	864	14.2	182	20.5
Crude protein g/Kg SU	236	22.8	84	20.9	220	14.0	195	32.3
NDF g/kg SU	94	20.2	548	22	90	20.4	510	14.8
ADF g/kg SU	189	14.1	278	20.8	170	18.2	240	8.5
Starch g/kg SU	250	2.3	160	24.2	-	-	-	-
Water soluble carbohydrates g/Kg SU	-	-	-	-	-	-	70	40.2
Raw energy MJ / Kg SU	16.2	2.2	18.8	9.9	18.8	2.4	11.0	0.38

* standard deviation; SU = dry substance; NDF = neutral detergent fibre; ADF = acid detergent fibre

The difference between the two fodder consumption was 2.3 kg Dry Substance per day and per cow. The difference in weight and, implicitly, the metabolic weight of the robust primipara resulted in a higher consumption of Dry Substance during the stall period. There were no restrictions on access to fodder or water to the two groups of animals.

The ideal cow for grazing systems is the cow that consumes large quantities of green mass and efficiently transforms it into high milk production per kg live weight. As shown in (Table 3), the group 2 primipara are somewhat more efficient at grazing than the robust primiparae (figure 1).

Table 3 Food behaviour during the stall and grazing period

Specification	Group 1 primipara	Group 2 of primipara	Standard error of difference	Signifi-cance
Period of stall				
Total Dry Substance consumed (kg/day)	19.5	17.2	0.67	*
Total feeding time (min/day)	256	236	0.005	
Time per kg of Dry Substance consumed (minutes)	13/12	13.7	18.2	
Duration of rumination (minutes/day)	466	490	28.2	*
Grazing period				
Total Dry Substance consumed (kg/day)	14.2	16.4	0.68	*
Total feeding time (min/day)	19	17.6	0.68	
Time per kg of Dry Substance consumed (minutes)	530	582	18.2	**
Duration of rumination (minutes/day)	350	366	21.0	

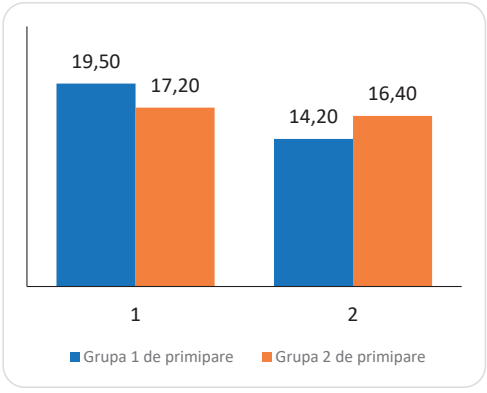

Figure 1. Dry Substance consumption during the year

Grass consumption was, on average, 16.4 kg Dry Substance in group 2 animals compared to group 1 animals 14.2 kg Dry Substance.

The difference between the two consumptions of 22.2 kg Dry Substance is statistically significant ($p<0.05$). The consumption of green mass at grazing is based on the length of time the pasture multiplied by the number of bowls swallowed by grass and the amount of grass per bowl swallowed by the animal. In most studies, the effects of environmental factors on grazing and less the genotypes of animals are examined. The duration of grazing was greater in group 2 of primipara (582 minutes/day) compared to group 1 of primipara (530 minutes/day). The same tendency was also observed regarding the duration of the rumination, 366 minutes for group 2 and 350 minutes for group 1 animals.

The higher fodder consumption in the robust primipara (group 1) observed in this study suggests a higher consumption capacity compared to the primipara in group 2, under feeding conditions in the stall. The selection for a higher weight at maturity results in a greater increase of the capacity of the rumen, which usually represents 0.022 of the body weight. Not the same thing was observed during the grazing period. This confirms that Holstein Friesian animals with large body growth do not behave as well as Holstein Friesian animals with lower body growth in terms of grazing. Further research is needed to clarify this aspect in terms of productive performance.

The results of this study suggest that the body weight and, in particular, the fodder consumption capacity are features with positive economic value. These features become even more important as the volume of fodder in the daily ration of cows is increasing. The use of concentrated fodder should not increase due to their costs and problems related to their consumption. The features related to the fodder efficiency of the cows should also be taken into account. The inclusion of features related to the efficiency of the dairy cow in the selection objectives makes possible the efficient use of fodder resources. For this purpose, the optimum body size of the dairy cow should be defined for the circumstances of a milk production system so that the use of volume fodder can be maximized. From this study it results that improving for body weight and greater ruminal capacity can further increase the profitability of a Holstein-Friesian cow.

The important interactions between breeding, nutrition, health and reproduction can be properly transposed into farm-level economic considerations.

The consumption capacity of dairy cows depends on three factors: fodder, management and animal. The factors related to fodder are

those related to composition and physical form. The management factors are those related to the restricted feeding strategy compared to the feeding at discretion, and the animal related factors are those related to the level of production, body size, age, physiological stage and genetic merit for the consumption of fodder. The ruminal capacity was defined as follows: maximum loading of the rumen with Dry Substance at any time. The dynamic model of digestion estimates the consumption capacity based on a coefficient of 0.021 multiplied by body weight. There are also some adjustments that take into account the level of production and the month of gestation.

The actual performance of a cow also depends on the feeding strategy. In this analysis it is assumed that cows have unlimited access to the consumption of volume fodder (alfalfa hay, green meal, corn silage) and limited for the consumption of concentrated fodder.

The concentrated fodder was allocated in a ratio of 1 kg concentrated fodder to 2.7 kg of milk.

CONCLUSIONS

The more robust primipara tend to consume larger quantities of fodder in the conditions of feeding at the stall. Their greater body growth results in greater gastrointestinal capacity in these animals.

Primipara with lower body development behave much better under grazing conditions, which suggests the creation of genotypes suitable for grazing systems.

Further research in other grazing seasons would also clarify these aspects in terms of productive performance.

The results of this study offer important information regarding the type of features to be considered within the objectives of genetic improvement in cattle specialized for milk production.

This study clearly shows the importance of the features related to the survival of the animals in the herd and the features related to the conception rate, both types of features are features related to the robustness of the dairy cows.

The robustness of dairy cows is a multi-character trait and reflects the combined success of several features (fertility, energy balance, longevity, docility, health, mobility, fitness). The economic value of these features is mainly determined by indirect effects, such as: reducing the percentage of replacement (and the costs of replacing animals in the basic herd); changes regarding the distribution of cows in age classes or changes in the number of reproductive days versus productive days.

During the life of a cow, managerial practices can disrupt the duration of behavioural times. A shortening by 3.5 hours of rest time, feeding or water consumption will force cows to give up other activities or shorten the time allocated to the above activities. Dairy cows need to perform daily behavioural activities and should not interfere with the management routine. Often the resting time and the feeding time are reduced with negative consequences on the productivity of dairy cows.

There is a sensitivity of economic values to changes in the price of milk components and the price of fodder. Changes of ± 20% as compared to the original values were taken into account in three situations: fixed herd, inputs with fixed concentrated fodder and total fixed milk production. The changes were made one at a time, keeping the other parameters to the original values.

The economic values have also been recalculated for all the characters assuming a change in the feeding strategy.

This strategy was selected on the basis of a study in which feeding strategies were compared on the basis of their efficiency to fully meet the nutritional needs of cows in a wider range of their productive status.

The strategy with the worst performance was selected for the recalculation of the economic values taking into consideration the assessment with fixed herd of livestock. In this case the cows were fed with fixed quantities of 6, 4 and 2 kg of concentrated fodder during the first 0-100 days, 101-200 and, respectively, over 200 days of lactation. Cows also consumed volume fodder without restrictions.

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STUDY ON HEMATOLOGICAL CHARACTERISTICS OF NATIVE RHODOPE SHORTHORN CATTLE BREED

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Abstract

We studied some hematological parameters in 36 cows from the autochthonous brachycephal Bulgarian breed - Rhodope short cattle. The blood for the study was taken twice - in spring and autumn, from cows bred in three different regions. We proved that the region and the season have a significant effect on hematological parameters. In the different regions, blood sugar ranges from 3.288 to 4.714 mmol/L, total protein from 50.67 to 58.48 g / L (P <0.001), cholesterol from 2.616 to 2.815 mmol/L, triglycerides from 0.258 to 0.595 mmol/L (P <0.001), creatinine from 217.8 to 254.1 µmol/L, Ca from 1.521 to 1.748 mmol/L, P from 3.073 to 3.218 mmol/L (P <0.05) and Mg from 0.649 to 1.497 mmol/L (P <0.001). Glucose, cholesterol (P <0.001), triglycerides (P <0.05) and minerals Ca, Mg (P <0.001) P (P <0.01) levels are higher in the fall compared to the spring while the levels of total protein (P <0.001) and creatinine (P <0.05) are lower in the fall compared to the spring.

Key words*: Rhodope short cattle; hematological parameters; season; regions.*

INTRODUCTION

The blood biochemical parameters, which are kept within certain reference values but at the same time are extremely sensitive to conditions leading to change in their functions (Ivanova et al., 2009), are used by the clinical practice as primary indicators of possible occurrence and development of pathological processes. By understanding the specificity and dynamics of the physicochemical and morphological blood changes within reference values and in case of pathology, there are ample opportunities to assess the condition of the individual.

According to Ježek et al., (2006, 2013), the blood serum biochemical analysis is a useful method for ascertaining the metabolic and health status of animals, however, when blood parameters are used, it is necessary detailed studies as they are influenced by the breed, age, physiological condition, and the lactation stage of the cow (Doornenbal, 1988).

In a study of glucose, total protein, hemoglobin, cholesterol, Ca and P levels in the blood of cows from the Bulgarian Rhodope Cattle breed during the autumn-winter period, Ivanova et al., (2009) found that the season had a significant influence only the phosphorus levels (P<0.05) which are lower during the winter when compared to the autumn. Apart from the hemoglobin, all other parameters examined in the Bulgarian Rhodope Cattle breed were higher when compared to that of the Bulgarian Black and White Cattle breed. The autumn-winter dynamics of the Bulgarian Rhodope Cattle parameters was less expressed than that of the Bulgarian Black and White Cattle.

Nikolov et al. (2009) report age differences in the cholesterol levels of cows from the Bulgarian Rhodope Cattle breed- its content is higher in younger animals. During the examination high ASAT/ALAT ratio, high hemoglobin and creatinine and low cholesterol levels, which are specific for the breed, were reported.

MATERIALS AND METHODS

The levels of major hematological parameters-glucose, total protein, triglycerides, cholesterol, creatinine, magnesium, calcium and phosphorus were examined during two critical periods-before and after the winter season. For this purpose, 36 blood samples were taken from *v. jugolaris*. The samples were taken twice - in spring and autumn, from cows with an equalized physiological status kept in three different regions, there were six blood samples per region. The hematological parameters were reported through a semi-automated analyser

with tests of ''BIOMED'' firm. The data were statistically processed via analysis of variance with the help of a specialized software (SPSS 21, IBM).

RESULTS AND DISCUSSIONS

The cows from the Rhodope Shorthorn Cattle breed are reared under harsh natural and climatic conditions and have a relatively poor diet. Taking the above mentioned in the account and searching for an explanation of the clearly exhibited significant seasonal differences in the reproductive ability, we examined some hematological parameters in cows before and after the period of mass calving- in autumn and spring- in different regions of the main areal.

The blood glucose level is a major interior index reflecting the balance of the carbohydrate-metabolism in the organism. In two of the regions, Smolyan and Haskovo, the cows from the Rhodope Shorthorn Cattle breed we examined exhibited values which were higher than the upper reference values (Table 1). The values were higher than that reported from Nikolov et al. (2012) with reference to the same breed in the same regions (2.40 nmol/l). The latter, however, were obtained during the winter but this study was carried out in autumn and spring.

Table 1. Serum blood sugar, total protein, cholesterol, triglycerides and creatinine content in the cows from different regions

Traits	Regions	N	LSM	± SE	SD	Min.	Max.
Blood sugar, mmol/L	Kurdzhali	12	3.288	0.181	0.937	1.940	4.920
	Smolyan	12	4.526	0.181	1.953	2.430	7.150
	Haskovo	12	4.714	0.181	2.039	2.110	7.460
Total protein, g/L	Kurdzhali	12	50.67	0.846	6.475	41.10	58.67
	Smolyan	12	55.28	0.846	3.598	49.70	62.65
	Haskovo	12	58.48	0.846	11.56	40.30	73.59
Cholesterol , mmol/L	Kurdzhali	12	2.616	0.139	0.870	1.390	4.100
	Smolyan	12	2.815	0.139	0.860	1.820	4.260
	Haskovo	12	2.640	0.139	0.549	1.910	3.650
Triglycerides, mmol/L	Kurdzhali	12	0.258	0.036	0.114	0.120	0.470
	Smolyan	12	0.595	0.036	0.227	0.190	0.920
	Haskovo	12	0.436	0.036	0.132	0.240	0.680
Creatinine, μmol/L	Kurdzhali	12	229.8	9.090	28.624	173.0	285.0
	Smolyan	12	254.1	9.090	45.231	170.1	325.0
	Haskovo	12	217.8	9.090	26.292	175.7	268.0

Note: LSM – average; SE – standard error; SD – standard deviation

Table 2 indicates that in spring, the average blood sugar levels are within reference values and are close to those typical for the winter period (Nikolov et al., 2012). Of all three seasons examined, apparent differences in the carbohydrate metabolism can be observed only during the autumn. The sseasonal changes can be clearly seen in Table 3.

Actually, the autumn is an unfavourable period for the cattle in the researched areal. The air temperature is high, the pastures are scarce with hay mostly, and the cattle is not supplementary fed by the farmers.

Shrikhande et al., (2008) have not found considerable seasonal variations in the blood glucose levels of lactating cows- they were respectively 2.46 and 2.67 nmol/L during the winter and spring period.

Table 2. Serum blood sugar, total protein, cholesterol, triglycerides and creatinine content in cows in the spring and the autumn

Traits	Season	N	LSM	± SE	SD	Min.	Max.
Blood sugar, mmol/L	Spring	18	2.712	0.148	0.453	1.940	3.600
	Autumn	18	5.640	0.148	1.353	3.390	7.460
Total protein, g/L	Spring	18	61.16	0.691	6.319	54.03	73.59
	Autumn	18	48.47	0.691	4.306	40.30	54.50
Cholesterol , mmol/L	Spring	18	2.108	0.114	0.299	1.390	2.510
	Autumn	18	3.272	0.114	0.610	2.340	4.260
Triglycerides, mmol/L	Spring	18	0.374	0.030	0.188	0.120	0.700
	Autumn	18	0.485	0.030	0.227	0.170	0.920
Creatinine, μmol/L	Spring	18	245.8	7.422	39.41	173.0	325.0
	Autumn	18	222.0	7.422	30.55	170.1	309.8

Note: N - Number of animals form each farm; LSM – average; SE – standard error; SD – standard deviation;

Table 3. Influence of the Season and the region on some hematological parameters of cows from the Rhodope Shorthorn Cattle breed

Traits	F- criteria and confidents level		
	Region	Season	Region of the season
Blood sugar, mmol/L	18.25***	195.40***	10.53***
Total protein, g/L	21.56***	168.72***	21.29***
Cholesterol , mmol/L	0.61	52.41***	1.28
Triglycerides, mmol/L	21.79***	7.08*	10.09***
Creatinine, μmol.l^{-1}	4.15*	5.13*	2.16
P, mmol/L	4.03*	9.44**	14.94***
Ca, mmol/L	2.06	968.78***	0.96
Mg, mmol/L	42.57***	178.77***	42.77***

Note: ***P<0.001; **P<0.01; *P<0.05; F-criteria and Sig – ration of the mean sums of squares of the regression equation and the residual and significance level

Figure 1 shows that in spring, the blood sugar levels of the animals from the three regions examined are practically the same. In autumn, however, the parameters of the cows from Kurdzhali region are more than 1/3 lower than those of the animals in the other two regions; the lowest levels are exhibited by the animals from Haskovo region.

Similar regional differences (Table 3) are also observed with reference to the serum triglycerides content (Figure 1).

As a whole, in the autumn their content is 29.6 % higher than that in spring (Table 2). Nevertheless, it is clear from Figure 1 that the above mentioned parameters refer two of the researched regions - Kurdzhali and Haskovo. The blood triglycerides content of the cows reared in Haskovo region is 35.1% higher in spring than in the autumn.

With reference to the cholesterol, the season is a source of variation ($P < 0.001$) while the region does not affect its values (Table 3).

According to Quiroz-Rocha et al. (2009), the total serum cholesterol concentration may be used as an index for energy balance of cows. The cholesterol is of vital importance for the proper function and the structure of the cell membranes. It is a compound of the steroid hormones, including estrogen, progesterone, testosterone and hormones related to the supra-renal function. Furthermore, the cholesterol acts as one of the organism natural barriers, protecting the cells from dehydration and infections. (Kaslow, 1997). In the light of the results we obtained, the latter is really interesting. Although they are within the established physiological references, during the less favourable autumn season, the blood cholesterol levels of the researched cows are 55.2% higher than in spring. The total protein levels are lower than the reference values for the cattle, being close to the minimum in spring. The feeding is proved to be the major factor influencing the blood protein levels. They are also affected by the condition of the liver, kidneys,

gastrointestinal tract, the stress, water loss and so on. During the less favourable autumn season, the total protein content in blood is (P<0.001) 20.4% lower, the decline being most considerable in Haskovo region (Figure 2)- 30.5%, and least noticeable in Smolyan region- 9.6%.

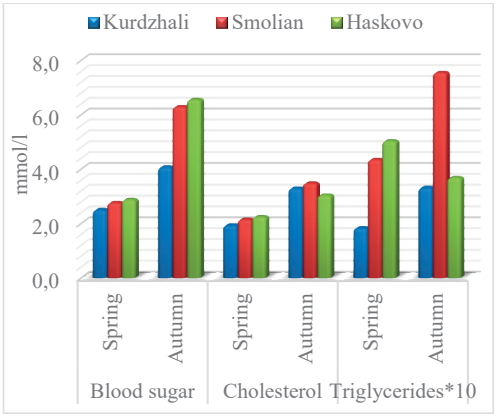

Figure 1. Seasonal dynamics of the blood sugar, cholesterol and triglycerides content in blood of cows from the Rhodope Shorthorn Cattle breed, reared in the different regions

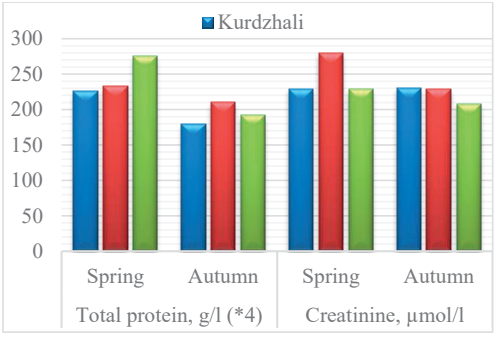

Figure 2. Seasonal dynamics of total protein and creatinine blood levels of cows from the Rhodope Shorthorn Cattle breed, reared in the different regions

Creatinine forms in muscles and usually has constant levels. In the case of the animals examined by us, the blood creatinine content during the two seasons is within close references but is significantly above the physiological norm (165 µmol/l). Even higher values were ascertained by Nikolov et al. (2012) upon examining the population during the winter period. Values higher than the upper reference levels were also reported with reference to the Bulgarian Rhodope Cattle, originating from the Rhodope Shorthorn Cattle (Nikolov et al., 2009).

Taking into account that the creatinine is formed in the muscles and that during intensive muscle activity the macroergic phosphate residue of the creatine phosphate is transferred to ADP forming ATP, and the creatine phosphate turns into creatinine, the above mentioned authors assume that the maintaining of high creatinine levels can probably be attributed to the energetic nature of the two breeds and the noticeable physical activity during grazing. Increased creatinine content after intensive muscle activity is also observed by Sato et al. (2001).

The Rhodope Shorthorn Cattle is extremely active and dynamic animal. Therefore, we can presume that the ascertained creatinine levels are probably close to the physiological ones which are typical of the breed. The region influences (P<0.5) the creatinine blood content of Rhodope Shorthorn Cattle, however, within the season, the regional differences are insignificant (Table 3).

Considerable (Table 4) and significant (Table 3) are the seasonal differences in the mineral blood content of the Rhodope Shorthorn Cattle breed. Such seasonal differences in the content of mineral substances in the blood of the cows are also observed by Ivanova et al. (2009).

All examined blood mineral values are higher in autumn than in spring. This can probably be accounted to the fact that in autumn, the concentration of mineral in the hay is higher.

The difference in phosphorus levels is minimal, while the calcium levels are two times higher in the autumn. In spring, the magnesium is within the lower physiological references and in autumn, it is 2.7 times higher. Although the minerals levels after the winter are considerably lower than those during the autumn, they are still within the physiological reference which indicates well functioning homeostatic mechanisms. According to Martens and Schweigel (2001), there are significant calcium and phosphorus reserves in the bones, but it is difficult for the organism to compensate the low magnesium content due to the lack of hormonal mechanisms of homeostasis.

Its content in the blood is mainly influenced by its intake with the food. At the same time the Mg-ATP complex is vital for all biosynthesis

processes in the body (glycolysis, energy dependent membrane transport, formation of cyclic AMP and transfer of genetic code) (Djokovic et al., 2014).

Our study ascertained significant regional differences regarding magnesium content in the blood of Rhodope Shorthorn Cattle breed (Table 5).

Table 4. Content of minerals in the blood of cows in spring and autumn

Traits	Season	N	LSM	± SE	SD	Min.	Max.
P, mmol/L	Spring	18	1.523	0.047	0.174	1.280	1.820
	Autumn	18	1.726	0.047	0.357	1.090	2.280
Ca, mmol/L	Spring	18	2.241	0.041	0.239	1.910	2.770
	Autumn	18	4.061	0.041	0.089	3.890	4.250
Mg, mmol/L	Spring	18	0.628	0.056	0.067	0.500	0.740
	Autumn	18	1.695	0.056	0.820	0.560	2.870

Note: N - Number of animals form each farm; LSM – average; SE – standard error; SD – standard deviation

Table 5. Content of minerals in the blood of cows reared in different regions

Traits	Reginos	LSM	± SE	SD	Min.	Max.
P, mmol/L	Kurdzhali	1.604	0.057	0.266	1.280	2.050
	Smolyan	1.748	0.057	0.307	1.370	2.280
	Haskovo	1.521	0.057	0.289	1.090	1.880
Ca, mmol/L	Kurdzhali	3.218	0.051	0.918	2.130	4.120
	Smolyan	3.073	0.051	0.940	1.990	4.030
	Haskovo	3.163	0.051	1.036	1.910	4.250
Mg, mmol/L	Kurdzhali	1.339	0.069	0.786	0.620	2.870
	Smolyan	0.649	0.069	0.073	0.540	0.760
	Haskovo	1.497	0.069	0.954	0.500	2.810

Note: LSM – average; SE – standard error; SD – standard deviation

Magnesium activates more than 300 enzymes. When its levels are too low, the organism defense systems decrease and the possibility of infections increases. Furthermore, the growth is slowing down and productivity and sexual activity diminish (Davoudi and Fazel, 2012).

In Smolyan region, its levels are two times lower when compared to the Mg levels in Eastern Rhodopes regions. They remain low in both examined periods (Figure 3).

Cattle have physiologically lower levels of inorganic phosphorus when compared to the other domestic animals due to the fact that the latter consume feeds which are richer in Ca. The phosphorus levels of the animals we studied are the highest in Smolyan region

where the lowest levels of calcium and magnesium are reported (Table 5). The lowest phosphorus level is reported in Haskovo region where the level of magnesium is the highest. With the exception of Mg no significant differences are observed between regions in different seasons with reference to all minerals examined (Figure 3). In magnesium, there was a general upward trend in its content in the blood during the autumn with the exception of Smolyan region where its levels were almost the same as those during the spring. The Mg content in the other two regions - Kurdzhali and Haskovo, during the autumn is 3 and 4 times higher.

The Ca content in all regions during the autumn is 75-85% higher than it is during the spring. Taking the quality of the food consumed during the two seasons into account, this is considered logical.

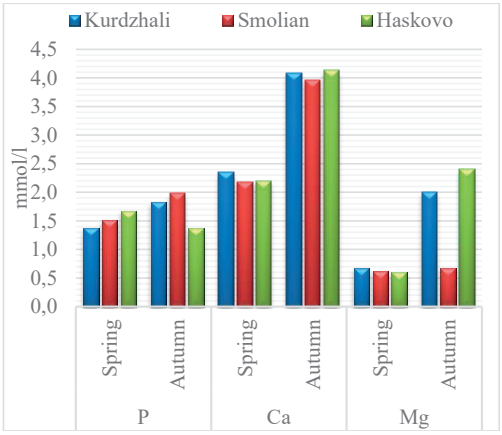

Figure 3. Seasonal dynamics of Ca, P and Mg content in the blood of cows from the Bulgarian Shorthorn Cattle breed reared in the different regions.

CONCLUSIONS

It can be concluded that the glucose, total protein, cholesterol, triglycerides, creatinine, Ca, P and Mg content in the Rhodope Shorthorn Cattle blood is seasonally affected.

There have also been regional differences ascertained regarding all parameters except the cholesterol and Ca.

The levels of total protein are lower and those of creatinine- higher than the physiological norms in all studied seasons and regions.

The blood sugar content is considerably above the reference values, the cholesterol and triglycerides levels are higher, and those of creatinine and total protein are lower in autumn.

In spring, the levels of minerals in the blood are lower than in the autumn; all of them are under the physiological norms.

As a whole, the mineral content is lower in Western Rhodopes, and more significant changes in the carbohydrate and protein metabolism are indicated in Haskovo region during the autumn.

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PRELIMINARY STUDY ON SOIL FAUNA AS A TOOL FOR MONITORING OF THE "SPRINGS COMPLEX OF CORBII CIUNGI" PROTECTED AREA -ROMANIA

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Abstract

The research objective was to demonstrate the use of soil fauna groups as a tool for monitoring of the "Springs Complex of Corbii Ciungi" protected area (the IUCN category 4), Dâmboviţa County, Romania. Some ecological indicators were quantified: taxonomic diversity, numerical abundance, dominance, evenness, equitability, Acari/Collembola ratio; Oribatida+Mesostigmata/Prostigmata+Astigmata ratio; correlations between the investigated communities and environmental factors (soil layer thickness, air temperature and humidity, soil temperature and moisture, soil pH, soil penetration resistance, exposure, slope, amount of organic carbon, total N, soil nutrients and the vegetation cover). Transects located near the water sources, were characterised by biological indicators with higher values. There was a significant interdependence between environmental variables. They influenced the abundance and distribution of the edaphic taxonomic groups. Statistical multivariate analysis showed that certain edaphic groups (from a total of 34 taxonomical taxa) are dependent especially on soil moisture e.g. Lumbricidae, Collembola and Oribatida.

Key words: *monitoring, soil fauna, environment factors, protected area.*

INTRODUCTION

Ecosystems that are maintained by direct or indirect access to groundwater, and are based on the flow or chemical characteristics of groundwater, are known as groundwater dependent ecosystems (GDE) (Aldous and Bach, 2011; Belvins and Aldous, 2011). GDEs provide valuable ecosystem services, such as supporting biodiversity (habitat for plant and animal species), providing basic river flows, water purification, flood control, water supply and recreational opportunities. Globally, GDEs are increasingly threatened because human exploitation often exceeds natural recharge rates (Gleeson et al., 2015). The types of surface GDEs are: spring, river / stream, wetland, estuary, accompanied by terrestrial vegetation, but they also occur in subterranean environments, such as aquifers, caves or hyporheic areas. From a water and ecological point of view, these are often connected to terrestrial and aquatic ecosystems, through transition zones (Tomlinson and Boulton, 2010). An important component of the biodiversity of this transition zones, as well as of terrestrial ecosystems that depend on groundwater is the soil fauna. Soil fauna include those animals that spend their entire life or only part of their development cycle in soil (Coleman and Hall, 2015).

The main objectives of the present study are the identification of some biological indicators within the soil fauna groups and description of the interrelationships between them and the analysed abiotic and biotic factors, in order to monitor the ecological quality of terrestrial ecosystems connected with GDEs (in our study the "Springs Complex of Corbii Ciungi" Protected Area).

MATERIAL AND METHODS

The study area

The Romanian protected area of Corbi Ciungi (*ca* 5 ha in extent) is of national interest and

corresponds to the IUCN category 4 (*i.e.* floral and faunal nature reserve). It was declared a protected area in 1966. Although located in Dâmboviţa County, it lies right on the border with Giurgiu County. The reserve focuses upon a complex of springs that feed two rivulets – Lisandru Vlăduţ and Cacaleţilor – which are themselves tributaries of the Neajlov River. The protected area comprises the springs and the floodplains of the rivulets together with adjacent grasslands and scrub, not only between Lisandru Vlăduţ and Cacaleţilor but also immediately adjacent to the west side of Lisandru Vlăduţ and the north and east side of Cacaleţilor. The Neajlov River forms the southern boundary of the reserve. It is located at $44^0$31'28.23" N and $25^0$30'43.66" E (the northernmost point); $44^0$31'01.47" N and $25^0$31'09.51" E (the southernmost point). Between the two streams, as well as above the terrace I of Neajlov, there is agricultural land cultivated with annual or biennial crops, generally wheat and maize. The area is predominantly agricultural, over 70% of the total area (Figure 1).

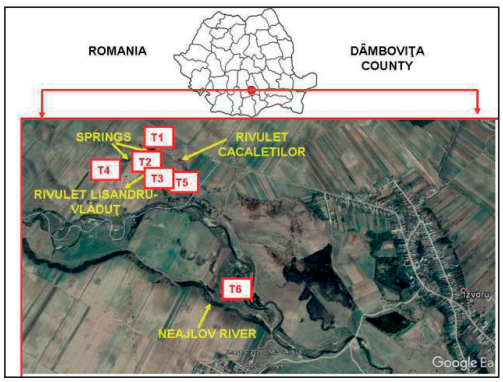

Figure 1. Geographical location of the "Springs Complex of Corbii Ciungi" Protected Area and of the investigated transects

In wooded and forest ecosystems, the dominant plant species were: *Salix fragilis L., Salix cinerea L., Alnus glutinosa (L.) Gaertn., Viburnum opulus L., Rhamnus frangula f. latifolia Dipp., Ligustrum vulgare L., Corylus avellana L., Euonymus verrucosus Scop., Cornus sanguinea L.* etc. Some species of bryophyte were identified: a) mosses *e.g. Cratoneuron commutatum (Hedw.) G. Roth* and *Brachythecium rivulare Schimp.*; and b) liverworts: *Aneura pinguis (L.)* and *Chiloscyphus polyanthus (L.).* Studies on invertebrates were made, especially on aquatic groups, but also on terrestrial ones, revealing the presence of 27 taxonomic units superior to the family (Botoşăneanu and Negrea, 1962; Ciubuc, 2007; Lotrean, 2012; The Official Monitor of Romania R.A., 2015).

Selected biological indicators

In order to monitor the conservation status and ecological diversity of the soil fauna, we selected the following biological indicators: number of taxa (Shannon diversity index), numerical abundance, dominance, evenness, equitability (Moretti et al. 2009); OM/PA index (Oribatida + Mesostigmata / Prostigmata + Astigmata) (Bedano et al., 2011); and Acari/Collembola ratio (A/C) (Perdomo et al., 2012; Yan et al., 2012; Deed, 2015). Some indexes were calculated using the statistical soft PAST (Hammer et al., 2001).

Bedano et al. (2011) found that natural areas have a higher OM / PA index value than those that are disturbed (or under stress). The A/C ratio is based on the numerical abundances of individuals from these two faunistic groups. In natural conditions this ratio has a value higher than 1. In contrast, where there is anthropogenic impact or soil degradation, this ratio changes in favour of Collembola and its value decreases (Bachelier, 1986; Visioli et al., 2013).

The soil fauna

The study was made using the transect method, following the soil moisture gradient. Six transects (T1-T6) were chosen, distributed in relation to the water source (Figure 1). The soil fauna was collected in July, 2019, using a square metal frame, with dimensions of 10X10X10 cm. In total, 30 soil probes were taken over an area of approximately 3000 s.q.m. (5 samples/ transect). The soil fauna groups were extracted using the Tullgren-Berlese method (by natural drying for 20 days) (Koehler and Melecis, 2010). Identification of soil fauna groups was performed on the Carl Zeiss stereomicroscope, and their preservation was made in ethylic alcohol pf 90^0. Taxonomic identification was done using the following keys (Dindal, 1990; Orgiazzi et al., 2016; Krantz, 2009). The statistical soft PAST was used in order to make a correspondence analysis (CA) and a canonical

correspondence analisys (CCA) (Hammer et al., 2001).

The six transects investigated were positioned as follows:

- transect T1 was located near the first spring (at 5 m distance) at 44.524361 N and 25.512138 E, at an altitude of 122 metres, on a slope of 20% and with South-east exposure. The investigated habitat is scrub. The dominant plant species were: *Euonymus verrucosus Scop., Rosa canina L., Cornus sanguinea L., Ligustrum vulgare L. and Prunus spinosa L.*
- transect T2 was located at 20 metres distance from a spring, at 44.524069 N and 25.512155 E, at an altitude of 120 meters, on a slope of 5% and with South-east exposure. The investigated habitat is a meadow. The dominant plant species were: *Alopecurus pratensis L.* and *Sanguisorba officinalis L.*
- transect T3 was located at 5 metres distance from the second spring, at 44.523185 N and 25.512108 E, at an altitude of 120 metres. The investigated habitat is scrub. The dominant plant species were: *Rosa canina L., Cornus sanguinea L., Euonymus verrucosus Scop., Ligustrum vulgare L.* and *Prunus spinosa L.*
- transect T4 was located at 20 metres distance from the second spring, at 44.523663N and 25.511809 E, at an altitude of 120 metres, on a slope of 5% and with South exposure. The investigated habitat is a meadow. The dominant plant species were: *Alopecurus pratensis L.* and *Sanguisorba officinalis L.*
- transect T5 was located at 1 metre distance from the rivulet Lisandru-Vlăduţ, at 44.521888 N and 25.511166 E, at an altitude of 123 metres, on a slope of 10% and with South exposure. The investigated habitat is scrub. The dominant plant species were: *Rubus caesius L., Viburnum opulus L., Cornus sanguinea L., Aegopodium podagraria L., Corylus avellana L.* and *Rosa canina L.*
- transect T6 was located at 2 metres distance from the Neajlov river, at 44.519222 N and 25.514583 E, at an altitude of 115 metres, on a slope of 15% and with South exposure. The investigated habitat is riverine scrub. The dominant plant species were: *Lysimachia vulgaris L., Cirsium arvense L., Salix fragilis L., Amorpha fruticose L., Urtica dioica L., Lythrum salicaria L., Carex riparia L.* and *Salix purpurea L.*

Abiotic environmental factors

In total, 30 soil samples were analysed for environmental and soil chemical variables. The environmental were: litter-fermentation layer thickness (OLF); humus layer thickness (OH); soil layer thickness (OS); air temperature (T^0C), air humidity (H%); soil temperature (T^0C), soil moisture (M%); soil pH (in aqueous suspension 1:2.5; SR 7184-13: 2001; PTL 04); soil penetration resistance -SPR (PSI), measured with penetrometer STEP Systems GmbH; exposure and slope. At the same time, quantified chemical analyses were: the amount of organic carbon (humus: wet oxidation; STAS 7184/21-82; PTL 12); total N (Kjeldahl method; STAS 7184/2-85; PTL 09); PAL (extractable phosphorus) was also analysed in ammonium acetate-lactate; STAS 7184/19-82; PTL 19P); KAL (potassium extractable in ammonium acetate-lactate; STAS 7184/18-80; PTL 22) and mobile forms of Zn, Cu, Fe, Mn (using atomic absorption spectrophotometry, SR ISO 11047: 1998, PTL 32).

The biotic factor investigated was vegetation coverage (%) on each soil sample.

RESULTS AND DISCUSSIONS

Analysis of the thickness of the soil layers from the six transects showed that the humus layer was the best represented. The highest value of this parameter was recorded in T5, but the litter and fermentation layer was well represented in T1, and the soil layer in T6. The quantification of the vegetation coverage indicates a maximum value of 100%, in T4 (Table 1).

Table 1. Thickness of the soil layers (cm) and the vegetation coverage (%) from transecs (T) in "Springs Complex of Corbii Ciungi" Protected Area, 2019

T	OLF (cm)	OH (cm)	Os (cm)	Veg.cov. %
T1	5 ±1.87	4.8 ± 1.48	0.2 ± 0.44	15.4 ± 4.56
T2	2.9 ±1.24	7 ± 1.22		72 ± 37.01
T3	3 ± 1.58	7 ± 1.58		32 ± 14.76
T4	3 ± 0.70	7 ± 0.70		100 ± 22.1
T5	1.8 ±1.89	8.2 ± 1.89		2 ± 0.44
T6	0.48 ±0.32		9.52 ± 0.32	28 ± 13.33

The highest values of the air and soil temperature were recorded in T4, and the lowest in T5. Air humidity and soil moisture were highest in T5, and lowest in T4 (Table 2).

Table 2. Air temperature and humidity, soil temperature and moisture from transects (T) in "Springs Complex of Corbii Ciungi" Protected Area, 2019.

T	Tair (^{0}C)	Hair (%)	Tsoil (^{0}C)	Msoil (%)
T1	22.82 ± 0.38	72.8 ± 2.28	16.78 ± 0.71	62.4 ± 4.62
T2	25.72 ± 2.29	63.1 ± 4.92	20.62 ± 2.94	41.18 ± 5.37
T3	26.7 ± 0.46	66.8 ± 2.16	19.42 ± 2.39	55.76 ± 6.30
T4	31.24 ± 0.811	55 ± 5.78	23.44 ± 6.37	28.68 ± 9.88
T5	21.8 ± 0.59	76.2 ± 2.58	16.58 ± 0.43	69.32 ± 3.88
T6	28.24 ± 1.10	62 ± 4.58	20.02 ± 1.18	49.24 ± 4.27

With regard to soil penetration resistance, the most resistant soils were in T4 and T2. The most acid soil was recorded at T1 and on opposite at T5 and T6, where the quantity of nutrients was higher in comparison with T4 and T2 (Table 3). Although soil pH is a good indicator of the balance of nutrients, electrical conductivity reflects the amount of nutrients available in the soil. Only nutrients that are water-soluble are "available" for plant absorption. In strongly acidic soils, Al and Mn become highly mobile and available to plants, being toxic to them, while Ca, P and Mg are not available to plants. In strongly alkaline soils, P and most micronutrients become less mobile (Pagani et al., 2014). Electric conductivity had high values in T5 and T6, but low values in T2 and T4 (Table 3).

Table 3. Soil penetration resistance (SPR), electric conductivity (CE) and soil acidity (pH) from transects (T) in "Springs Complex of Corbii Ciungi" Protected Area, 2019

T	SRP (PSI)	CE (µS)	pH (unit pH)
T1	170 ± 0.60	96.72 ± 24.16	5.62 ± 0.42
T2	250 ± 0.16	47.72 ± 11.15	6.54 ± 0.09
T3	66 ± 0.55	154.68 ± 97.32	6.86 ± 0.32
T4	270 ± 0.49	38.52 ± 7.76	6.58 ± 0.29
T5	94 ± 0.57	282.6 ± 112.30	7.1 ±0.43
T6	110 ± 0.33	301.8 ± 207.22	7.33 ± 0.38

For soil nutrients (macro and microelements), the following results were obtained: P had the highest concentrations in T5 and T6; K in T1 and T4; Zn in T3 and T5; Cu in T2 and T4; Fe in T1 and T6; and Mn in T1 and T6. In general, the lowest values of these nutrients were found in T2 and T4 (Tables 4, 5).

Table 4. Macroelements from soil transects (T) of "Springs Complex of Corbii Ciungi" Protected Area, 2019

T	$P_{AL\ (mg/kg)}$	$K_{AL(mg/kg)}$
T1	11.76 ± 6.64	230.3 ± 40.57
T2	7.92 ± 4.16	183.65 ± 29.48
T3	21.48 ± 9.96	106.18 ± 29.86
T4	8.36 ± 4.80	195.42 ± 36.01
T5	20.56 ± 13.20	102.53 ± 20.31
T6	76.36 ± 39.96	97.66 ± 36.20

Table 5. Microelements from transects (T) of "Springs Complex of Corbii Ciungi" Protected Area, 2019.

T	Zn(mg/kg)	Cu(mg/kg)	Fe(mg/kg)	Mn(mg/kg)
T1	2.18 ± 0.29	1.96 ± 0.42	260.28 ± 138.11	10.44 ± 1.64
T2	0.87 ± 0.25	2.61 ± 0.32	60.58 ± 16.36	4.42 ± 1.46
T3	4.09 ± 2.83	1.2 ± 0.47	123.54 ± 43.45	8.92 ± 6.5
T4	1.44 ± 0.40	2.11 ± 0.19	43.86 ± 15.43	5.42 ± 0.61
T5	3.55 ± 2.03	0.9 ± 0.36	87.2 ± 52.68	9.28 ± 1.60
T6	2.74 ± 1.20	1.99 ± 0.44	128.42 ± 61.23	27.72 ± 12.73

Samples from T1 and T5 were the most humus-rich (the quantity of organic carbon), whereas total nitrogen and the C/Nt ratio showed maximum values in T3 and T5 (Table 6).

The C/Nt ratio is a sensitive indicator of soil quality. It is considered an indicator of the mineralisation capacity of nitrogen. A ratio greater than 15 (C/Nt> 15) may slow the rate of decomposition of organic matter and organic nitrogen, limiting microbial activity, while a low ratio may accelerate the rate of decomposition. The presence of organic matter is a favourable factor for development of edaphic invertebrate populations (springtails, nematodes, enchytraeids, etc.), which in turn represent the food source for predator groups (such as mites) (Klarner et al., 2013).

Analysing the C/Nt ratio of the soil from this protected area, we found that a slower rate of decomposition of organic matter was recorded in T3 and T5, although this process is not very pronounced and the ratio did not greatly exceed the value of 15 (Table 6).

Table 6. The content of organic carbon (Corg) and of total nitrogen (Nt) from soil transects (T) of "Springs Complex of Corbii Ciungi" Protected Area, 2019

T	Corg.(%)	Nt (%)	C/Nt
T1	5.14 ± 0.55	0.42 ± 0.05	14.24 ± 1.04
T2	1.83 ± 0.28	0.17 ± 0.01	12.26 ± 1.26
T3	11.41 ± 6.9	0.83 ± 0.42	15.53 ± 1.69
T4	1.98 ± 0.28	0.2 ± 0.02	11.56 ± 0.67
T5	8.53 ± 3.63	0.61 ± 0.21	15.92 ± 1.75
T6	1.40 ± 0.61	0.15 ± 0.08	11.82 ± 2.58

From the taxonomic point of view, the biological material revealed the presence of 34 groups. These were classified in eight taxonomic classes: Oligochaeta (ord. Haplotaxida), Nematoda, Diplopoda (ord. Iulida), Chilopoda (ord. Lithobiomorpha, Geophilomorpha), Malacostraca (ord. Isopoda), Entognatha (ord. Collembola, Diplura), Insecta (ord. Hymenoptera, Coleoptera, Diptera, Hemiptera, Psocoptera), and Arachnida (ord. Trombidiformes, Sarcoptiformes, Mesostigmata, Araneae, Pseudoscorpionida) (Table 7). The numbers of insect and mite larvae were evaluated at the same time. In total, 4180 individuals were identified, from which 350 were immature mites and 107 individuals were insect larvae. The highest numerical abundance was recorded in the following taxonomic groups: Collembola (1108 individuals), Oribatida (969 individuals), Opiidae (540 individuals) and Mesostigmata (769 individuals); in contrast Nematoda, Chrysomelidae, Erytraeidae and Belbiidae were each represented by a single individual).

Those taxonomic groups most abundant in the protected area reflect soils rich in organic matter. From a trophic point of view, these taxonomic groups participate directly in the decomposition of organic matter, and the presence of a favourable habitat leads to high populations, which in turn are a source of food for other invertebrates (such as Mesostigmata).

Examining spatial variation over the study site, the highest numbers of soil invertebrates were recorded from transects located close to the different water sources (springs or rivulet), where the soil moisture was high (e.g. T1, T3, T5, T6). Similar trends were observed in the diversity of soil invertebrate groups. The lowest values of these two parameters were obtained in T2 (17 taxonomic groups with 306 individuals) and in T4 (20 taxonomic groups with 176 individuals).

The same trend was found in the totals of insect larvae and immature stages of mites (which is an indicator of the viability of the studied ecosystems), with maximums in T3 and T5 (Tables 7 and 8).

The numerical abundances of the soil taxonomic groups were evenly distributed in T2 and T4. In a few transects some taxonomic groups were notably dominant in terms of numerical abundance (in T5 and T6) e.g. Collembola, Oribatida, Opiidae and Mesostigmata. Dominance, evenness and equitability indices showed similar trends (Table 8).

The Acari/Collembola ratio was another useful biological indicator for monitoring the quality of terrestrial habitats dependent of groundwater and surface water. The recorded values of this ratio were higher than 1 in all six investigated transects, with the highest value (12.10) in T2 (Table 8).

Turning to the OM/PA ratio, small values were recorded if the mite communities were negatively influenced by an abiotic disturbance factor. The values of the OM/PA ratio were markedly higher in T1 and T5, and lowest in meadows (T2 and T4), where the soil humidity was lower and the distance to the water sources longer (Table 8).

Table 7. Numerical abundance of taxonomic groups from transects, in "Springs Complex of Corbii Ciungi" Protected Area, 2019

Taxa	Short name	T1	T2	T3	T4	T5	T6
Phylum Annelida							
Class Clitellata							
Subclass Oligochaeta							
Order Haplotaxida							
Family Lumbricidae	Lum	1		8	2	1	1
Family Enchytreidae	Enc	3	5		1	12	
Phylum Nematoda	Nem					1	
Phylum Arthropoda							
Sub-phylum Myriapoda							
Class Diplopoda	Dip	2		1	1	1	
Ord. Julida	Iul	1	5	14	1	5	
Class Chilopoda							
Ord. Lithobiomorpha	Lit		3	1	1	12	1
Ord. Geophilomorpha	Geo					2	
Sub-phylum Crustacea							
Class Malacostraca							

Ord. Isopoda	Iso		6		1		
Sub-phylum Hexapoda							
Class Entognatha							
Ord. Collembola	Col	103	20	146	28	621	190
Ord. Diplura	Dip				4		
Class Insecta							
Ord. Hymenoptera							
Family Formicidae	For	10	5	2	4	7	2
Ord. Coleoptera	Cole			4		3	
Family Staphylinidae	Sta	1	2	1		3	2
Family Chrysomelidae	Chr						1
Ord. Diptera	Dip			1		1	1
Ord. Hemiptera							
Superfamily Aphidoidea	Aph					20	
Ord. Psocoptera	Pso				2	2	26
Insect larvae	Ins l	12	18	12	7	28	30
Sub-phylum Chelicerata							
Class Arachnida							
Superorder Acariformes							
Ord. Trombidiformes							
Subord. Prostigmata							
Family Trombidiidae	Tro	1		1		3	
Family Bdellidae	Bde		2		1	1	6
Family Cunaxidae	Cun		17	4	8		
Family Johnstoninidae	Joh	2	3	5			2
Family Labidostommidae	Lab	4		2			
Family Tetranychidae	Tet	2					
Family Erythraeidae	Ery	1					
Ord. Sarcoptiformes							
Subord. Oribatida	Ori	105	35	212	33	545	39
Family Opiidae	Opi	102	92	18	12	219	07
Family Bellbiidae	Bel		1				
Subord. Astigmata							
Family Acaridae	Aca	2	5	37	6	6	2
Family Scutacaridae	Scu			2		3	2
Ord. Mesostigmata	Mes	67	19	222	42	371	48
Mites immatures	Mi im	32	68	95	20	118	17
Ord. Pseudoscorpionida	Pse	2		4	1		
Taxa	Short name	T1	T2	T3	T4	T5	T6
Ord. Araneae	Ara	1			1		
Numerical abundance (ind.)		454	306	792	176	1985	476
Number of taxa		20	17	21	20	23	17

Table 8. Soil biological indicators from "Springs Complex of Corbii Ciungi" Protected Area, 2019.

Soil fauna indicators	T1	T2	T3	T4	T5	T6	Total
No. of taxonomical groups	20	17	21	20	23	17	34
Numerical abundance	454	306	792	176	1985	467	4180
Dominance	0.18	0.17	0.20	0.14	0.22	0.24	
Shannon	1.94	2.14	1.89	2.27	1.74	1.82	
Evenness	0.35	0.50	0.31	0.48	0.25	0.36	
Equitability	0.65	0.76	0.62	0.76	0.55	0.64	
A/C	3.09	12.10	4.04	4.21	2.04	1.07	
OM/PA	22.83	5.65	8.86	5.80	87.31	15.33	

In order to explore how the invertebrate fauna is grouped according to the proximity of the analysed transects to water sources, we performed a correspondence analysis (CA), from which we obtained a classification of several taxonomic invertebrate groups related to the three types of water source (spring, rivulet and river):

- transects T2 and T4, located in meadows, were characterised by communities of Diplopoda, Formicidae, Cunaxidae and Araneae.
- transects T1 and T6, located closed to the water sources (rivulet and Neajlov River) were characterised by Staphylinidae, Erytraeidae, Insect larva, Lythobiomorpha and Opiidae.
- transects T3 and T5 were characterised by Oribatida, Mesostigmata, Coleoptera, Lumbricidae, Acaridae, Oribatida, Pseudoscorpionida and respectively by Collembola, Diptera, Scutacaridae, Nematoda (Figure 2).

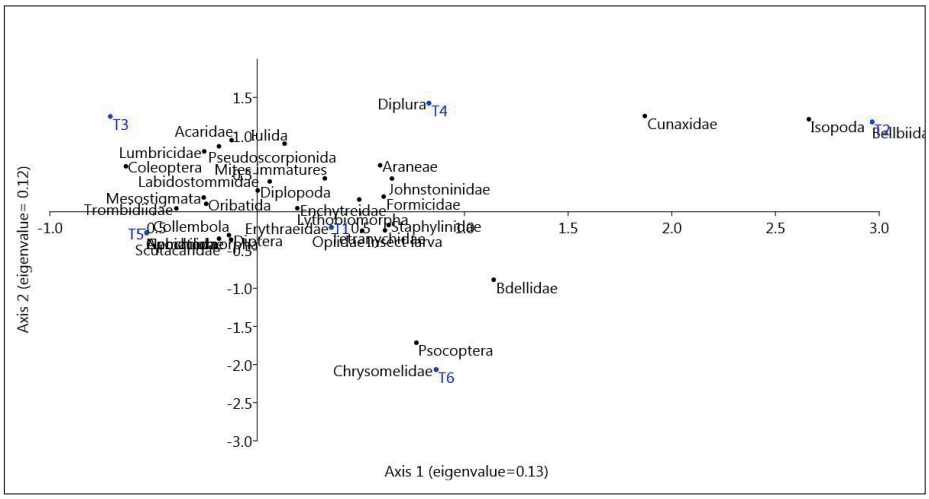

Figure 2. Correspondence analysis (CA) between identified taxonomical groups and analysed transects from "Springs Complex of Corbii Ciungi" Protected Area, 2019.

From analysis of the influence of abiotic and biotic environmental factors on the soil taxonomic groups, we observed that soil moisture, the decomposition rate of organic matter (C/Nt) and soil acidity positively influenced the numerical abundance of three invertebrate groups: Lumbricidae, Collembola and Oribatida (which are hydrophilous). These taxonomic groups are considered to be the most important edaphic invertebrates, participating directly inorganic matter decomposition (Figure 3).

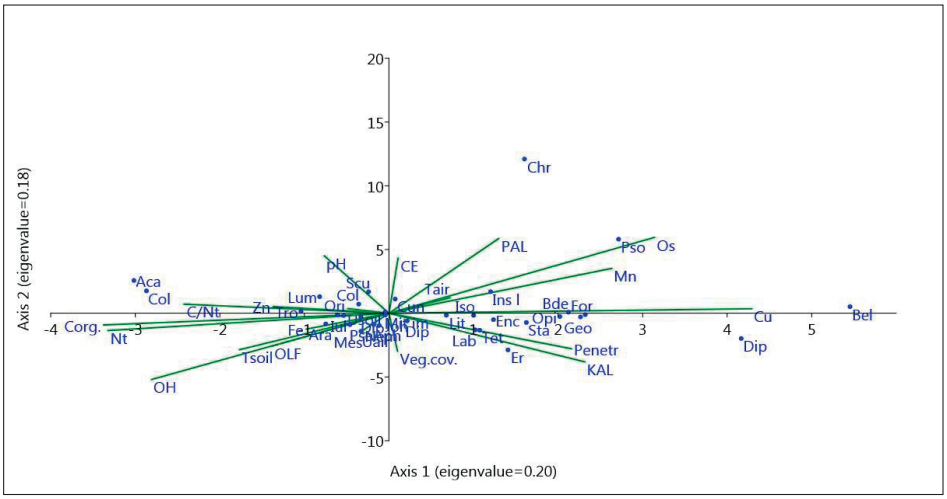

Figure 3. Canonical correspondence analysis (CCA) between identified taxonomical groups and abiotic – biotic factors, from "Springs Complex of Corbii Ciungi" Protected Area, 2019

CONCLUSIONS

Groundwater depended ecosystems are well represented in the "Springs Complex of Corbii Ciungi" Protected Area. Six transects were selected (T1-T6) on the basis of their proximity to water sources, in order to evaluate some biological indicators, that reflect the ecological status of the investigated ecosystems. Transects T2, T4 and T6 (the furthest from the water sources) were characterised by: a) their greater thickness of soil layers OLF and OH; b) higher

values of air temperature and humidity, soil moisture, soil penetration resistance, electrical conductivity, content of Nt, Corg and macronutrients; and c) lower values of soil temperature and a soil pH that is slightly acid to neutral.

The Springs Complex of Corbii Ciungi" Protected Area was investigated from the biological point of view, in as well. In total 30 soil samples were analysed.

Thirty-four taxonomic groups were identified, with a total numerical abundance of 4180 individuals. The results of this study indicated that transects located closed to the water sources (T1, T3 and T5) were characterised by the more numerous and diverse invertebrate populations than in T2 and T4. The results for T6, located near the Neajlov River, departed somewhat from this pattern. Although close to a water source, the combination of very variable ground level, and hence distance from the water surface (1.5 m), and the sandy substrate, led to lower values of taxonomic diversity and numerical abundance. The same phenomenon was observed with the environmental variables recorded at the site i.e. lower soil moisture, lower amount of organic matter, etc.

There is a significant interdependence between these environmental variables, influencing the abundance and distribution of edaphic taxonomic groups in the groundwater-dependent ecosystems. Multivariate analysis indicated that certain edaphic groups (e.g. Lumbricidae, Collembola and Oribatida) are dependent on soil moisture.

The present study demonstrated that soil invertebrate groups could be used as bioindicators for monitoring the ecological status of terrestrial ecosystems, which in this protected area are connected with groundwater dependednt ecosystems (GDEs).

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RESEARCHES REGARDING WEIGHT EVOLUTION CONSIDERING THE YOUNG FEMALE MATING'S AGE

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Abstract

In the researches carried out, several aspects were followed through which an objective analysis of the possibilities of early use for reproduction of the young sheep of the Karakul breed from Botoşani was pursued. In this sense, three batches were formed of young females who had an average age of 9 months (L1), 15 months (L2) and 20 months (L3). In order to eliminate the influence of external factors, all batches benefited from the same experimental treatment for three successive reproduction cycles. Finding the young ewes that were in heat was done with the help of testers rams and the fertilization was made in the autumn season, respecting the mating list. The data show that the early use of females of this breed did not affect the rate of body development at later ages. Thus, if at the time of the first reproduction season the average weight was only 72.38% of the adult weight, it increases to 87.99% in the second season and stands at 99.25% in the third season. Based on the data statistically processed, it is found that at the beginning of the third mating season an equalization of the data specific to the average live weight takes place in all experimental batches. Interestingly, at L1 it is found that early use in reproduction does not in any way affect the live weight in subsequent mating seasons. This batch had, after the third season of use in reproduction, a live weight greater with 0.25 kg compared to the batch who had at first mating season the age of 453.33 days and lower by 0.82 kg compared to the batch that had in the first season an average age of 20 months. At batch L2 the average body weight was 41.78± 0.87 kg, reaching the following season at 49.16± 0.25 kg which represented about 91% of the weight of adult sheep. By the fact that at the end of the third season the average body weight was 52.84± 0.38 kg it stated that this indicator reached 98.78% of the sheep weight that were over three years old.

Key words*: young sheep, Karakul of Botoşani, precocity, sheep body development.*

INTRODUCTION

The optimum age and weight for reproduction use of young sheep, for the first time, has a wide variability between breeds, being influenced, in particular, by the degree of their characteristic precocity.

Dyrmundson (1981), Sandu (1993), Pascal et al. (2010), studying the effect due to the use for breeding at different ages of young sheep, indicates for some breeds the age between 6 and 8 months, but also mentions situations in which the lambs from early lambing can conceive from the first autumn, even at the age of 4-7 months.

For the autochthonous races, slightly differentiated values are found in the specialized literature, the cause being their different precocity. For example, the Merinos of Palas lambs used early for breeding, when they had an average age of 308 days and a weight of 41 kg, had a fertility of 55-66% and the wool production was not negatively affected (Taftă et al., 1997; Mochnacs et al., 1978; Pascal et al., 1995).

Regarding body maturity, expressed by the weight in the reproduction season, in many publications it is specified that in the Merinos breeds the reproduction can be carried out without further negative effects if the weight, at the time of reproduction, represents at least 60-70% of the adult sheep (Moise et al., 2012; Sandu, 1993; Stăncescu, 2009; Pascal et al., 2005).

On the prolific breeds and in the English meat breeds, the early use in reproduction has no subsequent negative effects if at the time of fertilization the weight of the lambs represents 50-60% of the live weight of the adult sheep (Sandu, 1993; Pascal, 2006). Females from twin foetuses tend to reach puberty at an older age and at a lower body weight (Pascal et al., 2008; Pascal et al., 2006).

MATERIALS AND METHODS

The biological material was represented by the Karakul of Botoşani females breed at the Research and Development Station for Sheep and Goat Breeding Popăuţi-Botoşani.

To test the possibilities of early use at reproduction of young sheep, several experimental batches were set up that benefited of same experimental treatment.

The entire youth population used at mating was divided into three batches differentiated by age. At the date of mating, the females constituting batch 1 (L1) were 9 months old, those in batch 2 (L2) were 15 months old, and those in the third experimental batch (L3) were 20 months old.

The young sheep that were nine months old at the time of mating came from the early lambing of that year and the females in the other two batches came from the late lambing of the reproduction season of the previous year. The entire sheep population that was included in the three batches benefited from similar conditions of feeding, maintenance.

Finding the sheep in heat was done with the help of testers rams and the mating was done in the autumn season, respecting the mating list.

The collected data were processed using the MsExcel 2007 spreadsheet application. Thus, the database was prepared with the corresponding variation strings, each being coded according to the specific parameters studied.

To test the statistical significance of the differences between the average values of the studied parameters, as well as the correlations between them, the variable analysis algorithm (ANOVA Single Factor) and Pearson Correlation were used, both included in the MsExcel 2007 software package.

RESULTS AND DISCUSSIONS

For using at reproduction, as earlier as possible, of the female youth, without affecting further corporal development, a special attention must be paid to the applied rearing technologies for youth so, puberty to be installed as soon as possible, knowing the fact that those one occurs at the age at which reproduction is possible for the first time, being realisable when are fulfilled the following conditions: ovulation to be presented, ovules to be fecund, the conception product to be able to reach the term.

At Karakul of Botoşani breed after phenotype selection applied in the first neo-natal days and after establishing of the destination for the obtained lambs in each calving season, in the case of the ones kept for reproduction follows the growing period. During growing process the number of lambs initially kept could suffer also other modifications from numerically point of view function of growing rhythm and the way in which corporal development took place. So, till the age of 9-10 months if it is observed an improperly growing rhythm they could be redirected to capitalization for meat even if they have a valuable ascendance and recorded a high level of the score resulted after evaluation of production performances.

After this age, of 10 months, follow a new selection stage before youth to be introduced into the base flock. Function of corporal development at the age of 11-12 months females will be kept or will be eliminated from the flock destined for reproduction, being eliminated the weak and devoid of vividness ones, those which have deviations from the desired constitutional type, as well as the ones which have the corporal format with overhung and obvious bony angles.

Research conducted on the breed of Karakul of Botoşani female confirms, through the obtained results, that the early use in breeding can be a solution to obtain better results in the breeding and exploitation of this breed. This statement is based on the data obtained from the weighing of young females that were used on mating when they were 9 months, 15 months and 20 months. To verify how early use in reproduction had a certain influence on subsequent body development; all young females were weighed for two consecutive breeding seasons.

In the case of the batch consisting of females who had at the beginning reproduction cycle the age of only 272.20 days it was observed that this experimental treatment did not have a negative influence on the rate of body development at different ages. Thus, if we analyze the data from table 1 it is observed that at the time of the first mating the average weight was only 72.38% of the weight of adults, it increases to 87.99% in the second season and is very close to the live weight of the adult sheep in third reproduction

season. Basically, very early use in reproduction did not affect the rate of body development.

In the case of the batch consisting of females who had in the first mating season an average age of 15 months (L2), the average body weight represented 78.1%, increases to 91.90% and stands at 98.78% after the third cycle.

In the batch consisting of females with an average age of 20 months (L3), it is found that if in the first season it achieved only 85.45% of the average live weight of the adult sheep, in the following season it reaches an average living weight equivalent to about 94% and exceeds the body weight of adult sheep after the third breeding cycle.

Based on statistically processed data, it is found that at the beginning of the third mating season, an equalization of the data specific to the average live weight takes place in all the batches consisting of young females. It is interesting that in the first batch, that is, the one with an average age of nine months in the first breading season; it is found that the early use in reproduction does not in any way affect the live weight in the subsequent mating seasons.

This batch had, after the third season of use in reproduction, a live weight greater with 0.25 kg compared to batch that had in the initial breading season the age of 453.33 days and lower by 0.82 kg compared to the batch that had in the first season an average age of 20 months.

Statistical data processing confirms that these differences did not have any statistical significance for $P<0.01$ and $P<0.05$, which means that the use for reproduction of lambs, even from their first year of life, can be used upstream and will be an efficient solution and will contribute not only to reduce the lambing interval, but also to increasing the total number of lambs obtained each season and the total productive life of each female in the livestock.

In the case of the batches formed by the females that at the time of the first season were 15 and 20 months old, the same tendency is observed in the evolution of the live weight and during the other seasons analysed. If in the batch consisting of females that had initially 15 months the average weight was 41.78±0.87 kg in the following season it reached 49.16±0.25 kg which represented about 91% of the weight of the adult sheep. The fact that at the end of the third season the average body mass was 52.84±0.38 kg it was found that this indicator reached 98.78% of the sheep weight that were over three years old.

Research show that in case of batch of females that had an average age of 20 months when they were used for the first time for mating, after the end of the three consecutive breeding cycles, it is found that they exceeded by about 0.8% the live weight determined in sheep older than three years (Figure 1).

In order to have a complete picture of the data obtained from each batch used for the reproduction carried out in the three consecutive seasons, we determined the cumulative values resulted at the end of each season.

Table 1. Ewes age and weight considering the mating season (n = 35)

Batch	Body weight in the first mating season (kg)		Body weight in the second mating season (kg)		Body weight in the third mating season (kg)	
	$\bar{x} \pm s_{\bar{x}}$	% of adult sheep weight	$\bar{x} \pm s_{\bar{x}}$	% of adult sheep weight	$\bar{x} \pm s_{\bar{x}}$	% of adult sheep weight
L1 (272.2 days age)	38.72 ± 0.31^{dc}	72.38	47.07 ± 0.4^{cd}	87.99	53.09 ± 0.38^{ns}	99.25
L2 (453.3 days age)	41.78 ± 0.87^{ab}	78.10	49.16 ± 0.25^{ns}	91.90	52.84 ± 0.38^{ns}	98.78
L3 (606.2 days age)	45.71 ± 0.31^{ns}	85.45	50.12±0.11	93.96	53.91 ± 0.44^{ns}	100.78
Adults sheep age > 3 years	53.49±0.33					

Note: $^{a, b, c, d}$ – environments with different symbols show significantly different values ($P<0.05$);
ns – non-statistically significant differences ($P> 0.05$); * - statistically significant differences ($P<0.05$);
*** - statistically significant differences ($P <0.001$).

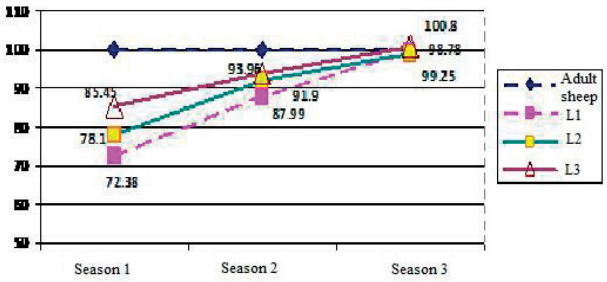

Figure 1. Body weight dynamics in young females used for mating considering sheep specific weight over three years age (%)

It was observed that at L1, out of the total of 35 females used for breeding, only in the first season was two that did not manifested heat and in the other seasons all were in heat and after mating became pregnant and produced at least a lamb (Table 2).

In L2 and L3 batches it is observed that, although, the number of females assigned for mating and those who manifested heat was identical, at the end of the three seasons the females from L3 produce 1.92% more lambs. This aspect is due to the fact that in this batch the females with twin lambs were higher.

The reproductive activity efficiency depends on many specific elements. For example, in England the efficiency of sheep breeding is based on determining a synthetic indicator represented by the number of products that enter the productive and reproductive circuit from each female in the livestock (Pascal, 2015). Therefore, we considered appropriate to carry out an analysis regarding the number of lambs obtained from each batch in each year of life from the respective females based on dividing the number of lambs obtained from the three seasons to the average age of the mother sheep after the end of the third breading cycle.

The efficiency of the early use for reproduction is also sustained by the fact that by dividing the total number of lambs obtained at the average age (expressed in years) recorded at the time of the third season, it results that from the same number of females for three reproductive cycles is obtained a different lamb number.

According to the analysis, it is observed that in the batch of females used early for breeding, during the three seasons the proportion of those who showed heat and the total number of lambs obtained had lower values (Table 2).

However, by comparing the total number of lambs obtained from this batch to the average age, expressed in years, recorded at the time of the third season, it turns out that every year of life 30.79 lambs are obtained (Table 3).

Table 2. Statistic of sexual heat manifest in young ewes and the number of obtained lambs

Batch	No.	First season		Second season		Third season		Total	
		Sheep in heat	Obtained lambs	Sheep in heat	Obtained lambs	Sheep in heat	Obtained lambs	Sheep in heat	Obtained lambs
L1	35	33	24	35	36	35	37	103	97
L2	35	34	31	35	36	35	35	104	102
L3	35	35	33	35	37	34	34	104	104
Total	105	102	88	105	109	104	106	311	303

In contrast, on the other two batches the total average number of lambs obtained per year of life was 27.94 and 25.61, being with 1.05 lambs less compared to the average lambs per year on life of L2 and with 5.18 lambs compared to L3.

The practical importance of these data is very great because it shows us that when applying a breeding technology based on the early use of females, that is, from their first year of life, the total number of lambs obtained each year increases by 9.25% compared with the use at the age of 15 months and 18.62% if the time of entry into the productive circuit is delayed until the age of 20 months.

This aspect is important for breeding and production because in the first three years three generations of lambs are obtained, the effects being positive and quantifiable during the economic growth period of each generation of young females that is introduced into the breeding nucleus.
In other research effectuated on Merinos de Palas breed was observed that batch formed by, which had a mean age at their first mating of 292.21±1.91 days, those ones had a corporal mass of 42.070±0.379 kg, which represented 75.5% from live weight specific for batch formed by adult sheep.
After utilization at first reproduction cycle fecundity was 100% and prolificacy was 107% (Pascal et al., 2005).

Table 3. The ration between the total lambs and the average age after three reproductive seasons

Average age at first breading season (days)	Average duration of the three breeding seasons (days)	Average age at the lambing from third breeding season		Born lambs/total average age from third season (no./years)
		days	years	
272.21±2.45 (L1 - around 9 months)	879.58±2.08	1151.79±3.58	3.15±0.87	30.79
453.36±1.97 (L2 - around 15 months)	880.26±2.78	1333.62±2.95	3.65±0.66	27.94
606.20±3.47 (L3 - around 20 months)	875.98±1.98	1482.18±2.77	4.06±0.51	25.61

Those values show the fact that when are assured optimal conditions for growing of young females, those ones could enter into the reproductive circuit starting with their first year of life, without being recorded negative effects on further corporal development or on the main indicators of reproduction function.

CONCLUSIONS

In the batch consisting of females who had at the beginning of the experiments the average age of 272.20 days it is observed that they had an average weight of only 72.38% of the adult weight, it increases to 87.99% in the second season and is situated close to the live weight of the adult sheep in the third mating season.
In the case of the batch consisting of females who had in the first mating season an average age of 15 months (L2) the average body weight represented 78.1%, increases to 91.90% and stands at 98.78% after the third cycle.
In the batch of females with the average age of 20 months (L3) it is found that if in the first season it achieved only 85.45% of the average live weight of the adult sheep, in the following season it reaches an average live weight equivalent to about 94% and exceeds the body weight of adult sheep after the third breeding cycle.
In the batch of females that had an average age of 20 months when they were used for the first time at mating, after the end of the three consecutive breeding cycles, it is found that they exceeded by about 0.8% the live weight determined in the ewes over three years old.

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EFFECT OF BOILING COCONUT WATER ON PHYSICAL, CHEMICAL AND ORGANOLEPTIC PROPERTIES OF REJECTED LAYING HEN

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Abstract

Rejected Laying Hen (RLH) meat has a low quality due to slaughtered at a relatively old age causing the tenderness of meat to be low and less desirable by the public. Improving nutritional quality and elevating the level of people`s preference for chicken meat that have been done by innovating and diversifying into RLH, one of which was using boiled coconut water. The purpose of this study was to determine the effect of boiled coconut water on physical, chemical and organoleptic properties of RLH. The Completely Randomized Design Method with four treatments (boiling time of 15, 30, 45 and 60 minutes) with four repetitions was used as an observation variable which included physico-chemical tests (tenderness, cooking losses, water holding capacity (WHC), water content, protein content and fat content) and organoleptic tests. Physical-chemical and organoleptic tests of this study showed that there were significant differences in the duration of boiling. The range of cooking losses was 33.60 - 38.92%; tenderness value 5.54 - 6.13; and WHC ranges from 38.21 - 40.42. While the water content ranges from 67.26 - 69.09%, Protein ranges from 16.24 - 16.89%, and Fat ranges from 10.11 - 11.72%. Organoleptic test values range from 4.05 - 5.05. The conclusion of this study shows that the physical-chemical and organoleptic properties of RLH with boiling coconut water for 60 minutes have been able to maintain the quality of RLH and organoleptic tests were acceptable.

Key words*: boiling, coconut water, Rejected Laying Hen (RLH)*

INTRODUCTION

Meat as a perishable food was very easily damaged. Rejected Laying Hen (RLH) has nutritional and high biological value were good as food source. The nutritional content was easily digested and absorbed by the body. Meat consumption and products have increased from year to year. The rejected chicken was known as a laying hen that was no longer productive has a body weight range from 2 to 2,5 kilogram and the age was ranged between 18 to 20 months old. The low carcass quality with relatively high fat content, but has good connective tissue (Murtidjo, 2003), was confirmed by Purnamasari (2012) that the low quality was due to late cutting time so that the tenderness of the meat becomes less and was not liked by the public. Therefore, to increase consumer interest in RLH, there needs to be innovation and diversification by boiling chicken meat into coconut water.

Old coconuts contain high calories in the amount of 359 calories per 100 grams; Half-aged coconut contains 180 calories per 100 grams and young coconut meat contains 68 calories per 100 grams. The average calorie value of coconut water was 17 calories per 100 grams where green coconut water contains tannin or antidote (anti-poison) higher than other types of coconut. The composition of chemical substances found in coconut water includes ascorbic acid or vitamin C, protein, fat, carbohydrate, calcium or potassium with mineral content such as iron and phosphorus and sugars such as glucose, fructose and sucrose (Alexia et al., 2012). While coconut has a water content of 95.5 per 100 grams of coconut meat (Directorate of Nutrition of the Ministry of Health Republic of Indonesia, 1981).

According to Kailaku et al. (2016), states that coconut water has a total sugar content of 6.06%, potassium levels 1,736.46 mg/kg, sodium levels 14.17 mg/kg and clarity of 97.4%. Coconut water has good nutritional value and is efficacious in reducing the frequency of heart rate and blood pressure which states that the blood pressure of hypertensive patients who

routinely consume coconut water drops to 71% (Paramitha and Budiman, 2018), as a refreshing drink, anti-rehydration and as a natural drink with the main content of sugar and minerals (Chathuri et al., 2018). In addition, according to Geetha et al. (2016), coconut water functions as an antioxidant also contains 5.2% protein, 53.5% carbohydrates, 2.6% phenolic and 1.4% flavonoids. Furthermore, Yong et al. (2009) states that coconut water contains acetic acid and citric acid.

Based on the above considerations, the study entitled 'Effect of boiling coconut water on physical, chemical and organoleptic properties in Rejected Laying Chicken Meat, was conducted'.

This study aims to measure cooking losses, tenderness, water holding capacity, water content, protein content, fat content and organoleptic properties of rejected chicken meat after boiling with coconut water.

MATERIALS AND METHODS

The thighs and breasts of the RLH that were clean from feathers and offal weighed 1000 gr for each treatment into 3000 ml of coconut water immersion, then boiling for 15, 30, 45 and 60 minutes.

Knives, measuring cups, analytical scales, stopwatches, thermometers, ovens, desiccators, Erlenmeyer tubes, panic, gas stoves and stationery are tools used in this study.

This study uses a completely randomized design (CRD) with 4 levels of treatment with 4 replications. The treatment was the length of boiling meat in coconut water with 4 levels of treatment, namely:

A1 = Cook meat for 15 minutes
A2 = Cook meat for 30 minutes
A3 = Cook meat for 45 minutes
A4 = Cook meat for 60 minutes

Retrieval of data from the physical-chemical test observation variables were tenderness, texture, water holding capacity, cooking losses, water content, protein content, fat content, carbohydrates and organoleptic tests.

Rejected laying chicken meat that has been cleaned from feathers and offal was taken 1000 grams of thighs and breasts for each treatment and soaked with 3000 ml of coconut water, then boiled for 15, 30, 45 and 60 minutes at a temperature of 90 to 100 degrees Celsius for further analysis.

RESULTS AND DISCUSSIONS

A. Physical Characteristic.

The results of the study on the effect of coconut water boiling time on cooking losses, tenderness, WHC were shown in Table 1.

The results of the statistical test of cooking losses shown in table 1 show that the boiling time in coconut water showed a significant difference ($P<0.05$) to the cooking losses of the rejected chicken meat. With the Honestly Significant Difference test (BNJ) with the aim to see the difference in each treatment, namely between chicken meat which was boiled for 15, 30, 45 and 60 minutes. According to Soeparno (2005), cooking losses were closely related to protein content, juiceness and water holding capacity. The higher the protein, the lower cooking losses where meat with low cooking losses will be better.

The percentage of cooking losses in this study ranged from 33.60-38.92%. The effect of boiling on the water content causes shrinking of the meat because the water was released from the meat also because of evaporation during boiling. The effect of increasing the temperature and cooking time makes the value of cooking losses as an indicator of meat nutrition associated with meat juice levels, namely the amount of water bound in and between the muscle fibers. Meat with lower cooking shrinkage values illustrates better nutrient quality due to the low nutrient loss during the cooking process. The high value of the water holding capacity (WHC) will result in low cooking losses. The longer the cooking time with the same temperature, causing an increase in cooking losses and tenderness. Animal age factors affect meat tenderness, namely the muscle, meat composition, myofibril structure, connective tissue content and the degree of cross-linking in addition to the ability of the WHC by protein and meat juice (Soeparno, 2005).

Tenderness of rejected chicken meat in this study ranged from 5.54 to 6.13. Meat quality after cooking affects the physical character of chicken meat. Aberle et al. (2001) stated that the connective tissue group, the meat fiber group,

and the fat group related to muscle were the components that affect the tenderness of meat but Wahyuni et al. (2018), states that beef marinated with coconut water in 90 minutes has not been able to influence water holding capacity and tenderness.

Table 1. The mean values of cooking loses, tenderness and WHC

Parameter	Length of Boiling Meat (minutes)			
	15	30	45	60
Cooking Loss (%)	33.60 ± 0.20^{a}	37.52 ± 0.12^{c}	37.78 ± 0.02^{b}	38.92 ± 0.24^{d}
Tenderness (mm/g/s)	5.54 ± 0.20^{a}	5.76 ± 0.12^{b}	5.94 ± 0.15^{c}	6.13 ± 0.12^{d}
WHC (%)	38.21 ± 0.05^{d}	39.28 ± 0.05^{c}	41.13 ± 0.05^{b}	40.42 ± 0.05^{a}

Remarks: different notations show significant differences between treatments ($P<0.05$)

Water holding capacity in this study varied 38.21-40.42%. Duration of boiled chicken meat with coconut water affects the water holding capacity. The longer the boiling time, the more the binding capacity of the water. The process of cutting, heating, grinding and pressure can affect the holding capacity of the water. Low WHC causes the meat to lose a lot of water, resulting in weight loss and dissolved components of the water. Warming at a temperature of 600 degrees Celsius results in almost complete denaturation of sarcoplasmic proteins (Lawrier, 2005).

B. Chemical Characteristic

The mean value of the results of chemical analysis of rejected chicken meat was shown in Table 2.

Based on the data in Table 2. the water content of rejected chicken meat obtained an average value of 67.26%-69.09%. Coconut water has the ability to maintain the water content of rejected chicken meat, this was due to the presence of organic acid content, namely acetic acid (Othaman, 2014). Meat moisture content has decreased due to heating or boiling. Decreased water content due to shrinkage of meat causes much water to be released out of the meat to be strengthened by the influence of coconut water which contains acetic acid which was able to pull water out of the meat tissue so that the water content of the boiled meat drops. Boiling using coconut water can form a gel layer on the meat.

Based on the data in Table 2, the results of the analysis of fat content during boiling with coconut water obtained an average value of 10.11-11.72%. Laying chicken meat during boiling with coconut water at high temperatures was able to hydrolyze the fat content that causes meat fat levels to fall. The longer the boiling time with coconut water, the lower the fat content of meat. According to Sundari et al., (2015), boiling affects the decrease in fat content because the nature of the fat that cannot stand the heat will melt and evaporate (volatile). The content of acetate in coconut water coupled with heating at high temperatures was able to hydrolyze the fat content in meat.

Test the protein content in RLH meat obtained an average value of 16.24-16.89%. presented in Table 2. The results of ANOVA showed that the boiling time with coconut water had a significantly different effect ($P<0.05$) on the protein content of rejected chicken meat. Changes in protein after boiling were denaturation, loss of enzyme activity, changes in solubility, discoloration and breaking of peptide bonds. (Sundari et.al. 2015).

Table 2. The mean value of the results of chemical analysis of RLH

Parameter	Length of Boiling Meat (minutes)			
	15	30	45	60
Water Content (%)	69.42 ± 0.17^{a}	69.09 ± 0.08^{b}	68.69 ± 0.21^{b}	67.26 ± 0.08^{c}
Protein (%)	16.24 ± 0.37^{a}	16.84 ± 0.09^{a}	16.68 ± 0.12^{c}	16.89 ± 0.13^{d}
Fat (%)	11.72 ± 0.02^{d}	10.93 ± 0.20^{c}	10.23 ± 0.02^{b}	10.11 ± 0.24^{a}

Remarks: different notations show significant differences between treatments ($P<0.05$)

C. Organoleptic Test

The results of the organoleptic test analysis of rejected chicken meat with boiling water 15, 30, 45 and 60 minutes were shown in Table 3.

The panelist rating score for organoleptic is 7 (very likes), 6 (likes), 5 (somewhat likes), 4 (neutral), 3 (somewhat dislikes), 2 (dislikes), and 1 (very dislikes).

Organoleptic assessment was used as a determinant test of quality in consuming rejected chicken meat. The results of the analysis showed no significant difference in the length of boiling of color, aroma and taste (Table 3). According to Lawrie (2005), one of the parameters in assessing flavor was the odor or aroma test. Soeparno (2005) also states that flavor was one of the factors that influence the level of liking. Long boiling chicken meat with coconut water gave significantly different results ($P<0.05$) on texture and tenderness. Boiled water can cause hydrolyzed protein and fluid exudation (free water, mobilized water and gaps of meat liquid) so that the meat becomes hard and dry (Nuhriawangsa, 2004). The pale color of chicken meat due to the amount of free water released from meat fibers in the form of the ability of high extracellular water content can reflect light so that the level of light absorption and color intensity also decreases (Miskiyah et al., 2017).

The results of the study reported by Prasetyo (2014), that the length of boiling chicken meat with coconut water made the best processed shredded meat in 30 and 45 minutes. Immersion of coconut water on chicken carcasses tends to affect the texture of the chicken becomes softer at room temperature storage (Mishiyah et al., 2017).

Processing of RLH with coconut water was able to overcome the problem of toughness to become more tender without reducing the nutritional value of meat. According to Guan et al. (2013), stated that 72 weeks old chicken meat was harder than other types of poultry and has a lower sensory score. The longer the boiling time with coconut water, the smoother the texture and tenderness level.

Table 3. Mean of RLH meat organoleptic value

Parameter	Length of Boiling Meat (minutes)			
	15	30	45	60
Colour	4.52 ± 0.02^{a}	4.43 ± 0.02^{a}	4.33 ± 0.02^{a}	4.14 ± 0.02^{a}
Aroma	4.95 ± 0.20^{a}	4.90 ± 0.01^{a}	5.19 ± 0.14^{a}	5.05 ± 0.20^{a}
Taste	4.57 ± 0.02^{a}	4.70 ± 0.10^{b}	4.57 ± 0.10^{c}	4.71 ± 0.10^{d}
Texture	4.67 ± 0.02^{a}	5.05 ± 0.20^{a}	4.19 ± 0.02^{a}	4.81 ± 0.20^{a}

Remarks: different notations show significant differences between treatments ($P<0.05$)

CONCLUSIONS

The results of the physical and chemical values of the RLHs on cooking or boiling in 60 minutes can maintain the quality of RLH meat and organoleptically acceptable.

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RESEARCH ON THE ECONOMIC EFFICIENCY OF FARMS IN THE FUNCTION OF THE MILKING SYSTEM

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Abstract

The economic efficiency of dairy farms is influenced by many factors and depends on their management. Some research shows that the conventional milking system (CMS) is more cost effective than the automatic milking system (AMS). The data collected for carrying out this research come from the accounting records of the dairy cow farms (from 2018). From the current study it appears that the capital expenditures were significantly higher in the case of farms with AMS than in the case of farms with CMS and the total incomes for the farms with AMS and CMS were 1745.2 RON/t milk and respectively 1785 RON/t milk, and the net productions were 1117.61 RON/t milk for farms with AMS and 1155.14 RON/t milk for farms with CMS. In terms of personnel costs, they are higher in farms with CMS, 512.3 RON/t milk, whereas in AMS farms staff costs do not exceed 484.1 RON/t milk. Following the observations, the two milking systems have similar profitability in the market conditions of the Romanian economy.

Key words*: costs, milking system, profitability, revenues.*

INTRODUCTION

The number of dairy farms exploited for milk production, using the automatic milking system (AMS), is constantly growing, especially in Eastern Europe. In 1987, the Dutch company Lely, invented the milking robot, starting its marketing in countries with advanced animal husbandry (Huijps et al., 2008). In Romania, about 6% of farms use AMS, this percentage is constantly increasing. It is recognized that, in agriculture, zootechnics is a basic sector, mostly as an economic weight in the countries considered developed, being the main source of high biological value proteins indispensable to human nutrition (Butler and Holloway, 2016). In terms of feeding the population, specialized international bodies (FAO and OMS) consider that optimal nutrition is the result of the combination of 2/3 foods of plant origin and 1/3 foods of animal origin, especially milk, meat and eggs (FAO, 2015). Moreover, a direct and significant correlation was established between increasing milk production in the future and reducing infant mortality, but also between increasing the availability of milk for consumption and reducing the consumption of alcoholic beverages. There are approximately 250 million cows supplying milk all over the world. There are about 10 million dairy cows in North America, 23 million in the EU and 6 million in Australia and New Zealand. Milk production is on the rise in Asia, including in countries that are not traditionally known for their milk consumption, such as China, which now has more than 12 million cows producing milk (FAO, 2015). Research on the economic efficiency of the use of AMS and conventional milking systems (CMS) has been mainly based on normative models, focusing on the return on investment (Daily Nation, 2017). Conventional milking systems represent those with vacuum system, the first such milking device was designed in 1851 by Hodges, but it included the whole uterus and which later (1860) was developed with rubber glasses and vacuum pipes, which led to the creation of milking machines, whose operating principles are still valid today, only that they have been improved and diversified (Rossing and Hogewerf, 1997).

The CMSs currently used in our country are organized in milking rooms, specially designed for this purpose and are represented by: horizontal milking platforms in parallel, frontal, in tandem, arranged in a broom, roller type and unilactor type (Maciuc, 2006). Moving from an CMS to AMS requires a new management approach and a change in the workload for the staff (Oude Lansink et al., 2002). The use of AMS eliminates the milking process, but includes new tasks such as controlling and cleaning AMS, periodic visual control of animals in order to observe whether or not there have been flocks, etc. (Stokes et al., 2007). In the CMS, the key interactions between lactating cows and humans occur during milking and occasional herd health practices. The practical and social advantages of such technologies include the availability of extra time for dairy farmers and improved work flexibility (de Koning, 2010). Because farmers often have little time flexibility and often have difficulties in securing reliable labor in some areas, it is not surprising that researchers have reported positive adoption of AMS (Mathijs, 2004) improved labor efficiency, increased flexibility, shortened working hours and lower physical workloads. The advantages and appeal of AMS to a farmer's lifestyle are clearly evident (with no proof of negative effects); however, in order to ensure economic viability and future long-term survival, it is important to investigate the ability of cows to adapt and perform in such a framework (Dearing et al., 2004). The objective of this paper was to compare the profitability of dairy cows, depending on the milking system used. This objective was achieved by analyzing the accounting data of the studied farms. Hyde and Engel (2002) found that the investment in AMS was profitable. In contrast, in a study by Dijkhuizen et al. in 1997 it was concluded that the investment in a AMS was not profitable, and Rotz et al. (2003) concluded that the use of AMS does not provide economic benefits to most farms in the United States. The only empirical comparison, from an economic point of view, between farms with AMS and CMS was conducted by Bijl et al. (2007), who concluded that farms with CMS were more profitable than farms using AMS. Since then, no sub-food comparisons have been published economically. In a study conducted on 105 farms in Belgium, Denmark, Germany and the Netherlands (Mathijs, 2004) there was a 20% reduction in the costs of physical labor in the case of AMS, but in the short term a low profitability can be recorded due to the expenses of capital, which are raised in this case. Previous research has not determined whether adopting a AMS can replace the cost of staff work (Tse et al., 2017). Farms that are based on milk production, as the main source of income, are more likely to try to improve their organization's management in order to make the farm more efficient (Bravo-Ureta et al., 2007). The stress response of cattle in various environments has been extensively studied in an attempt to understand the changes in their emotional state. Milking in AMS compared to CMS has been shown to minimize cow stress as indicated by lower cortisol levels by Maina et al. (2018). The cost of feeding dairy cows constitutes the highest percentage of the total cost of milk, and in this case, significant progress has been made in which the reduction of food expenditure is almost impossible. Thus, the manager of a dairy cow farm can act on the technical details to obtain the desired profit (Coleman and Hemsworth, 2014). One of the main reasons for investing in an automatic milking system is the desire for more flexible working hours, but also more free time (Holloway et al., 2014). Indeed, some previous research has reported savings in labor following the adoption of AMS, but at the same time it has been hypothesized that capital expenditures would increase due to high maintenance costs but also to a much larger investment (Svennersten - Sjaunja and Pettersson, 2008). The modification of the milking system coincides with a change of daily work activities, which requires more attention for the verification of dairy cows. Maina et al. (2018) found that the adoption of new technologies in the dairy sector requires considerable investment with high capital expenditures. Efficiency is a major problem in the economy of agricultural production and is measured by comparing the value obtained with the expected one. The economic efficiency in the case of farms, is a product of technical efficiency together with the allocation efficiency (Wilson, 2008).

MATERIALS AND METHODS

The data collected to carry out this study come from the accounting records of the dairy cow farms, registered in 2018. In this research, 10 farms using automatic milking system and 20 farms using conventional milking systems were studied. The database included information on incomes (for example, milk production revenues and other agricultural activities), fixed costs (for example, building and machinery maintenance costs), variable expenses (e.g. feed costs, reproduction, energy, water) and general information about the respective farm regarding the number of hectares of land used, the number of animals, etc. All farms were fetching and milking their cows twice daily. All incomes and expenses are expressed in one tonne of milk (using the total quantity of milk quota), and subsequently these results were analyzed with a t test to compare farms with CMS with those with AMS. Statistical calculations were performed with the IBM SPSS V.22 software.

RESULTS AND DISCUSSIONS

Annual economic reports are generally of interest to farmers who pay particular attention to the finances that represent the performance of the respective farm. The farms in this sample can thus be characterized as farms wishing to obtain and keep track of financial performance, their purpose being to help in making the best management decisions.

Table 1. Overview of information on farms studied

	AMS (n=10)		CMS (n=20)		
Elements	**Average**	**Standard deviation**	**Average**	**Standard deviation**	**p-value**
Total land used (ha)	115	27.8	110	28.2	0.77
Milk production/animal/year (kg)	9142	980	8964	816	0.11
Fat (%)	4.38	0.12	4.31	0.12	0.17
Protein (%)	3.45	0.08	3.49	0.10	0.0006

Table 2. Description of the input and output variables used to analyze the efficiency and average values (with the std deviation) for farms with automatic milking system (AMS) and those with conventional milking system (CMS)

		AMS (n=10)		CMS (n=20)		
Variable	**Data from farm accounting**	**Average**	**Standard deviation**	**Averag**	**Standard deviation**	**p-value**
Animals	Total number of cows	176	27.9	165	28.2	0.76
Capital expenditure (RON/tons milk)	Expenses for buildings	202.2	84	184	79	0.91
	Expenditure on machinery and equipment	401.5	170	304.4	150.2	0.0028
	Total capital expenditures	603.7	254	488.4	229.2	0.0030
Personnel expenses (RON/tons milk)	Total staff costs	484.1	78	512.3	176.47	0.60
Expenditure on materials (RON/tons milk)	Feed for animals	50.23	15.43	51.4	12.82	0.28
	Concentrates + premixes	329.32	26.74	328.46	28.5	0.98
	Fertilizers	48.65	9.32	49.88	9.72	0.18
	Pesticid	12.3	2.78	12.1	3.14	0.096
	Reproduction	43.42	12.4	42.98	11.8	0.68
	Medication	64.2	4.5	64.4	4.2	0.16
	Energy and water	74.67	8.3	75.8	9.1	0.001
	Elimination of manure	4.8	1.25	4.84	1.4	0.46
	Total expenditure on materials	627.59	80.72	629.86	80.68	0.12
Revenue (RON/tons milk)	Milk income	1600	130	1645	129	0.74
	Income from the sale of animals	120	24.5	116	24.4	0.61
	Revenue from other activities	25.2	4.7	24	4.7	0.45
	Total revenue	1745.2	159.2	1785	158.2	0.90
Net production (RON/tons of milk)	Total revenue - Expenditure on materials	1117.61	78.48	1155.14	77.52	0.77

Table no. 2 presents an overview of the inputs and outputs for the 30 farms studied in total. Expenditure and revenue were reported on a tonne of milk to allow comparison between farms on lactation (305 days), and the weight of milk in fat and protein. As expected capital expenditures were significantly higher for AMS farms than for CMS farms. These higher costs are due to the higher costs of maintaining and constructing buildings and installations, because in the case of AMS farms the rate of equipment replacement is higher. However, estimates of the economic life of a AMS are not available, so it would be beneficial to assess the lifetime of the AMS to allow for a reliable comparison with the farms where CMS is used. In terms of personnel costs, they are higher in the farms with CMS, 512.3 RON/t milk, as more employees are needed to perform the milking of the cows, while in the farms with AMS the personnel costs do not exceed 484.1 RON/t milk. The costs of the materials are about the same in both situations because the animals benefit from the same feed ration, the same drug treatment, etc. No differences were observed between the farms with AMS and those with CMS on the sold productions, the net income resulting from the sale of the productions registering near values. The total revenues for the farms with AMS and CMS were 1745.2 RON/t milk and 1785 RON/t milk respectively, and the net yields were 1117.61 RON/t milk for the farms with AMS and 1155.14 RON/t milk for farms with CMS.
Therefore, from table no. 2 it can be observed that the farms with AMS have higher capital expenditures, but the net production is not different between the farms with AMS and those with CMS. This shows us that the small, insignificant differences in personnel and material expenses observed in CMS farms, offset the capital expenditures of AMS farms. Hyde and Engel (2002) have found that the investment in AMS is profitable. In comparison, a 1997 study by Dijkhuizen et al. concluded that investment in AMS was not beneficial, and Rotz et al. (2003) concluded that the use of AMS does not offer economic benefits to most farms in the United States. The results of previous studies showed that investments in AMS were not profitable for farms in the Netherlands (Dijkhuizen et al. 1997) and the United States of America (Rotz et al., 2003). The results of the current study show that the net production does not differ depending on the milking system, indicating that the economic efficiency of the farms with AMS and CMS is similar. However, farms that use milking robots are expected to be more profitable in the future, with increased labor and energy and water spending (Dijkhuizen et al., 1997). Maina et al. (2018) found that the implementation of new technology in the dairy sector requires significant investment with high capital expenditure. A major disadvantage of AMS is that it can make milking a limited number of cows / day, and in large farms such as some in the US, AMS is not cost effective because it requires a large number of milking robots, which leads to a high investment, while labor costs are lower in the US than in Europe (de Koning, 2010). Research into cow health and physiology has been conducted to investigate the possible long-term effects of the type of milking device. Improvements or no significant changes in cow health have been reported in the AMS when farms are well managed (Svennersten-Sjaunja and Pettersson, 2008), especially when cows remain managed on pasture. In addition, previous research compared the two different brands (Lely and DeLaval) of milking equipment and found that one brand was correlated with more restless activity than the other, which is a limitation of the present study. As the AMS changes the way cows are handled and communicate with humans, especially with the introduction of voluntary cow traffic, it is important to consider how this change affects the relationship between humans and animals (Bravo-Ureta et al., 2007).

CONCLUSIONS

The current study was based on the accounting records of dairy farms in Romania that use automatic milking systems and conventional milking systems, in order to investigate whether the economic efficiency differs depending on the milking system. Farms with AMS had higher average capital costs (603.7 RON/t milk) compared to CMS farms (488.4 RON/t milk).
Sustainability issues play an increasingly important role in milk production, so innovative technologies are also present in this area, in order to streamline specific production

processes in order to achieve the best economic results.

Total labor costs and net output were not significantly different between AMS farms and CMS farms. Thus, the economic efficiency of the farms with AMS and those with CMS was similar.

With the overall health and behavior of cows showing small and inconsistent variations between system types, the welfare of cows in both system types is likely to be similar and dependent on good management. Management is highly dependent on humans, indicating that the human element in the milking system is likely to have the greatest effect on cow output and welfare.

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COMPARATIVE STUDY ON THE RESULTS OF SLAUGHTERING SEPARATELY BY SEX IN QUAILS OF THE BROWN JUMBO MEAT POPULATION AND IN QUAILS OF THE MIXED EGGS - MEAT POPULATION BALOTEȘTI AT THE AGE OF 42 DAY

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Abstract

The purpose of the research was to determine comparatively the results of slaughter separately by sex, in the case of two populations of quails (meat and mixed), at the age of 6 weeks. The average weight of the eviscerated carcass for meat quails was 205.00 ± 8.82 g/head in females, while in mixed eggs-meat female quails it was 135.20 ± 3.29 g/head (34.05% higher as in females in the mixed population) and 173.80 ± 6.37 g/head in males of the meat population (26.24% higher than in males of the mixed population, 128.20 ± 3.98 g/head). Studied Jumbo meat quails can be used with good results in meat production, chickens being raised for meat production, while quails from the Baloteşti mixed egg population can also be used in the direction of meat production, if desired of smaller carcasses compared to those of meat quails. For both populations, the breeding for meat is conditioned by a high performing breeding lot and without directing the females to egg production.

Key words*: quails, slaughtering, meat, egg.*

INTRODUCTION

Currently, quail raising in Romania is an activity with an upward development, in the market of the quail egg products, appearing several producers that have farms with tens of thousands of quails, millions of eggs and tens tons of meat, who also accessed European funds of hundreds of thousands of euros (Ioniță et. al., 2019). However, under the conditions of this development, there is a need to know the interaction of these birds with environmental factors, rigorous programming of productions in close correlation with the preferences of consumers and the market of these products (Popescu Micloșanu, 2007). In general, farms are mixed, in the sense that they produce eggs and meat also, which means that the growth performance of the quail population, its direction of specialization and its interaction with environmental factors must be known very well (Almeida et al., 2002).

The purpose of the experiment was to determine the results of slaughter in quails from a meat population (brown Jumbo) and a mixed population (Balotești) and to establish comparative data that can be useful to both researchers and quail of 42 days aged producers, considering that in most quail meat production systems the age of slaughter is between 40 and 45 days (Sari et al., 2011; Silva et al., 2013). Also, the sex of birds is particularly important in obtaining superior results when sacrificing quails (Perez and Slaes, 1997; Okamoto et al., 1989).

MATERIAL AND METHOD

The investigations were carried out within the quails exploitation Ioniță T. Lucian Individual Enterprise in Gherghița Commune, Jud. Prahova and a total number of 300 quails were investigated, of which 150 quails (females and males) from the brown Jumbo meat population and 150 quails (females and males) from the mixed egg-meat population "Balotești". The environmental conditions in which the birds grew up were within the limits provided by the specialized literature (Ioniță, 2014). During the experiment, two combined feed recipes were administered, namely quail starter (1 - 3 weeks)

and quail grower (4 - 6 weeks). The quail starter compound feed recipe had the following calculated nutritional value: 3010 kcal metabolizable energy/kg compound feed, 24.80% crude protein, 5.10% crude fat, 0.97% methionine, 1.58% lysine, 0.96% calcium and 0.75% phosphorus. The compound feed specific for the second growth phase of the quail youth had the following calculated nutritional value: 3140 kcal metabolizable energy/kg compound feed, 22.50% crude protein, 6.10% crude fat, 0.98% methionine, 1.33% lysine, 0.86% calcium and 0.70 % phosphorus (source: https://www.ibna.ro/pdf/Furaje-pentru-curci-prepelite-si-fazani.pdf (Compounds feeds-for-turkey-quail-and-pheasant), Institute of Biology and Animal Nutrition, Balotești, Romania).

The birds were weighed at the age of one day and, after individualization, at 42 days. After slaughter, the weight of the after bleeding carcass, the plucked carcass and the eviscerated carcass were determined. The average data were calculated for both the male and female carcasses. The same has been done for the component parts of the housing. Finally, the proportions were established for the different components of the carcass.

In order to establish the significance of the differences between the averages, the Student test was used.

RESULTS AND DISCUSSIONS

At the age of 1 day, the average weight of chicks (weight valid for both males and females, since at this age sexing is not done) in the meat population was 9.47 ± 0.07 g/head, while in the chickens from the mixed population was 8.76 ± 0.33 g/head, 7.5% lower.

In a study conducted in Romania (Popescu-Micloșanu et al., 2006) on two batches of mixed and meat quail in which the determination of the growth parameters up to the age of 42 days was sought, in the chicken lot of meat quail is mentioned at the age of 42 days an average weight of 244.9 g/head (as average for males and females), while in the present study the average weight determined in females and males is 8.55% higher (267.80 g/head).

Also, the same authors determined in the quails of the mixed population an average weight for females and males at 42 days of 205.30 g/head, 3.46% lower than that determined in the present experiment in the mixed quails (198.20 g/head).

Stoica et al. (2001) mentions an average living weight in the Balotești quails at 42 days of 208.00 g / head in females and 202.00 g/head in males.

Table 1. Average live weight at 1 day and 42 days in females and males of quail from the mixed eggs-meat population of Balotești and brown Jumbo meat quail (g)

Specification	Meat quails		Eggs-meat mixed quails	
	Females	Males	Females	Males
Live weight at 1 day of age	9.47 ± 0.07		8.76 ± 0.33	
Live weight at 42 days of age	290.60 ± 8.38***	245.00 ± 7.90***	206.60 ± 3.95***	189.80 ± 6.85***

Note. Between the values noted *** the differences are very significant at the same age

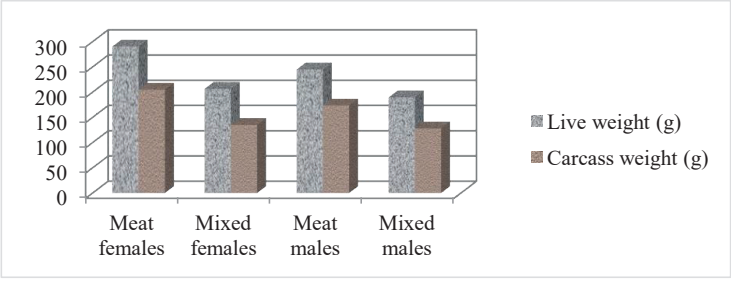

Figure 1. Average live weight and average carcass weight in females and males in the two populations aged 42 days

Table 2. Average results at slaughter at age 42 days in females and males of brown Jumbo meat quails and mixed eggs - meat Balotești quails (g)

Specification	Meat quails		Egss-meat mixed quails	
	Females	Males	Females	Males
ALW (g)	290.60 ± 8.38 aaabbb	245.00 ± 7.90 aaaddd	206.60 ± 3.95 bbbcc	189.80 ± 6.85 ccddd
CBW (g)	279.80 ± 8.14 aaabbb	235.80 ± 8.41 aaaddd	199.40 ± 4.37 bbbcc	183.80 ± 6.25 ccddd
PCW (g)	260.20 ± 6.38 aaabbb	220.00 ± 7.34 aaaddd	176.00 ± 3.51 bbbcns	164.80 ± 6.02 cnsddd
ECW (g)	205.00 ± 8.82 aaabbb	173.80 ± 6.37 aaaddd	135.20 ± 3.29 bbbcns	128.20 ± 3.98 cnsddd
SY (%)	70.45 ± 1.46 ansbb	70.98 ± 1.89 ansdns	65.55 ± 0.70 bbcc	67.60 ± 0.58 ccdns
BW (g)	10.80 ± 0.58 ansbbb	9.20 ± 0.80 ansdns	6.80 ± 0.49 bbbcns	6.00 ± 0.84 cnsdns
FW (g)	30.40 ± 3.38 ansbns	25.00 ± 5.76 ansdns	30.20 ± 1.53 bnscc	25.00 ± 1.05 ccdns
OIW (g)	44.40 ± 1.94 aaab	30.20 ± 2.89 aaadns	39.30 ± 0.97 bccc	27.80 ± 1.02 cccdns
BP (%)	3.72 ± 0.18 ansbbb	3.79 ± 0.41 ansdns	3.32 ± 0.28 bbbcns	3.13 ± 0.42 cnsdns
FP (%)	10.40 ± 0.98 ansb	10.11 ± 2.35 ansdns	14.64 ± 0.67 bcns	13.17 ± 0.32 cnsdns
OIP (%)	15.30 ± 0.63 aaab	12.24 ± 0.80 aaadns	19.02 ± 0.41 aaab	14.71 ± 0.69 cccdns
Statistical significance Student values	t calculated < 1.994 – insignificant differences- ns t calculated = 1.994 – 2.648 – significant differences * (t0.05 = 1,994) t calculated = 2.648 – 3.416 – distinct significant differences ** (t0.010 = 2.648) t calculated > t 0.001 = 3.416 – very significant differences *** (t0.001 = 3.416)			

Note: ALW-average live weight; CBW- carcass after bleeding weight; PCW-plucked carcass weight; ECW - eviscerated carcass weight; SY – slaughter yield; BW - blood weight; FW – flakes weight; OIW – organs and intestines weight; BP – blood proportion; FP – flakes proportion, OIP - organs and intestines proportion. Significance of differences: a - meat females vs. meat males; b - meat females vs. mixed females; c - mixed females vs mixed males; d - meat males vs. mixed males.

The results of slaughter at the age of 42 days in the quails of the two populations

In the current experiment, the mean live weight at 42 days of age in the quail in the meat population was 15.70% significantly higher in females (290.60 ± 8.38 g/head) compared to males (245.00 ± 7.90 g/head). In the case of quail in the mixed population, the difference between the weight of females (206.60 ± 3.95 g/head) and of males (189.80 ± 6.85 g/head) was smaller, of 8.13%, but distinctly significant. The body weight of females in the meat population was 28.91% higher compared to that recorded in females in the mixed population, the difference being statistically very significant. The live weight of males in the meat population was 22.53% higher compared to that of males in the mixed population, the difference being statistically very significant.

The weight of the after bleeding carcass in the quails in the meat population was 15.73% higher in females (279.80 ± 8.14 g/carcass) compared to males (235.80 ± 8.41 g/carcass). In the case of quails in the mixed population, the difference between the weight of females (199.40 ± 4.37 g/carcass) and of males (183.80 ± 6.25 g/carcass) was smaller, of 7.82%.

The weight of the plumaged carcass in the meat quail was 15.45% higher in females (260.20 ± 6.38 g/carcass) compared to males (220.00 ± 7.34 g/carcass). In the case of quail in the mixed population, the difference between the weight of females (176.00 ± 3.51 g/carcass) and of males (164.80 ± 6.02 g/carcass) was smaller, of 6.36%.

The weight of the eviscerated carcass in the quails of the meat population was 15.22% higher in females (205.00 ± 8.82 g/carcass) compared to males (173.80 ± 6.37 g/carcass). In the case of quail in the mixed population, the difference between the weight of females (135.20 ± 3.29 g/carcass) and of males (128.20 ± 3.98 g/carcass) was smaller, by 5.18%. The average

weight of the eviscerated carcass was 34.05% higher in females in the meat population compared to females in the mixed population, the difference being statistically very significant. The average weight of the eviscerated carcass of males in the meat population was 26.24% higher compared to that of males in the mixed population, the difference being very significant statistically.

In a study conducted in Brazil on a quail flock from a meat population (Dumont et al., 2017), the authors mention an average weight of the eviscerated carcass between 171.00 g and 182.00 g, similar to those recorded in carcasses from males in the meat population of this experiment.

Carcass yield in females from the meat population, of 70.45±1.46%, was 4.90% distinctly significant higher compared to females in the mixed population. Also, the carcass yield in males in the meat population, of 70.98 ± 1.89%, was higher by 3.38% compared to that of males in the mixed population, the difference being statistically insignificant.

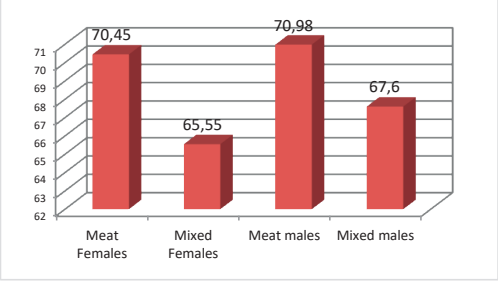

Figure 2. Carcass yield (%) in females and males in the two populations aged 42 days

The average proportion of blood was approximately the same in both females (3.72 ± 0.18%) and males (3.79 ± 0.41%) in the meat population, as well as in females (3.32 ± 0.28%) and males (3.13 ± 0.42%) in mixed population.

The average proportion of flakes recorded in the case of the meat population was 10.40 ± 0.98% in females and 10.11 ± 2.35% in males, while in the quail of the mixed egg-meat population it was 14.64 ± 0.67% in females and 13.17 ± 0.32% in males.

The average proportion of organs and intestines was higher for females both in the meat population (15.30 ± 0.63%) and in the mixed population (14.64 ± 0.67%) compared to that of males (12.24 ± 0.80% in males from the meat population and 14.71 ± 0.69% in males from the mixed egg-meat population).

The average breast weight in females of the meat population was 116.80 ± 6.11 g/chest, while in males it was 82.40 ± 4.65 g/chest, 29.11% higher in females, the difference between sexes being statistically very significant. In the case of quails in the mixed population, this was 69.60 ± 2.01 g/chest in females and 64.60 ± 2.75 g / chest in males, 7.18% higher in females, the difference being statistically insignificant.

In the same study from Brazil, the authors mention an average chest weight of 76.00 g, a weight that is closer to that recorded in the males in this experiment.

The average chest weight in females from the meat population was very significant, 40.41%, higher compared to that recorded in females from the mixed population. Also in males from the meat population the chest weight was distinctly significantly higher, by 20.60%, compared to that of males from the mixed population.

The average thighs weight was higher in females (49.40 ± 2.50 g/thighs) in the meat population by 17.00% compared to males (41.00 ± 1.22 g/thighs). In the case of quails in the mixed population, the average weight of the thighs was approximately the same in both sexes (33.40 ± 0.75 g/thighs in females and 32.80 ± 1.02 g/thighs in males).

Table 3. Weight and proportion of the component parts of the eviscerated carcass at the age of 42 days in females and males of mixed Balotești egg-meat quail and brown Jumbo meat quail

Specification	Meat quails		Eggs-meat mixed quails	
	Females	Males	Females	Males
ABW (g)	116.80 ± 6.11 aaabbb	82.40 ± 4.65 aaadd	69.60 ± 2.01 bbbcns	64.60 ± 2.75 ddcns
ALW (g)	49.40 ± 2.50 aabbb	41.00 ± 1.22 aaddd	33.40 ± 0.75 bbbcns	32.80 ± 1.02 dddcns
ABW (g)	41.40 ± 1.17 aaabbb	29.20 ± 1.74 aaad	26.00 ± 0.77 bbbcns	25.20 ± 0.80 dcns
ABW (g)	12.60 ± 0.51 ansbbb	11.60 ± 0.55 ansddd	8.00 ± 0.31 bbbcns	8.60 ± 0.51 dddcns
ABP (%)	57.05 ± 2.20 aabb	47.42 ± 2.00 aadns	51.54 ± 1.47 bbcns	50.60 ± 2.75 dnscns
ALP (%)	24.12 ± 0.79 ansbns	23.64 ± 0.50 ansdns	24.73 ± 0.53 bnscns	25.70 ± 1.23 dnscns
ALP (%)	20.26 ± 0.46 aaabbb	16.78 ± 0.56 aaaddd	19.30 ± 0.88 bnscns	19.70 ± 0.65 dddcns
ABP (%)	6.19 ± 0.39 ansbns	6.68 ± 0.19 ansdns	5.92 ± 0.17 bnscns	6.71 ± 0.35 dnsc
Statistical significance Student values	t calculated < 1.994 – insignificant differences- ns t calculated = 1.994 - 2.648 – significant differences * (t0.05 = 1,994) t calculated = 2.648 – 3.416 – distinctly significant differences ** (t0.010 = 2.648) t calculated > t 0.001 = 3.416 – very significant differences *** (t0.001 = 3.416)			

Note: ABW-average breast weight; ALW-average legs weight; ABW-average back weight; AWW-average wings weight; ABP-average breast proportion; ALP-average legs proportion; ABP-average back proportion; AWP-average wings proportion. Significance of differences: a - meat females vs. meat males; b - meat females vs. mixed females; c - mixed females vs mixed males; d - meat males vs. mixed males.

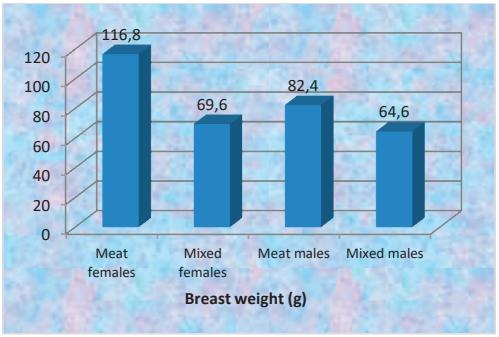

Figure 3. Average weight of the breast (g) in females and males fom the two populations at the age of 42 days

The average weight of the back was 29.47% higher in females (41.40 ± 1.17 g/back) compared to that of males (29.20 ± 1.74 g/back) in the quails of the meat population, the differences being very significant statistically, while in the case of quails from the mixed population this was approximately the same in both females (26.00 ± 0.77 g/back) and in males (25.20 ± 0.80 g), the difference being statistically insignificant.

The average weight of the wings was approximately the same in both females (12.60 ± 0.51 g/wings) and the males (11.60 ± 0.55 g) of the meat population, as well as in the females (8.00 ± 0.31 g/wings) and males (8.60 ± 0.51 g) from the mixed population, the difference being statistically insignificant.

The average proportion of the breast registered significant differences in the case of quails from the meat population, which was 9.63% higher in females (57.05 ± 2.20%) compared to that of males (47.42 ± 2.00%). In the quails of the mixed population, the average proportion of the breast was approximately the same in both females (51.54 ± 1.47%) and males (50.60 ± 2.75%).

The average proportion of the thighs was approximately the same in the case of quails in the meat population (24.12 ± 0.79% in females and 23.64 ± 0.50% in males) and in the case of quails in the mixed population (24.73 ± 0.53% in females and 25.70 ± 1.23% in males), the differences being statistically insignificant.

The average proportion of the back was higher in females (20.26 ± 0.46%) by 3.48% than in males (16.78 ± 0.56%) in the meat population, while in the quails in the mixed population it was approximately the same in both females (19.30 ± 0.88%), as well as in males (19.70 ± 0.65%).

The average proportion of wings was higher in males (6.68 ± 0.19%) than in females (6.19 ± 0.39%) in both the meat quails and mixed quails population (6.71 ± 0.35% in males and 5.92 ± 0.17% in females), the difference being statistically significant.

CONCLUSIONS

The present paper aims to determine the results of slaughter in quails from a meat population (brown Jumbo) and a mixed population (Baloteşti) and their comparison.

The average live weight was 290.60 ± 8.38 g in females of the meat population and 206.60 ± 3.95 g in females of the mixed population, 28.91% higher in females in the meat population, the differences being statistically very significant.

The average live weight of males in the meat population was 245.00 ± 7.90 g/head, 22.53% higher compared to that of males in the mixed population (189.80 ± 6.85 g/head), the difference being statistically very significant.

The average live weight at 42 days of age in the quails of the meat population was 15.70% very significantly higher in females compared to males. In the quails of the mixed population the difference between the weight of females and males was smaller, of 8.13%, but distinctly significant.

The average weight of the eviscerated carcass was 205.00 ± 8.82 g/carcass, 34.05% higher in females in the meat population compared to females in the mixed population (135.20 ± 3.29 g), the differences being very significant statistically.

The average weight of the eviscerated carcass of males in the meat population was 173.80 ± 6.37 g / carcass, 26.24% higher compared to that of males in the mixed population (128.20 ± 3.98 g), the differences being statistically very significant.

The weight of the eviscerated carcass in the quails of the meat population was 15.22% higher in females compared to that of males. In the case of quails in the mixed population, the difference between the weight of females and males was smaller, of 5.18%.

Carcass yield in females from the meat population was 70.45 ± 1.46%, with 4.90% distinctly significant higher compared to that of females in the mixed population (65.55 ± 0.70 %).

Also, the carcass yield registered in the males of the meat population, of 70.98 ± 1.89 %, was higher by 3.38 % compared to that of the males in the mixed population (67.60 ± 0.58 %), the differences registered being statistically insignificant.

The average breast weight in females from the meat population was 116.80 ± 6.11 g, 40.41% higher compared to that recorded in females in the mixed population (69.60 ± 2.01 g). Also in males from the meat population the breast weight was 82.40 ± 4.65 g, higher by 20.60% compared to that of males in the mixed population. The average breast weight in females of the meat population was 29.11% higher than in males, and in the case of quails in the mixed population, it was only 7.18% higher in females.

The slaughter results at 42 days were higher in the meat population compared to the mixed egg population, in females compared to males in the same population and among females in both populations than in males from these, regarding the weight of the carcass, the cutting yield, the weight of the chest and of the other component parts of the carcass.

Following the researches carried out, it is recommended to use in the meat production the quails from the brown Jumbo meat population, specifying that a breeding lot should be formed that is specially raised for this direction, and the eggs from this lot incubated and the chickens raised for meat, without directing the females to egg production. This will result in superior production and high quality carcasses. You can also opt for breeding separately by sex, the carcasses from females being larger and more attractive.

The quails from the mixed eggs - meat population of Baloteşti can also be used in the direction of meat production, if it is desired to obtain smaller carcasses compared to those from meat quails, depending on the preferences of consumers and the logistics of producers (breeding areas, types of compound feed, lighting programs, etc.).

In the future, the growth parameters for meat quails should be studied in correlation with the growth factors, as being bigger, a smaller density should be applied to the unit area

compared to that of mixed quails and other nutritional parameters of the combined food recipes should be applied.

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IMPACT OF PRECISION LIVESTOCK FARMING ON WELFARE AND MILK PRODUCTION IN MONTBELIARDE DAIRY COWS

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Abstract

The concept of "precision livestock farming" represents the optimization of the contribution of each animal to the integrated economy of the farm. During more than three decades of applied research, precision zootechnics has gone through several stages, namely from process automation, developed to reduce human labor, to focusing attention on monitoring animal needs (health, well-being). All information collected at the farm level has the role of supporting the farmer in making optimal and fast decisions, in accordance with the animal's condition and the efficiency of the farm. Sensor technologies are being used to monitor the production and physiological condition of the animals, thereby contributing to animal welfare, animal health and food safety. Farm management is assisted by sensor systems collecting information about milk production, reproduction and animal health status. Increasingly, these innovations are leading to a more efficient performance of dairy farms, in terms of both physiology and profitability.

Key words*: microclimate, milk production, precision livestock farming.*

INTRODUCTION

Modern world's challenges are growingly diverse, ranging from the increase in the number of people on the planet, to the higher food demand (70% increase amid a rapid urbanization and the surge in the purchasing power / revenues), to climate changes, which will affect relatively large areas of the Globe (extreme weather events, water shortage, soil depletion) and price volatility, as a result of regional conflicts or enzootics / pandemics (African swine fever, Avian influenza, Foot and Mouth Disease, Bluetongue to Covid19, which affects the entire planet). In this general context, farmers must follow a strategic plan to optimize resources and reach the estimated yield potential. The EU-level average milk production is expected to increase up to 8,340 kg milk/cow until 2030, compared to 7,300 kg milk/cow in 2010.

The expected growth rate is +1.2% / year, lower than in the previous 2009-2019 period, namely +1.9% per year.

Due to this efficiency requirement, the number of dairy cows could be reduced by 1.4 million heads (21.2 million heads in 2019). Additionally, production systems will have an increasing influence on the total milk production (thus, the organic milk production will increase to 7% of the total milk production until 2030, compared to 3% in 2017) (EU Agricultural Outlook for markets and income, 2019).

The National Institute of Statistics data indicate a lower number of dairy farms, namely less by 14.8% in 2016 compared to 2013. At the same time, the number of dairy farms with capacity of 10-30 milk cows seems to have increased by 16.6%. Although commercial farms in Romania accommodate 11.8% of the total number of dairy cows, yet, the average yield per cow is 8,741 kg of milk.

Precision livestock farming (PLF) is an instrument available to farmers is which focuses on real-time monitoring and management of livestock and production factors, in order to improve livestock life, quality and productivity, alerting whenever problems occur, so farmers

can take immediate action. PLF's main purpose is to combine existent hardware and software to obtain a wide range of information that will produce added value, to monitor health, well-being, productivity and environmental impact (Berckmans, 2014).

Sensor technologies are being used to monitor the production and physiological condition of the animals, thereby contributing to animal welfare, animal health and food safety. (Kelemen, A. et al, 2016) Farm management is assisted by sensor systems collecting information about milk production, reproduction and animal health status. Increasingly, these innovations are leading to a more efficient performance of dairy farms, in terms of both physiology and profitability (Lokhorst, 2018; Vidu et al., 2016).

In dairy farms, sensor-based technologies are mainly used to:

- identify each animal;
- detect physiological changes in reproduction and monitoring the health status;
- record the activity and location of each animal;
- monitor feeding and ruminating;
- automate milking and feeding;
- monitor milk quality.

MATERIALS AND METHODS

The purpose of the paper is to conduct a study on the impact and benefits of using precision farming means on milk production and on the welfare of dairy cows.

The researched material consists of the lot of lactating cows from the Moara Domneasca teaching farm. The cow breed is Montbeliarde, a breed with very good environmental adaptability and resilience. The study was conducted over a 4-month period, respectively (1 March 2019 - 30 June 2019). The experimental group consisted of 28 cows at different stages of lactation.

The working methodology is based on two precision farming systems, namely system to monitor microclimate parameters in dairy cow shelter and the second one is system to monitor milk quantity and quality. (for microclimate monitoring in the cow shelter (*SMART Zoo Tech*; to identify animals in the milking parlor, to monitor reproduction and rumination - *Dairyplan C21*) *SMART Zoo Tech* solution for measuring, logging, monitorig the temperature, humidity, dew point, carbon dioxide (CO_2) concentration, ammonia (NH_3) and dust particles (PM 2.5).

To evaluate the welfare, analyses were conducted in the shelter, at animal level, in the milking parlor.

The evaluation was performed based on the methodology included in the *ANI 35 system*. The findings were processed and interpreted in order to define the welfare and the influence on production. Specifically, 5 groups of factors were analyzed:

- freedom of movement;
- social interactions;
- type and characteristics of outdoor floors and surfaces;
- lighting, air quality and noises;
- conditions of care and maintenance.

The *ANI score* is calculated by cumulating the scores awarded for each of the 5 groups of factors separately. An individual sheet is drawn up for each of the 5 groups of factors.

The evaluation of animals focused on:

- separate evaluation of welfare for the cattle category;
- determination of welfare in the period (March-June 2019).

In the ANI system, animal welfare is studied based on 5 groups of factors and details are presented below on how each group of factors can influence the cattle welfare. According to the ANI 35 system, the cattle welfare, at the end of the evaluation, falls into the following categories:

- below 16 points - very poor welfare;
- between 16 and 20 points - poor welfare;
- between 20 and 25 points - average welfare;
- between 25 and 28 points - satisfactory welfare;
- between 28 and 32 points - full welfare;
- above 32 points - optimal welfare (Gavrilă, 2015).

The dairy cow breeding technology is modern, offering European standard tools and equipment for the maintenance, feeding and milking of dairy cows.

The maintenance of dairy cows is carried out in a free maintenance shelter. Indoors, there are delimited areas for animal feeding (with feeding front), area for movement and manure disposal (with delta-shaped plough), individual cubicle-type resting areas.

The floor is covered with various types of rubber mats (fig. 1).

Figure 1. Cows rest in individual cubicle with rubber mat flooring

Cow feeding is done with optimized feed based on the nutritional needs, production level and physiological state, without being differentiated seasonally. The distribution of feeds is done by technological trailer. The milking technology is based on the use of a herringbone milking parlor, 1x3 places. The milk produced at farm level is marketed in fresh form through milk vending machines.

RESULTS AND DISCUSSIONS

The studied data indicated that, during the experimental period (March-June), the total milk production at farm level was as follows: in March, the total production was 10,226 kg milk, with a daily average of 342.77 kg; in April, the total production was 10,058.3 kg, with a daily average of 335.27 kg, in May, the total production was 10,533 kg and the daily average 339.77 kg and, in June, the total production was 8,541.09 kg, with a daily average 284.7 kg (fig. 2).

As for the link between individual production of cows and microclimate parameters, findings were that there is a close correlation between them. Thus, on the first day of the experiment, the average milk production per dairy cow was 11.79 kg milk, the air temperature was 16°C on that day, according to the National Meteorological Administration (ANM). At shelter level, the temperature was recorded on hourly basis and the data were transmitted, thus, the variation interval ranged between 10.80°C at 9:00 a.m. and 15.78°C at 5:00 p.m. Fig. 3 illustrates the variation of all the microclimate parameters. The CO_2 (ppm) and NH_3 (ppm) concentration had the most interesting dynamics, with very high values during nighttime. Thus, the peak of NH_3 concentration was reached at 9:00 p.m., when the CO_2 concentration was maximum, as well (fig. 4).

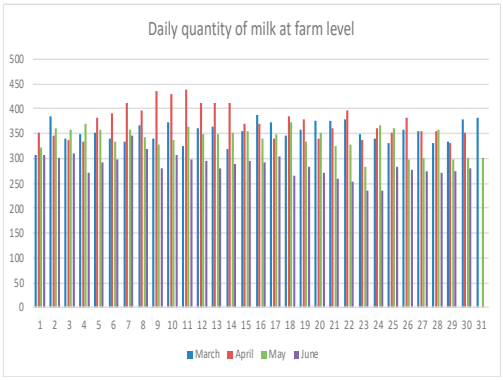

Figure 2. Total daily milk production during March-June

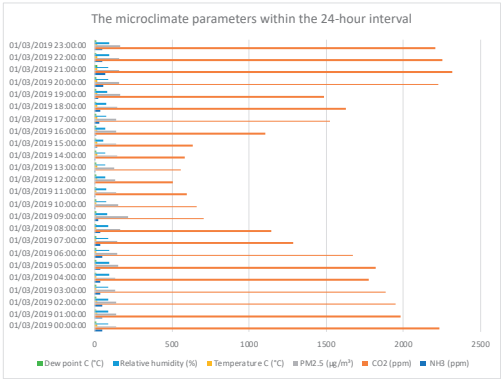

Fig. 3 Microclimate parameter values on 1 March 2019

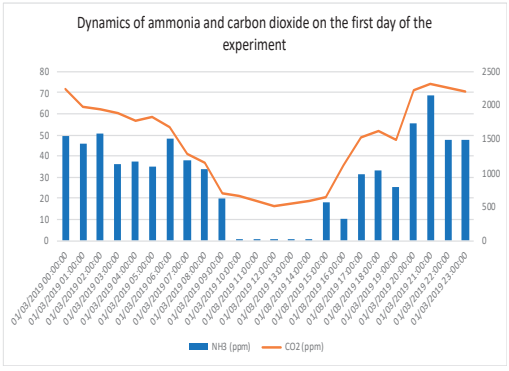

Fig. 4 Dynamics of ammonia and carbon dioxide concentration on 1 March 2019

From the entire experiment period, we have selected to show as an example the day of 13

June 2019, which was the warmest day of that period. Air temperature on that day was 34°C and the shelter-level temperature varied between 23.45°C at 4:00 a.m. and 31.04°C at 5:00 p.m. Moreover, the dew point followed the same dynamics. The relative humidity had several fluctuations after 4:00 pm. After that time, the ammonia level maintained high until the end of the day. During that day, the farmer received individual alerts on the mobile phone for each microclimate parameter on hourly basis in the second part of the day (fig. 5, 6).

As for milk production, it is known that the optimal temperature range for dairy cow is between 9 and 16°C. June had the lowest milk production of the 4 experimental months. On June 13, cows' milk production was 20 kg less than two days before (fig. 7).

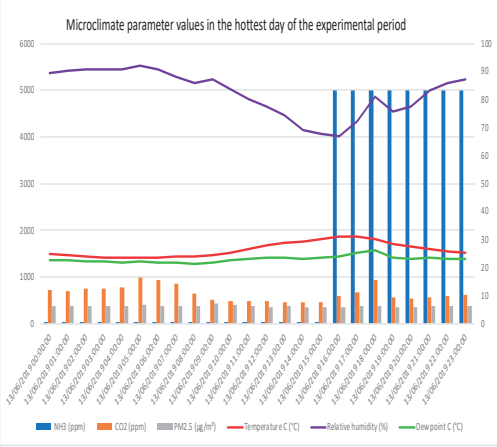

Fig. 5 Microclimate parameter values on 13 June 2019

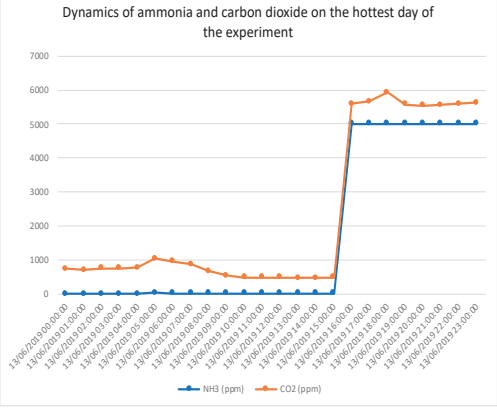

Fig. 6 Dynamics of ammonia and carbon dioxide concentration on 1 March 2019

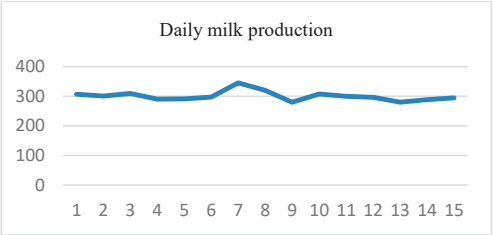

Fig. 7 Dynamics of milk production in the first 15 days of June

As for the ANI 35 system-based analysis of the welfare of Montbeliarde cows from the experimental farm, the evaluation sheets were completed and the scores obtained were reported at grid reference intervals.

For the freedom of movement criterion, the main deficiency of the farm identified during the study was the fact that the farm does not have access to a pasture, therefore, the score awarded to this chapter is 0. as for the access to paddock, it was found that the animals have access to pasture for a period of at least 230 days per year. Out of the maximum 12.5 points that can be obtained for this criterion, the farm received 10.5 points (Table 1).

As for the second criterion - social interaction, the evaluation showed that the farm received 7 points out of maximum 10 points possible. The access to pasture is one of farm's drawbacks in that case, as well. Additionally, the access to paddock is restricted to over 230 days per year. (Table 2)

Table 1. Freedom of movement

Group of Factors	Score awarded
Minimum surface area available m²/head	3
Comfort during rest day	3
Comfort provided by tie-stalls	1
Amplitude of the possible movements in tie-stalls	1
Access to the paddock days / year	2.5
Access to pasture days / year	0
TOTAL Score	10.5

Table 2. Social Interaction

Group of Factors	Score awarded
Minimum surface area available m²/head	3
Group structure	1
Calf and young cow management	0.5
Access to paddock days / year	2.5
Access to pasture days / year	0
TOTAL Score	7

The floor characteristics in terms of elasticity, cleanliness are analyzed within criterion 3.

Table 3. Type and characteristics of the floor and outdoor areas

Group of Factors	Score awarded
Elasticity of the rest area	1.5
Cleanliness level in the rest area	1
Risk level in terms of sliding in the rest area	1
Floor quality in activity areas	1
Type and characteristics of paddocks	0.5
Type of pastures	0
TOTAL Score	5

We can conclude that the welfare in terms of the type and the characteristics of the floor and outdoor surfaces is very good and receives 5 points out of maximum 8 possible points. But the points are lost due to the lack of pasture and paddock surface (fig.8 and Table 3)

Fig. 8 Aspect of the paddock surface

Table 4. Lighting, air quality and noise

Group of Factors	Score awarded
Natural light	1.5
Air quality	1.5
Ventilation in the rest area	1
Noises in the shelter	1
Access to outdoor areas day / year	2
Access to outdoor areas hours / day	2
TOTAL Score	9

Criterion 4 analyzes shelter's lighting, air quality, ventilation in the rest area, noises and access to outdoor areas during the whole year and during an entire day. The farm received a score of 9 points out of a maximum of 9.5 for this criterion. (Table 4)

Table 5 lists the items considered to assess the animal health, as well as the health of skin and nails. The farm received 7.5 points out of a total of 8 possible points for this criterion.

Table 5. Tending and maintenances conditions

Group of Factors	Score awarded
Sanitation of surfaces in accommodation, feeding, milking areas	1
Equipment wear and tear	1
Skin health	1
Animal body hygiene	0.5
Trotter health	1.5
Incidence of technopathies	1
Livestock health	1.5
TOTAL Score	7.5

The farm cumulated a total of 39 points, which indicates optimal welfare conditions for the dairy cows. That means that the farm management is high-level and special attention is paid to the welfare of the animals, understanding that welfare and productive performance are closely connected.

CONCLUSIONS

The farm of the Moara Domneasca teaching station accommodates Montbeliarde cows, brought in from France 10 years ago, this breed being characterized by good adaptability, resistance and hardiness.

The analysis of milk production over 4-month experimental period indicated that milk production was lower in the month with the highest temperature, although, physiologically, the lactation curve was ascending.

The use of precision livestock farming proves useful at farm level, because it records the microclimate parameter values throughout the day and, in case of excess level, alerts are sent to farm administrator's phone / email so he can take immediate action.

In terms of welfare, through the ANI 35 system-based evaluation showed that the farm received a good score, which places it at the "optimal welfare" level.

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INFLUENCE OF THERMAL PROCESSES ON DONKEY MILK COMPOSITION

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Abstract

Currently, the extinction of donkeys (Equus asinus) worldwide is very limited, but donkey milk is recently getting popular in many countries due to its nutritional properties and chemical composition. For this reason, in our country, the farmers are encouraged to keep, to raise and increase the number of donkeys in their farms. Milk and donkey products are niche products, consumed by a small number of people, especially people suffering from various diseases (e.g. food allergies, children to whom breast milk should be substituted with milk similar in composition). Donkey milk brings benefits to consumers due to its chemical composition. Donkey milk storage depend on the chemical composition, antioxidant capacity and the biochemical degradation under heat treatment.

Key words*: donkey milk, fat, protein, lactose, antioxidant capacity*

INTRODUCTION

Donkey milk has a high antioxidant capacity, delays the aging process and is rich in various compounds that stimulate the body's immunity (Ma et al., 2008). Milk is an important nutritious food with many essential components (vitamins, minerals, acids, amino acids, minerals, lysozyme) and compounds such as antioxidants. Antioxidants are a particular importance due to their positive effect by neutralizing and eliminating free radicals (Saxelin et al., 2003; Yazdanparast et al., 2007). At present, people pay a lot of attention to natural products that have antioxidant capacity. Antioxidants have ability to neutralize and eliminate free radicals, and bring benefits to the human body (Khan, 2019).The antioxidant capacity of donkey milk is higher than cow milk. For children, people with allergic problems, and cardiovascular diseases and diabetes the administration of donkey milk and powdered milk are better than cow's milk due to their nutritional properties and chemical composition (Ling et al., 2018). Donkey milk can substitute the breast milk, when this is not enough due to its chemical composition and the antioxidant capacity that reduces oxidative stress (Beghelli et al., 2016).The antioxidant capacity is also shown in the study reported by (Anuradha et al., 2019) on Halari and French Poitu donkeys. The chemical composition and antioxidant capacity of milk are influenced by processes applied to milk (e.g. processing, pasteurizing, boiling, packing, storage conditions) (Zygoura et al., 2004). Antioxidant capacity is a very important parameter and it should be analyzed in milk and dairy products. There are studies that highlight the presence of oxidants and the mechanism of defence of antioxidants (Halliwell, 1996). Pozzo et al. (2019) evaluated the activity of human milk, cow's milk, donkey milk, raw and after pasteurization. He observed a significant variations depending on species and the treatment applied. Human milk minimized the intake of food oxidative compounds compared to other infant formulas. Cloetens, 2013 used different methods to measure the total antioxidant capacity (TAC), similar compounds in human and animal milk, and the effect of antioxidants in vitro and in vivo studies.

The main components of donkey's milk play a very important role in improving the defence system for new-borns and young children, who cannot be fed with breast milk or have an allergy to milk from other animal species (e.g. cows). The importance of donkey milk due to due to its content in antimicrobial components was reported in various studies. In the last decade, the interest for donkey milk consumption has grown as a source of human food. The large amounts of antimicrobial components and defense factors present in milk provide protection against microbial infections. These characteristics make it different from other types of milk (Carminati et al., 2014; Sies et al., 2007). Clinical studies showed that donkey milk can be used in in treating allergies to cow's milk proteins or in food intolerance. In addition, it has benefits in terms of energy balance, lipid metabolism and disease prevention, which is considered a functional food (Martini et al., 2014). The purpose of this study was to evaluate the influence of thermal processes on the composition (fat, protein, lactose, pH, antioxidant capacity) of donkey milk. The milk was obtained of donkeys in the Transylvania area.

MATERIALS AND METHODS

Sterile containers were used for sample collection.

The samples were collected from donkeys maintained in traditional system. Farms were situated in Cluj and Salaj area. Milk production per animal/day was 900 ml -1.5 litters. Milk samples were collected from donkeys in lactations 1 and 2 and were manually milled. Physico-chemical analysis (fat, protein, lactose and pH) was performed by using Lactoscan. The antioxidant capacity of donkey milk was analysed by using the ACL (antioxidant capacity of lipid soluble compounds) method. Photochem was used to determine antioxidant capacity.

RESULTS AND DISCUSSIONS

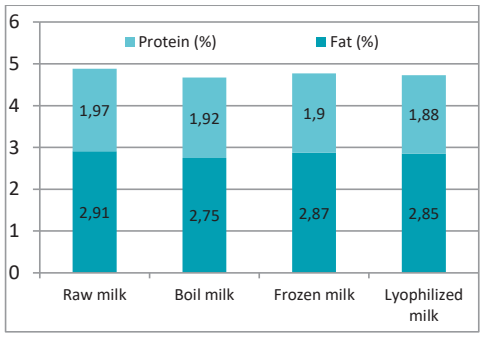
Donkey milk due to its antioxidant capacity, has the ability to prevent lipid peroxidation and has a positive effects on people will consume this milk. Donkey milk is considered a nutritional and functional food due to its property and the general chemical composition (Beghelli, 2016). In our study, the correlation between fat content and TAC was observed in the analysed raw milk. In addition the whole milk and whey had a significant influence on TAC, caseins and albumin. Similar results with our study were obtained by Zulueta (2009), who observed a significant differences for TAC, whey and deproteinized samples when compared pasteurized milk and milk treated with UHT. In addition, Ling et al. (2018) informed in his study that the total antioxidant capacity of donkey milk ranged between 13.933±1.387 and 16.578±0.291 U/m, which are in the same range with values obtained in the present study.

Antioxidant activity increased significantly when increased the fat content in the milk. This result may be influenced by lipid involvement and antioxidant reactivity (Alaa et al., 2020). The antioxidant activity in cheese increased during the ripening period, and this was correlated with the degree of proteolysis (Gupta et al., 2009).Similar findings with our study was obtained by Khan (2019), who evaluated the antioxidant and nutritional capacity of milk and dairy products; determines the total antioxidant capacity of pasteurized milk and (UHT). TAC in donkey milk was not affected by lactation did, but this content may vary depending on the quality of the feed in the diet (Beghelli et al., 2016). Lipko-Przybylska et al. (2012) evaluated antioxidant activity in colostrum and mil, and reported dynamic changes regarding antioxidant capacity in the postnatal period. Protein in raw donkey milk was 1.97%, decreased to 1.92% in boiled milk and 1.90% in frozen milk. The lowest value of protein was registered in lyophilized milk (figure 1.a).

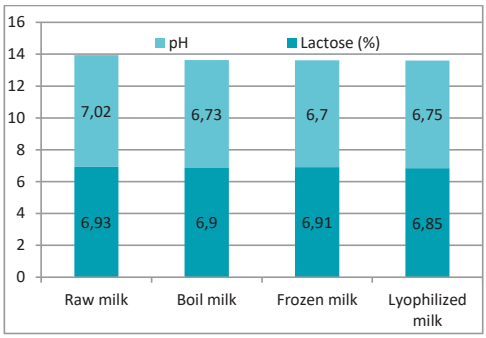
Fat showed the lowest values in boiled milk 2.75%, and in lyophilized milk 2.85% (figure 1.a). Lactose had the lowest values in lyophilized milk 6.85% and in boiled milk 6.90% (figure 1.b). Protein is a very important parameter in the composition of donkey's milk.

Protein is a very important parameter in the composition of donkey's milk, and an important factors in the diet and development of organisms (El-Hatmi et al., 2015). Ibrahim, 2015, realized study about the chemical composition of camel milk (fresh, dried and frozen). The content of total protein, casein, lactose and ash from skimmed and freeze-dried milk was higher

compared to camel milk completely freeze dried. The results indicated that the moisture content of completely dried and frozen camel milk was lower due to the freeze-drying process (Ibrahim, 2015). Similar results about the physico-chemical parameters were reported by Kumar and Mishra, 2004.



a



b

Figure 1. a., b. Physico-chemical composition of donkey milk (raw, boiled, frozen and lyophilized)

Figure 2 shows the results of TAC (U/ml) of donkey milk according to the applied process (boiling, freezing and lyophilization).

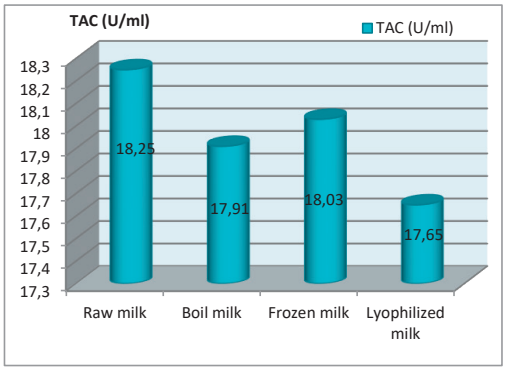


Figure 2. TAC (U/ml) of raw, boiled, frozen and lyophilized donkey milk

TAC - total antioxidant capacity;

The TAC values obtained in our study were higher than the values reported by Ling et al. (2018) on donkey milk. Khan (2017) reported the following values for TAC (%): for cow's milk and buffalo milk in raw milk 42.1%, pasteurized 41.3%, and boiled 40.7%. TAC (%) in buffalo milk presented the following values: in raw buffalo milk: 58.4%, pasteurized: 57.6%, and boiled 66.5%. We observe for TAC, significant changes also in milk that comes from other species.

Mann et al. (2016) studied the antioxidant capacity at different stages of lactation in cattle and buffaloes. TAC in milk was higher during early lactation compared to other lactation periods. The data suggested that they are removed from lactation to make time for dairy cows and buffaloes to have a higher content of antioxidants compared to other stages of lactation. Polidori and Vincenzetti (2013) investigated the protein profile of donkey milk, considered safe for the prevention and treatment of various human diseases. The influence of heat treatment on the protein fractions of donkey milk was also evaluated. Lyophilization is a method that can maintain the nutritional characteristics of milk (Polidori and Vincenzetti, 2013).

Silvestre et al. (2008) reported changes in the total antioxidant capacity of milk, when it was pasteurized at different temperature and different intervals of time. The antioxidant activity of cheese increased during the maturation period, and the antioxidant activities were correlated with the proteolysis degree (Gupta et al., 2009). Total antioxidant capacity also gives information about the antioxidant state of biochemical compounds, which has the ability to fight the free radicals produced. Antioxidant capacity is used as a new marker to evaluate oxidative stress (Sies et al., 2007). Vora et al. (2017) in his study recommended the use of natural antioxidants in order to extend the shelf life of products. For the human body, the natural antioxidants cause fewer side effects compared to synthetic. In the case of dairy products, such as ginseng yogurt, antioxidant capacity decreased during storage. These aspects were related in realized by Jung et al. (2016). Consumers prefer natural antioxidants obtained from various food sources instead of synthetic antioxidants, which can have toxic

effects on the human body (Zambonin et al., 2012; Abdel-Hamed et al., 2014). People increase the interest on the milk and dairy products obtained from donkey, buffalo, cow and goat due to their potential for antioxidant capacity, the diversity of milk caseins and whey proteins (Pihlanto, 2006; Suetsuna et al., 2000). TAC has also been studied for cow's milk, camel, cow's and camel's yogurt (Niero et al., 2016; Santos et al., 2012; Shori, 2013; Shori and Baba, 2011).
Table 1 shows the average values for TAC in milk and colostrum of donkey obtained in the present study compared to other species.

Table 1. TAC for colostrum and donkey milk compared to other species

Sample	TAC[1]	References
Donkey milk (Italy)	31.2 (mmol/L)	Simos et al., 2011
Donkey colostrum (µ mol neut. HClO/ml)	L.P.1st (221.7) – L.P. 5th (210.9)	Beghelli et al., 2016
Human milk	816.3 (µmol/L)	Živković et al., 2015
Human colostrum	1061.6 (µmol/L)	Živković et al., 2015
Cow milk (pasteurized)	1246 (µmol/L)	Chen et al., 2003
Cow colostrum	3.92 (µmol/g)	Albera et al., 2011
Murrah buffalo milk	360.49	Mann et al., 2016
Murrah buffalo colostrum	393.42	Mann et al., 2016
Ewe milk	6.69 (µmol/g)	Lipko-Przybylska et al., 2010
Ewe colostrum	4.78 (µmol/g)	Lipko-Przybylska et al., 2010
Goat milk	35.8 (mmol/L)	Simos et al., 2011

TAC[1] - total antioxidant capacity; L.P. - lactation period

CONCLUSIONS

Antioxidant capacity is a very important parameter, because it gives us information about the benefits of donkey milk depending on the amount of TCA in milk. TAC in donkey milk was influenced by the boiling and lyophilization process. Donkey's milk can be used to obtain innovative and value-added products due to its nutritional and functional properties. This information can bring important financial benefits to both producers and consumers.

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RESEARCH REGARDING THE SLAUGHTERING OUTPUT, TISSUE STRUCTURE AND THE QUALITY OF THE CARCASSES AT THE HYBRID LAMBS FOR MEAT COMPARATIVELY TO THEIR CONTEMPORARIES FROM THE PARENTAL BREEDS

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Abstract

The present work aims to present the superiority of the hybrid lambs for meat regarding the slaughtering output, the tissue structure and the quality of the carcasses, comparatively to their with contemporaries from the parental breeds. The research was made on lambs fattened until the living weight 40 kg/head from the breeds of Palas Merino, Palas Meat Breed and F1 hybrids of Palas Meat Breed x Palas Merino and F1 Suffolk x Palas Merino. The highest slaughtering output was that of the F1 hybrids Suffolk x Palas Merino, out of them R1 having the value of 51,21 % and R2 of 55,94 % besides 44,25 % and respectively 49,54 % at the contemporaries of Palas Merino. Also, these hybrids had also the best carcasses, being categorized by conformation in U class (very good carcasses) and by fattening degree in the 3rd class 3 (medium fat carcasses).

Key words*: hybrid, slaughtering output, carcass.*

INTRODUCTION

In Romania the meat of youth sheep is produced through fattening the lambs from the breeds of Merino, Tsigaie and Tsurcana and less through producing and fattening meat hybrid lambs.
Less research was made regarding the slaughtering output, the tissue structure and the quality of the carcasses at the local sheep breeds and at the hybrids with the meat breeds (Ciolcă et al., 1973; Vicovan, 2008; Vicovan, 2009).
This paperwork aims to show and to promote the superiority of the hybrids comparatively to their contemporaries from the three Romanian breeds, regarding the slaughtering output and the quality of their carcasses.

MATERIALS AND METHODS

The research was made on young male sheep that have been fattened until the living weight of 40 kg/head, from Palas Merino Breed, Meat Breed Palas, F_1 hybrids of Meat Breed Palas x Palas Merino and F_1 Suffolk x Palas Merino.
From each breed and crossbreeding variant three exemplars were slaughtered.
The slaughtering output was calculated through two methods as follows:

- Output1 (R_1) by reporting the weight of the cooled carcass to the living weight before slaughtering;
- Output2 (R_2) by reporting the weight of the cooled carcass to the empty living weight, which results after from the living weight it was subtracted the content of the digestive tube.

The tissue structure of the carcasses was determined by the dissection of the right semi-carcass (separation of the muscles from bones and from the intramuscular covering fat).
The appreciation of the quality of the carcasses was made by conformation and the degree of fattening through comparison with the European grid of classification of the carcasses (S.E.U.R.O.P).
All data was processed and statistically interpreted through the Fisher test (Snedecor, 1968).

RESULTS AND DISCUSSIONS

Table 1 is presenting the slaughtering output at the lambs from the 2 breeds and at the studied hybrids for meat.

Table 1. The values of the slaughtering output at the hybrid lambs comparatively to their contemporaries from Palas Merino breed and Palas Meat Breed

Breed/ Hybrid	The slaughtering output (%)					
	R_1			R_2		
	n	X ± sx	V%	n	X ± sx	V%
Palas Merino	3	44.25 ± 0.8290	3.21	3	49.54 ± 1.3700	4.79
Meat Breed - Palas	3	47.54 ± 0.5100	1.85	3	53.98 ± 0.4700	1.51
F_1Meat Breed - Palas x Palas Merino	3	48.12 ± 0.4988	1.79	3	54.74 ± 1.1800	3.74
F_1 Suffolk X Palas Merino	3	51.21 ± 1.2280	4.12	3	55.94 ± 0.9100	2.83

It is noted that the Palas Merino had the lowest values of the slaughtering output, these being 44.25% for R1 and 49.54% for R1 and R2, followed by the Palas Breed Meat with 47.54% respectively 53.98% (Table 1).

The hybrid F_1 Meat Breed Palas x Palas Merino values were 48.12% to 54.74% for R1 and R2, and the highest values being for F1 hybrid Suffolk x Palas Merino with 51.21% and respectively 55.94%.

Table 2 (a.b) is presenting the differentiation of slaughtering output between genotypes, are shown the differences of R1 and R2 related to the F1 hybrid between Meat Breed Palas x Palas Merino as compared to PalasMerino and Meat Breed Palas. It also shows the values of R1 and R2 in the Meat Breed Palas from the Palas Merino and Hybrid F1 Suffolk X Palas Merino compared with PalasMerino and Hybrid F1 Meat Breed Palas x PalasMerino.

It can be noticed that there were significant differences ($p< 0,05$) both between F1 Meat Breed Palas x Palas Merino and Palas Merino and also between Meat Breed Palas and Palas Merino, but also between F_1 Suffolk x Palas Merino and Palas Merino (Table 2). Between the two hybrids the differences were insignificant ($p> 0.05$).

Table 2a. Differentiation of slaughtering output

Differences between	Output 1 (R_1)	
	Percent points	Significance of differences
F_1Meat Breed – Palasx Palas Merinoand Palas Merino	+ 3.87	p< 0.05
F_1Meat Breed – Palasx Palas Merino and Meat Breed - Palas	+ 0.58	p> 0.05
Meat Breed – Palasand Palas Merino	+ 3.29	p< 0.05
F_1 Suffolk x Palas Merinoand Palas Merino	+ 6.96	p< 0.05
F_1 Suffolk x Palas Merinoand F_1Meat Breed – Palasx Palas Merino	- 3.09	p> 0.05

Table 2b. Differentiation of slaughtering output 1.

Differences between	Output 2 (R_2)	
	Percent points	Significance of differences
F_1Meat Breed – Palasx Palas Merino and Palas Merino	+ 5.20	p< 0.05
F_1Meat Breed – Palas x Palas Merino and Meat Breed - Palas	+ 0.76	p>0.05
Meat Breed – Palasand Palas Merino	+ 4.44	p< 0.05
F_1 Suffolk x Palas Merinoand Palas Merino	+ 6.40	p< 0.05
F_1 Suffolk x Palas Merinoand F_1Meat Breed – Palasx Palas Merino	- 1.20	p> 0.05

Table 3 is presenting the tissue structure of the carcasses at Palas Merino, Meat Breed Palas and the two hybrids.

Table 3a. The tissue structure of the carcasses at the lambs that had been experimentally slaughtered

Breed /hybrid		Tissue Structure (%)	
		muscles	bones
Palas Merino	n	3	3
	X ± sx	60.77 ± 2.0800	23.32±1.0200
	V%	5.85	7.57
Meat Breed - Palas	n	3	3
	X ± sx	64.34±0.6700	21.69±1.1700
	V%	1.80	9.35
F_1Meat Breed - Palas x Palas Merino	n	3	3
	X ± sx	62.03±0.0156	21.31±0.6300
	V%	4.37	5.14
F_1 Suffolk x Palas Merino	n	3	3
	X ± sx	59.13±1.4100	22.45±0.5100
	V%	4.12	3.95

Table 3b. The tissue structure of the carcasses at the lambs that had been experimentally slaughtered

Breed /hybrid		Tissue Structure (%)	
		fat	meat*
Palas Merino	n	3	3
	X ± sx	15.95±2.8700	76.71±0.9900
	V%	31.17	2.24
Meat Breed - Palas	n	3	3
	X ± sx	14.20±0.5000	78.53±0.9600
	V%	6.07	2.11
F_1Meat Breed - Palas x Palas Merino	n	3	3
	X ± sx	16.70±1.9800	78.73±0.6500
	V%	20.56	1.43
F_1 Suffolk x Palas Merino	n	3	3
	X ± sx	18.45±1.8100	77.59±0.5100
	V%	17.02	1.13

* The meat is considered as muscles together with the covering and inter-muscle fat

It can be noticed that the proportion of the muscles in the carcass was between the limits of 59.13% at F_1 Suffolk x Palas Merino and 64.34% at Meat Breed –Palas, with 60.77% at Palas Merino and 62.03% at F_1 Meat Breed - Palas x Palas Merino.

The proportion of bones was between the limits of 21.31% at F_1 Meat Breed - Palas x Palas Merino and 23.32% at Palas Merino, with 21.69% at Meat Breed –Palas and 22.45% at F_1 Suffolk x Palas Merino.

The fat was between the limits of 14.20%at Meat Breed - Palas and 18.45% at F_1 Suffolk x Palas Merino, with 15.95% at Palas Merino and 16.70% at F_1 Meat Breed - Palas x Palas Merino.

The meat was in proportion of 76.71% at Palas Merino, 77.59% at F_1 Suffolk x Palas Merino, 78.53% at Meat Breed - Palas and 78.73% at F_1 Meat Breed - Palas x Palas Merino.

Doing the tests of significance of the differences for the five combinations between the 2 breeds and the two hybrids regarding the proportion of muscles, bones, fat and meat, all the differences were insignificant ($p > 0.05$).

Table 4 is presenting the classification of the carcasses after SEUROP grid.

The results are showing that at the hybrids of F_1 Suffolk x Palas Merino all carcasses were in U class by conformation (very good carcasses) and by the fattening degree in the 3rd class (medium fat carcasses).

The carcasses of F_1 Meat Breed Palas x Palas Merino hybrid were classified by conformation in a proportion of 67% in U class (very good carcasses) and 33% in R class (good carcasses), and by the fattening degree in the 2nd class (week carcasses).

At the Meat Breed of Palas all carcasses were from U class by conformation and by the fattening degree 33 % were in the 2nd class (weak carcasses) and 67 % in the 3rd class (medium fat carcasses).

The carcasses from PalasMerinos totally correspond by conformation to R class (good carcasses) and by the degree of fattening, 33% were from the 2nd class and 67 % from the 3rd class (Table 4).

Table 4a. Classification of the carcasses after EUROP grid, at the hybrids for meat comparatively to their contemporaries of Palas Merino and Meat Breed Palas

Specification	Class by conformation			
	U (very good carcasses)		R (good carcasses)	
	no.	%	no.	%
F_1 Suffolk x Palas Merino	3	100.0	-	-
F_1Meat Breed – Palasx Palas Merino	2	67.0	1	33.0
Meat Breed - Palas	3	100.0	-	-
Palas Merino	-	-	3	100.0

Table 4b. Classification of the carcasses after EUROP grid, at the hybrids for meat comparatively to their contemporaries of Palas Merino and Meat Breed Palas

Specification	Class by the fattening degree			
	2 (weak carcasses)		3 (medium fat carcasses)	
	no.	%	no.	%
F_1 Suffolk x Palas Merino	-	-	3	100.0
F_1Meat Breed – Palas x Palas Merino	1	33.0	2	67.0
Meat Breed - Palas	1	33.0	2	67.0
Palas Merino	1	33.0	2	67.0

CONCLUSIONS

The Palas Merino had the lowest values of the slaughtering output, these being 44.25% for R1 and 49.54% for R1 and R2, followed by the Palas Breed Meat with 47.54% respectively 53.98%.

The hybrid F_1 Meat Breed Palas x PalasMerino values were 48.12% to 54.74% for R1 and R2, and the highest values being for F1 hybrid Suffolk x Palas Merino with 51.21% and respectively 55.94%.

The differentiation of slaughtering output between genotypesare shown the differences of R1 and R2 related to the F1 hybrid between Meat Breed Palas x PalasMerino as compared to PalasMerino and Meat Breed Palas. It also shows the values of R1 and R2 in the Meat Breed Palas from the PalasMerino and Hybrid F1 Suffolk X Palas Merino compared with PalasMerino and Hybrid F1 Meat Breed Palas x PalasMerino.

There were significant differences ($p<0.05$) both between F1 Meat Breed Palas x Palas Merino and Palas Merino and also between Meat Breed Palas and Palas Merino, but also between F_1 Suffolk X Palas Merino and Palas Merino. Between the two hybrids the differences were insignificant ($p>0.05$).

The proportion of the muscles in the carcass was between the limits of 59.13% at F_1 Suffolk x Palas Merino and 64.34% at Meat Breed –Palas, the proportion of bones was between the limits of 21.31% at F_1 Meat Breed-Palas x Palas Merino and 23.32% at Palas Merino. The fat was between the limits of 14.20% at Meat Breed-Palas and 18.45% at F_1 Suffolk x Palas Merino. The meat was in proportion of 76.71% at Palas Merino, 77.59% at F_1 Suffolk x Palas Merino, 78.53% at Meat Breed - Palas and 78.73% at F_1 Meat Breed - Palas x Palas Merino.

Doing the tests of significance of the differences for the five combinations between the 2 breeds and the two hybrids regarding the proportion of muscles, bones, fat and meat, all the differences were insignificant ($p>0,05$).

At the hybrids of F_1 Suffolk x Palas Merino all carcasses were in U class by conformation (very good carcasses) and by the fattening degree in the 3rd class (medium fat carcasses).

The carcasses of F_1Meat Breed Palas x Palas Merino hybrid were classified by conformation in a proportion of 67% in U class (very good carcasses) and 33% in R class (good carcasses), and by the fattening degree in the 2nd class (week carcasses).

At the Meat Breed of Palas all carcasses were from U class by conformation and by the fattening degree 33% were in the 2nd class (weak carcasses) and 67% in the 3rd class (medium fat carcasses).

The carcasses from Palas Merinos totally correspond by conformation to R class (good carcasses) and by the degree of fattening, 33% were from the 2nd class and 67 % from the 3rd class.

From the presented data it certainly results the superiority of the hybrid lambs obtained through the crossbreeding the breed of Palas Merino with two meat breeds, comparatively to the lambs of Palas Merino, regarding the output at slaughtering and the quality of the carcasses.

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SECURITY OF PORK MEAT AFFECTED BY AFRICAN SWINE FEVER AND ITS IMPACT ON THE STATE BUDGET AND E.U. BUDGET

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Abstract

The legitimacy of a state is the capacity to provide food security, that is to have sufficient availability of agricultural products and foods to cover the food needs for all inhabitants within its borders. Food security aims to ensure that everyone has access to as healthy a food as possible and ensures and respects the right of every person to a healthy and nutritionally balanced diet. Vulnerabilities in agriculture and the food industry can also be highlighted by identifying hazards, threats and risks, with African Swine Fever (ASF) in our case. ASF is a viral disease of domestic and wild pigs, with a rapid evolution and mortality of up to 100% for sick pigs. The occurrence and evolution of ASF (14779 outbreaks in the two years studied: 2018 and 2019) led at national level to the risk of the safety of pig meat being affected by the high number of animals that died, were euthanised or were slaughtered (approx. 2 million head), with a decrease of 31,6% in pig numbers in 2019 compared to 2017, it also helped to destabilize the pig meat market in quantitative terms, with the production of live-weight pigs falling by 210.777 tons (31,7%) in 2019 compared to 2017.To these losses are added significant costs for implementing control and quarantine measures, compensation (payment to breeders), etc.

Key words: *African Swine Fever Epidemic (ASF), ASF outbreaks, food security.*

INTRODUCTION

African Swine fever is a rapidly evolving infectious disease, where mortality is 100% where it occurred, without prevention and control treatment. The first disease outbreaks occurred in Romania in the Danube Delta Biosphere, Tulcea county, in the wild pig (wild boar) from which it spread rapidly to households and livestock complexes specialized in pig farming. In Tulcea county ASF has expanded to neighboring counties and from here to all over the country, namely in 38 counties.

The purpose of this study is to quantify the economic impact of the occurrence and evolution of this disease in Romania, in its counties, in order to justify the economy and to establish the necessary control measures.

The appearance of the ASF required preventive control measures to be put in place:

- the notification of the relevant structures at national and European level as well as the World Organization of Animal Health about the confirmation of the outbreak of African swine fever on Romania's territory;

-informing the CNSSU, MADR, MAP, MAI, MDRAP, Hunting Associations, Pig producers' Associations, the European Commission, Member States and neighboring Third Countries of the confirmation of the outbreak of ASF;

- specific measures have been taken to combat ASF, establish restricted zones: Protection (3 km) and surveillance (10 km);
- the local veterinary authorities reinforced border controls;
- training has been carried out at local offices and the veterinarian, several grades of tasks have been issued from central to local level, stressing the importance of early warning and the importance of specific awareness: farmers of all types, hunters, carriers, sales agents, etc.;
- information campaigns were targeted at farmers, hunters, the general public on the clinical signs of ASF and biosecurity measures;
- MADR press releases on the ASF hazard;

Food security is the capacity to provide or have sufficient availability of agricultural products and foodstuffs capable of meeting food needs for all inhabitants within its borders, and to ensure and respect the right of every person to a healthy and nutritionally balanced diet.

MATERIAL AND METHODS

The study was carried out on the basis of data collected from the representatives of the county

veterinary departments and the local councils (mayors) both in terms of the number of animals and the amounts allocated per head of animal slaughtered or dead and of the data collected from the county agricultural Departments, Institution subordinate to the Ministry of Agriculture and Rural Development on the evolution of livestock and meat production provided by them.

The data have been processed statistically into national animal herds, their meat production based on their processing, outbreaks of ASF disease, economic operators and private households. The data was collected over a period of two years (2018 – 30 November 2019), i.e. from 2018 when this epidemic occurred to date.

RESULTS AND DISSCUSIONS

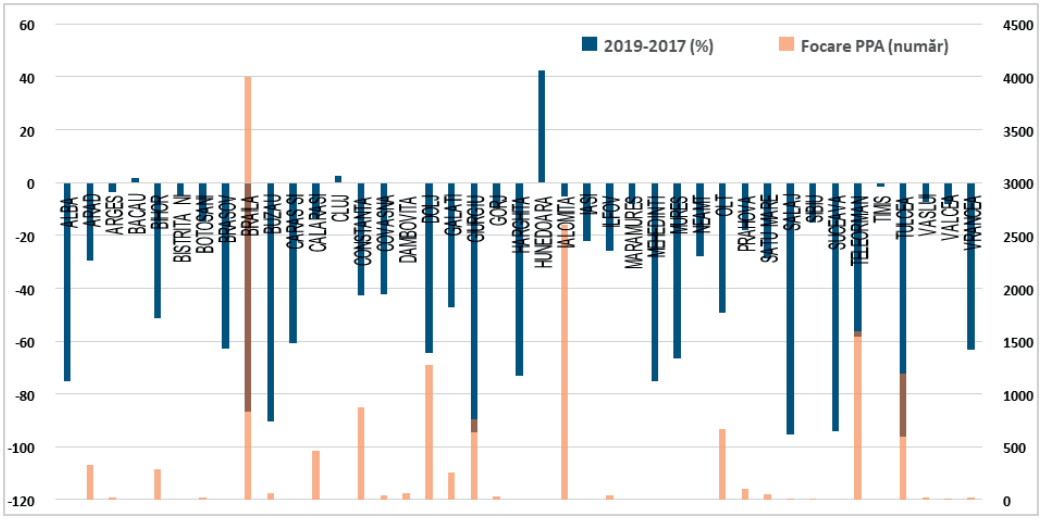
From the data presented in Table 1, Picture 1, it can be observed that the number of animals decreased significantly in 2019 compared to 2017 by 1933,88 thousand head.

The counties that have significantly contributed to the reduction of livestock numbers are: Braila with 176,136 heads, Brasov with 157,490 heads, Vrancea 145,755 heads, Tulcea 114,593 heads, Arad 108,656 heads, Suceava 100,555 heads and less The County of Dambovita with 0,130 thousand heads, but there are also counties where the number has increased as compared to the year taken as a reference, Hunedoara county with 37,773 heads, Cluj County with 1,830 heads and Bacau County with 1,779 heads.

As an evolution of the ASF we can say that the number of pigs herds suffered heavy losses where outbreaks occurred. Of the total pigs killed and slaughtered by 530,919 heads as a result of the occurrence of ASF outbreaks, both in the sector of large complexes and in private households, i.e. 431,804 heads in complexes and 99,115 heads in private households. The counties most affected by the ASF are Braila with 234,861 heads, Tulcea with 72,368 heads, Calarasi with 42,078 heads, Ilfov with 37,395 heads, Arges with 29,376 heads, Giurgiu with 6,978 heads, Olt with 3,774 heads, Dambovita with 3,587 heads and Vaslui with 1,387 heads.

The mathematical calculation shows that the Romanian State lost 6.371.028 lei, respectively 1.332.851 euros.

Evolution of production, presented in Table 2, Picture 2, of live weight pigs has suffered heavy losses, i.e. 210,777 thousand tons liveweight.



Picture 1. Quantification of African swine Fever (ASF) influence on the evolution of number of pigs in Romania's counties in 2019 as compared to 2017

Table 1. Quantification of African swine Fever (ASF) influence on the evolution of number of pigs in Romania's counties in 2019 as compared to 2017

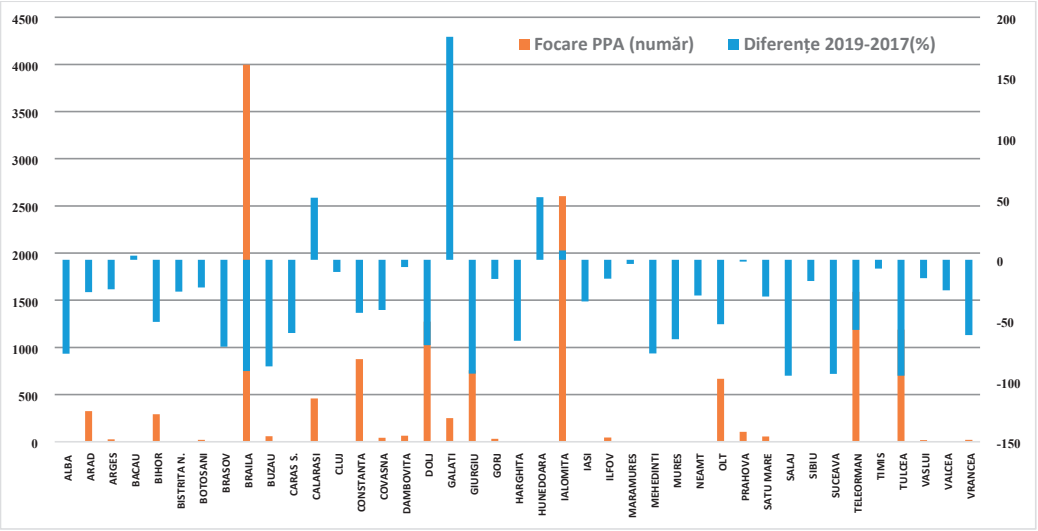
Specification	Number of pigs 2017 (thousand heads)	Number of pigs 2019 (thousand heads)	Number of ASF outbreaks extinguished 2018 + 2019	Differences 2019 - 2017	
				Thousand heads	%
Alba	112,000	27,900		- 84,100	- 75.1
Arad	367,316	258,660	326	-108,656	-29.6
Argeș	279,375	269,040	25	-10,335	-3.7
Bacău	95,531	97,310		+1,779	+1.9
Bihor	186,597	90,954	292	-95,643	-51.2
Bistrița N.	79,580	75,688		-3,892	-4.9
Botoșani	136,155	116,121	19	-20,034	-14.7
Brașov	250,171	92,681		-157,490	-63.0
Brăila	202,844	26,708	3.996	-176,136	-86.8
Buzău	47,600	4,550	58	-43,050	-90.4
Caraș S.	155,817	60,996		-94,821	-60.9
Călărași	241,240	208,884	458	-32,356	-13.4
Cluj	68,069	69,899		+1,830	+2.69
Constanța	225,512	129,479	875	-96,033	-42.6
Covasna	86,828	50,016	43	-36,812	-42.4
Dâmbovița	28,830	28,700	65	-0,130	-0.5
Dolj	46,590	16,530	1.273	-30,060	-64.5
Galați	63,839	33,581	252	-30,258	-47.4
Giurgiu	50,416	2,692	759	-47,724	-94.7
Gorj	29,537	26,598	32	-2,939	-10.0
Harghita	21,907	5,867		-16,040	-73.2
Hunedoara	88,903	126,676		+37,773	+42.5
Ialomița	145,091	137,699	2.604	-7,392	-5.1
Iași	250,888	194,835		-56,053	-22.3
Ilfov	23,061	17,059	45	-6,002	-26.0
Maramureș	319,082	293,212		-25,870	-8.1
Mehedinți	30,053	7,495		-22,558	-75.1
Mureș	131,260	44,087		-87,173	-66.4
Neamț	146,418	105,647		-40,771	-27.8
Olt	97,190	49,338	667	-47,852	-49.2
Prahova	196,802	160,892	105	-35,910	-18.2
Satu Mare	75,630	54,000	55	-21,630	-28.6
Sălaj	88,508	3,981	4	-84,527	-95.5
Sibiu	66,614	56,533	7	-10,081	-15.1
Suceava	106,780	6,225		-100,555	-94.2
Teleorman	100,464	41,658	1.590	-58,807	-58.5
Timiș	1.029,206	1.013,827		-15,379	-1.5
Tulcea	119,263	4,670	1,188	-114,593	-96.1
Vaslui	21,400	19,820	18	-1,580	-7.4
Vâlcea	76,180	69,913	2	-6,267	-8.2
Vrancea	230,148	84,393	21	-145,755	-63.3
TOTAL RO	**6.118,696**	**4.184,814**	**14.779**	**-1.933,880**	**-31.6**

The counties that contributed to the drop in the production of live weight are the same counties that had a small number of swine heads in 2019 (Arad, Bihor, Brașov, Brăila, Caraș Severin, Constanța, Galați, Iați, Mureș, Suceava, Tulcea, Vrancea). Losses caused by ASF by the reduction in the number of animals and the consequent reduction in meat production have affected national food security.

Table 2. Quantification of African Swine Fever (ASF) influence on the evolution production of live weight pigs in Romania counties, in 2019 as compared to 2017

Specification	Swine Production 2017 (thousand tons live weight)	Swine Production 2019 (thousand tons live weight)	Number of ASF outbreaks extinguished 2018 + 2019	Differences 2019 - 2017	
				Thousand tons live weight	%
Alba	13,620	3,065		-10,555	-77.5
Arad	40,447	29,716	326	-10,731	-26.5
Argeș	22,934	17,349	25	-5,585	-24.4
Bacău	11,559	11,958		+0,399	+3.5
Bihor	20,808	10,175	292	-10,633	-51.1
Bistrița N.	7,782	5,745		-2,037	-26.2
Botoșani	14,977	11,554	19	-3,426	-22.9
Brașov	36,622	10,427		-26,195	-71.5
Brăila	22,584	1,871	3996	-20,713	-91.7
Buzău	4,488	0,546	58	-3,942	-87.8
Caraș S.	18,111	7,193		-10,918	-60.3
Călărași	16,133	24,400	458	+8,267	+51.2
Cluj	6,216	5,599		-0,620	-10.0
Constanța	24,968	14,036	875	-10,932	-43.8
Covasna	9,141	5,364	43	-3,777	-41.3
Dâmbovița	3,594	3,384	65	-0,210	-5.8
Dolj	6,337	1,875	1273	-4,462	-70.4
Galați	6,473	18,377	252	+11,904	+183.9
Giurgiu	5,564	0,350	759	-5,214	-93.7
Gorj	3,250	2,739	32	-0,511	-15.7
Harghita	2,677	0,892		-1,785	-66.7
Hunedoara	8,894	13,479		+4,585	+51.6
Ialomița	14,449	15,556	2604	+1,107	+7.66
Iași	32,963	21,625		-11,338	-34.4
Ilfov	2,384	2,015	45	-0,369	-15.5
Maramureș	33,232	32,078		-1,154	-3.5
Mehedinți	3,584	0,820		-2,764	-77.1
Mureș	15,303	5,286		-10,017	-65.5
Neamț	17,264	12,176		-5,088	-29.5
Olt	10,588	4,974	667	-5,614	-53.0
Prahova	16,992	16,746	105	-0,246	-1.4
Satu Mare	8,308	5,800	55	-2,508	-30.2
Sălaj	9,293	0,417	4	-8,876	-95.5
Sibiu	7,195	5,935	7	-1,260	-17.5
Suceava	11,250	0,678		-10,572	-94.0
Teleorman	11,330	4,791	1590	-6,539	-57.7
Timiș	113,212	105,051		-8,161	-7.2
Tulcea	12,271	0,550	1188	-11,721	-95.5
Vaslui	2,570	2,180	18	-0,390	-15.2
Vâlcea	9,901	7,417	2	-2,484	-25.1
Vrancea	25,316	9,621	21	-15,695	-62.0
TOTAL RO	**664,586**	**453,810**	**14779**	**-210,777**	**-31.7**

At national level, during the period under study, 14779 files were submitted per holding for compensation for the mortality or killing of pigs in the ASF outbreaks worth 392801,44 lei, out of this total number of files submitted for compensation, 13701 files were paid for 330641,27 lei, with the remaining 341 files worth 62160,17 lei being on the work, to be paid when they were completed.



Picture 2. Quantification of African Swine Fever (ASF) influence on the production evolution of live weight pigs in Romania counties, in 2019 as compared to 2017

CONCLUSIONS

Losses caused by the occurrence of ASF have led to a decrease in pig numbers in households and the closure of pig rearing complexes. The Romanian pig is grown in households and therefore the synthetic (high genetical level) pig raised in the livestock units cannot be raised at family level.

The swine sector is depleted by biological material, and as a result, the import of young pigs for growth and fattening is increasingly used.

The losses caused by the ASF by the very high mortality caused, have increased the import of meat in a massive way and also increased the cost per kg of meat.

Romania through ASF, has lost in zootechnical terms animal losses, but also in economic terms, namely costs of disinfection, rendering and compensation for livestock farmers (pigs), etc.

REFERENCES

Decisions of National Committee for Special Emergency Situations (CNSSU):

Decision number 1 - which states that Ministry of Defense will provide help in meaning of transporting two mobile incinerators (big capacity) for the National Sanitary Veterinary Authority for Food Safety, from Timiș county to Tulcea county, which was the most hit by ASF. Ministry of Environment, is rolling out campaigns, through Reservation Danube Delta, to identify and collect the corpses of wild boars. Ministry of Environment is supporting National Sanitary Veterinary Authority for Food Safety, to identify places, where the corpses of pigs and wild boars would be buried, if the capacity of mobile incinerators is exceeded.

Decision number 2 - National Committee for Special Emergency Situations was empowered to ensemble Local Center for Fighting Diseases, under the authority of minister of internal affairs, and also was assigning responsibilities for central and local authorities in fighting against ASF. Imposing measures to stop spreading the disease and combating its effects within national territory.

Decision number 3 - National Committee for Special Emergency Situations approved the Additional Plan for actions to fight against ASF.

Government Decision nr.1214/2009 - on the methodology for determining and paying the compensation to be paid to the owners of animals slaughtered, killed or otherwise affected with a view to rapid liquidation of outbreaks of disease transmissible to animals.

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FARM SUPPLY CHAIN MANAGEMENT IMPROVEMENT THROUGH THE USE OF LEAN, AGILE AND DEVOPS METHODOLOGIES

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Abstract

Farmers need to manage better the increasing demand of food by scaling their services according to the required capacity and through the use of IT products and services. The process needs to be efficient and sustainable in order to perform safely. Lean, Agile and DevOps are leading workflow models and concepts which can be included in the process to improve the time and quality of product delivery. Agile optimizes the development of software. DevOps combines development with operations, while shortening the life cycle of software development and improves its quality. Lean enhances focus and optimizes the value stream by identifying automation opportunities and collaboration. The article presents the purpose, methodology and goals for supply chain management in farms. Farmers start from ideas and they apply practices and principles until they get the value out of the supply chain. The Lean principles are the basis of the product development flow. The practices start from finding and validating ideas for the Lean product and how it fits in the market. DevOps incorporates many lean principles and focuses on the enhancement of the collaboration between the employees who are responsible for software development and operations. Software development is based on Agile and it has the Scrum and Kanban methodologies for processes, while engineering is based on extreme programming. Methodologies in the farm supply chain management can be considered to be diagnosis instruments that allow evaluation. Farms identify and implement activities which aim to augment the benefits gained through the use of software and methodologies. Additionally, these principles can be used by academics and practitioners in complementary domains.

Key words*: farms, practices, principles, supply chain, management.*

INTRODUCTION

Food production needs to keep up with the increase of the world population. The COVID-19 pandemic caused tough market conditions that greatly influence farmers, as well as the business environment. The consequences of the COVID-19 pandemic affect the global economy, but also the behaviour of business and consumers.

The supply chain management in farms requires good decisions at all levels due to the exposure to financial risks. During the past years, software products and services aimed to bring efficiency and accuracy, while lowering expenses and time consumption. Digitalization allows payments to be done online. Many grocery stores started to sell their products online and to offer delivery services due to the COVID-19 pandemic (Baig et al., 2020). The president of dairy farms of Canada stated that the dairy sector needs to align to the needs of the consumer, because bottlenecks will appear in the supply chain due to the demands from alimentary stores (Dairy Farmers of Canada, 2020). The trajectory of dairy products was also affected due to the shipments that need to undergo several procedures in order to reach many locations around the world. The stock of milk increases, but the price of it will reduce worldwide in the long-run (Uddin, 2020). During the COVID-19 pandemic time, farms undergo a digitalization process that is connection to the Lean, Agile and DevOps workflow models.

An organization which uses the Lean methodology, comprehends the value of customers and concentrated on the key processes that it possesses in order to increase them (Mahajan et al., 2019). Agile project management fits well when the companied need to be innovative and proactive in order to reach the expectations of

their customers. Customers demand until their need is satisfied after the communication process with the support team leads to the successful accomplishment of the associated activities and goals (Loiro et al., 2019). DevOps comprises software development and information technology operations.
Short term goals need to be reached in a short period of time, like a couple of weeks and even days. Agile project management fits well to this situation and value is delivered fast. A successful example is represented by the Scaled Agile which was tested by scaling the business with a number of teams that are placed at multiple locations, leading to the test of novel opportunities for the business to rely on (Romanukha, 2020).According to Dunn-Krahn, a survey done by xMatters digital service platform on 300 participants showed that 90% of them use digital services for buying food, goods, and using banking facilities. DevOps will always be in demand, even in farms, because digital services have to be offered and the consumer's expectations need to be met.
The current paper presents an analysis of the workflow models which are used in farms and how they can mitigate the problems which appear due to the COVID-19 pandemic.

MATERIALS AND METHODS

The Lean, Agile and DevOps workflow models can be introduced in farms dairy farms in order to meet the needs of the consumers. According to Gartner, the world leading research and advisory company, by 2021 there will be up to 25 billion gadgets which will be connected via wireless networking (Gartner, 2018).
Adrian Cockcroft from Netflix explained in 2015 to chief information officers from various goat farms how DevOps, microservices, Cloud can be used in industry, with the scope to make them adopt these new concepts into their business(The Goat Farm, 2015).
Validation and testing of DevOps embedded projects is done in test farms to select the best approach based on the demands which require the adaption of digitalization (Rajkin, 2020). The link between Lean, Agile and DevOps is given by the fact that Agile is a part of DevOps and DevOps is an extension of Lean. A holistic view is needed to monitor all the components of the farm processes and to have multiple retrospectives to improve as time passes.
Larry Maccherone presented at the 2018 Nexus User Conference the concept called DevSecOpsthat joins development, security and operations throughout integration and delivery of the desired outcomes (Addo, 2020). In this direction, Libelium, a Spanish Plug&Sense platform provider based on sensors for cow feeding, has reported an increase of milk equal to 18% (Curry, 2019).
More farm management solutions, along with their results are presented in Table 1. In Romania, there does not exist any similar software solution which is dedicated to farm management based on the Lean, Agile and DevOps methodologies.

Table 1. Existent farm management solutions

Farm Management Solution	Country	Results
High-quality dairy product development (Roald, 2020) Amazon Web Services (AWS)	Norway	Suggestions for data-driven innovation by tailoring dietary decisions Milk production and cow delivery predictions
Machine Learning		Multi-phase approach for planning the dairy farms logistics
Dairy herd management (BoviSync, 2020) DevOps	USA	Labour cost reduction based on the records generated by cow sensors Decisions can be automated
Cloud		
Smart herd management software (MilkingCloud, 2020) DevOps Cloud	USA	Herd, reproductive, reports, feed, health, milking, cost management Mastitis and calving detection devices integrated in the system
Agri-tech and herd improvement (Livestock Improvement Corporation, 2019) DevOps – Microsoft Azure, Azure Stack Cloud	USA	Infrastructure scaling according to customer demand Data management using massive computing tasks

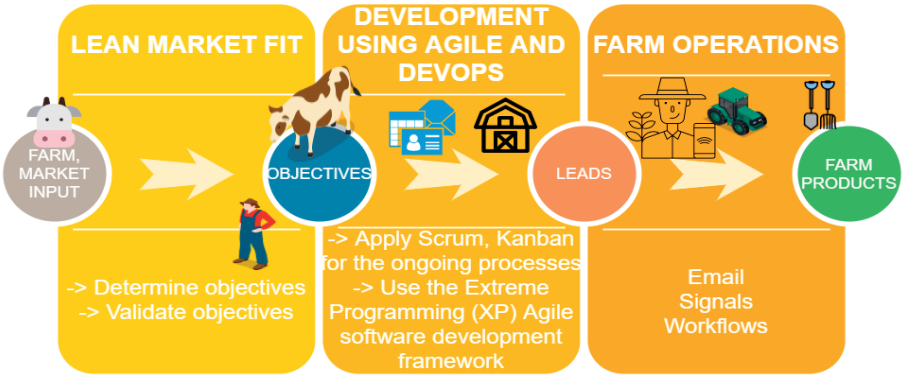

Figure 1. Proposed farm management based on Lean, Agile and DevOps methodologies

The current paper aims to help farmers to sustain the efficient usage of data systems in relationship with the supply chain management, because processes can be delayed and there may not be enough capacity for the farm business goals. The Lean methodology is extended by the use of the Agile project management and DevOps methodology. Software created using DevOps incorporate the Scrum and Kanban methodologies for the decided processes which are part of the Agile project management.

The Scrum methodology consists in dividing the work of programmers into several tasks which are finished in sprints with determined duration (Hidalgo, 2019). The Kanban methodology appeared as part of the Toyota Production System to enhance the production flow through the usage of tickets (Belu et al., 2018). Every production stage corresponds to a Kanban ticket. The process is improved by monitoring the route between stages.

Within the digitalized farm, the centralized nodes are responsible with data acquisition from the farm sensors. The equipment usage pattern is determined in order to improve the power consumption within the farm. A Kanban ticket is produced inside the system when an alarm is triggered. The tickets can be visualized by the user in a dashboard through a web interface.

RESULTS AND DISCUSSIONS

The proposed farm management system based on the Lean, Agile and DevOps practices is depicted in Figure 1. The input concerning farm sensors, herd reproductive values, feed, health, milking and costs are analyse din order to determine objectives and to validate them based on Lean market fit. After setting the objectives, the development of the desired platform is done using the Agile and DevOps methodologies. The process consists in including the Scrum and Kanban methodologies for the processes which run. A Scrum sprint is set for maximum one month and it leads to a clarified scope and the quality of the goals is improved. If the duration would be longer, complexity and risk can rise. Inspections and adjustments are done throughout the process. Along with this, extreme programming is done for the development which is done based on the Agile project management. By involving the DevOps software development methodology, the integration is done between development and farm operations.

The platform enables the farmers and their collaborators to manage the farm, while analysing the signals coming from sensors and controlling the workflows that exist in the farm. The system aims to allow farmers to be aligned to their business context and to have access to information and assistance. This also leads to decreased costs and effort, because initiatives will be reconsidered when going through the whole process which was previously presented.

The management needs to set goals and to analyse the progress towards the desired destination. In this way, the shift is done from seeing vulnerabilities into visualizing the positive aspects that characterize the dairy farm.

The farm management solution developed based on the above principles will help the user understand the impact of factors and how to use

them. The COVID-19 pandemic influenced the life and work style of everyone. By keeping the farm operations updated and in close connection to the user's needs, the success of the business will be higher. Everyone needs to have access to information at a distance of a click and the cloud environment is a proper solution.

CONCLUSIONS

The COVID-19 pandemic triggered a negative impact upon dairy farms. As a consequence, the farms should adopt new methodologies in their work process and namely the supply chain management, because the user needs change and competition are rarely done by autonomous farms. The supply chain involves several companies throughout the process flow.

The paper presented existent farm management solutions, as well as it proposed a new one which includes the Lean, Agile and DevOps workflow models. The pandemic caused the client behaviour to shift and this triggered the absolute need to analyse it, along with the operations of the farm itself. The risks that exist have to be determined and predicted for the times which will come. This leads to having information and assistance better organized and managed. Moreover, the time will be efficiently used and the focus will be put on product delivery quality.

Academics and practitioners can get involved in determining the Lean market fit, development of the farm management using Agile and DevOps, and farm operations. In this way, the specialists of tomorrow will be empowered by getting closer to the business context.

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ANALYTIC DETERMINATION OF THE ENERGY CAPACITY OF THE MIXING FORMATION IN THE STEP-TYPE MIXER

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Abstract

The analysis of mixing devices for the preparation of dry mixtures and technological lines used for this determines the process of formation of mixtures as the interaction of metering and mixing devices. One of the effective options for mixing is step mixing. As a result of the analysis of the process, we clarified the operational sequence of actions for stepwise mixing of material, taking into account the activities of not only the mixer, but already taking into account the entire mixing unit, i.e. during the interaction of the mixer and the multicomponent batcher. The components of the energy intensity of mixture formation are clarified. The operational sequence diagram for the stepwise preparation of dry mixes is analytically determined. It made it possible to establish the dependences of the total work on the preparation of the mixture, carried out by the mixing unit as part of a multicomponent batcher and mixer, mixer performance, the duration of individual cycles and the entire cycle of the mixer.

Key words*: mixing devices, mixture formation, multicomponent batcher.*

INTRODUCTION

The economic efficiency of animal husbandry requires high productivity of farm animals. To solve this problem animals are provided with the necessary nutrients. For this purpose, an animal feeding ration is developed taking into account their species, age and productivity. According to the recipe given to the animal feed, feed mixtures should be prepared in such a way. For the preparation of mixtures, mixers of different designs and with different working parts are used. For liquids, it is possible to use circulation mixers (Habchi et al., 2018; Soni et al., 2019; Yaraghi et al., 2018). However, they are problematic for bulk mixtures used in the concentrated type of animal feeding. Drum mixers with a rotating container prepare bulk mixtures with low energy consumption, however, it is difficult to evenly distribute all components throughout the entire volume of the cooked (Teryushkov et al., 2019; Li et al., 2017). Mixers with a working auger require more energy, but a better mix is possible (Emeljanova et al., 2018; Celik et al., 2019). Paddle mixers spend a little more energy they are able to prepare a uniform mixture (Ebrahimi et al., 2018; Chupshev et al., 2019). The reduction of energy consumption for the preparation of the mixture is realized by improving the design of the mixer, as well as improving the process (Fomina et al., 2016). For example, using stepwise mixing in an increasing volume of material being mixed.

To determine the energy intensity of mixing by a batch mixer of a step type with 4 mixing stages E (J/kg) it was proposed to use the formula (Chupshev et al., 2018):

$$E = \frac{\sum A_{j,i}}{M} = \frac{\sum_i [P_{j,i} \cdot Txx_j + P_{j,i} \cdot Tz_j/2 + P_{j,i} \cdot Tx_j + P_{j,i} \cdot Tc_i + P_{j,i} \cdot Tv_j/2]}{M}$$

$A_{j,i}$ – work spent on performing j-x operations in the i-x capacity, J; M is the mass of the prepared portion of the mixture, kg; Txx_j - idle movement time of the working body (j = 1), s; Tz_j, Tv_j - the duration of the loading of components (j = 2) and unloading of the finished mixture in the i capacity (j = 5), s; Tx_j, Tc_i - the duration of idle mixing (before applying the controlled component, /j=3/) and working mixing (j = 4), s; $P_{j,I}$ is the power required to drive the working body in the i capacity during the j operation, W.

In the technology of operation of stepwise batch mixers was proposed, which reduces energy costs by preparing the mixture at the preliminary stages of mixing to a uniformity of 80%, and only at the last stage (k) of mixing is the main mixing ensured that the quality of the mixture is maintained according to technological (zootechnical) requirements - 90 or 95% (Konovalov et al., 2015).

Therefore, studies were carried out to reduce the number of mixer capacities and to determine the energy intensity of stepwise mixing in the minimum number of capacities.

The purpose of the research is to justify the expression of the energy intensity of stepwise mixing with a minimum number of capacities of the mixing device with the definition of the expressions necessary for calculation.

MATERIALS AND METHODS

The research methodology provided an analytical justification for expressing the energy intensity of stepwise mixing with a minimum number of mixing containers based on known theoretical expressions, as well as the establishment of missing functions. Parameters that could not be found analytically were determined experimentally. For this, 4 mixer tanks were used with the corresponding proportional to the working bodies. The influence of the duration of mixing of the components (from 0.5 to 15 minutes) was studied with a change in the proportion of the laid control component from 1 to 10%. The number of samples weighing 100 g with each measurement - 20 pcs with three iterations.

A previous review analysis made it possible to establish a list of the required calculated indicators. Using literary sources, we define the necessary expressions.

The duration of the loading of components (s) in the capacity of the mixer is determined:

$$T_Z = {M_{i,n}}\Big/{Q_n},$$

Q_k – productivity of the nth dosing device, kg/s; $M_{i,k}$ is the mass of the *n*th component loaded into the *i* capacity, kg.

$$M_n = M \cdot d_{k_n},$$

d_{kn} – the proportion of the *n*th component according to the recipe of the mixture (Konovalov et al., 2015).

The discharge time of the mixture can be determined on the basis of data. If the mass of the mixture components *M* (kg) is loaded in the mixer with a diameter of *D* (m), then the height of the feed in the mixer will be *H* (m). In this case, the coordinate of the position of the center of gravity of the X_C of the radial elementary sector at the time *t* of rotation of some mixer blade rotating on the shaft 4 around the vertical axis of the vertical cylindrical tank 2, relative to the beginning of the discharge hole 1 on its side surface (at which *t*=0), m, will be determined relative to the axis of rotation:

$$X_C = C_1 \cdot e^{\lambda_1 \cdot to} + C_2 \cdot e^{\lambda_2 \cdot to} + \frac{g \cdot f_1 \cdot H}{\omega^2 \cdot S_l},$$

g – acceleration of gravity, m/s^2; f is the coefficient of friction; ω - angular speed of rotation of the mixer, rad/s; S_l is the height of the discharge opening, m (Konovalov et. al., 2014). When unloading materials, the sector shifts in the radial direction have no time to be filled up. Radial sectors constitute in total the entire surface of the bottom of the mixing tank.

The radial velocity of the center of gravity of the radial sector will be a function of the rotation time of the blade (Figure 1) (respectively, the location of the blade relative to the beginning of the discharge hole), m/s (Konovalov et. al., 2014):

$$\vartheta = \dot{X}_C = C_1 \cdot \lambda_1 \cdot e^{\lambda_1 \cdot t} + C_2 \cdot \lambda_2 \cdot e^{\lambda_2 \cdot t},$$

The supply of material through the discharge opening in the form of a rectangular slit is determined, kg/s

$$Q = Q_V \cdot \rho = (S_l \cdot S_O \cdot \vartheta_{cp}) \cdot \rho$$

$$\text{or} \quad Q = S_l \cdot S_O \cdot \rho \cdot \frac{\sum_i \vartheta_i}{N_i},$$

S_O – discharge hole width, m; ϑ_{cp} - average speed of material exit from the mixer through the discharge hole in the radial direction, m/s; ϑ_i is the speed of material exit from the mixer in the radial direction on the *i* section, m/s; N_i - the number of i-x sections of the discharge opening, pcs; ρ is the density of the heap of material in the mixing tank during operation of the mixer, kg/m^3.

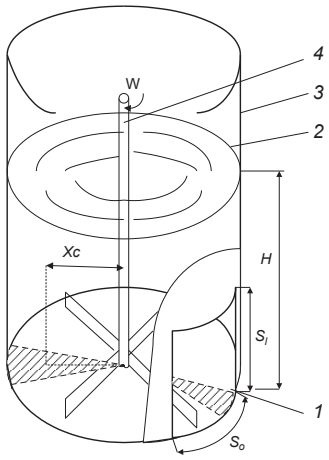

Figure 1. Layout of dimensions when unloading materials from the mixer tank: 1 - discharge hole; 2 - top layer of material; 3 - mixer capacity; 4 - rotating shaft with a mixer

During unloading, for an infinitely short time ΔT, the feed mass M_1 (kg) and the mixture volume V_1 (m3) are unloaded from the mixer:

$$M_1=\Delta T \cdot Q. \qquad V_1=M_1/\rho$$

Power when the stirrer is moving in the *i* capacity with a diameter of D_i is written, W:

$$P_i = K_1 \cdot \sigma_i \cdot Z \cdot \left(\frac{D_i}{2}\right)^3 \cdot \sin(\alpha) \cdot \omega/6$$

K_1 is the coefficient of proportionality of the width of the blade and the diameter of the tank; Z - the number of blades, pcs; α - angle of attack when installing the blades, rad; σ - stresses in the mixture during blade motion at K_1 = D/6:

$$\sigma = 10125{,}86 \cdot M \cdot D^{-4} \cdot n^{-0{,}185863} \cdot Z^{-0{,}606518} \cdot \sin(\alpha)^{-0{,}653631} \cdot L^{0{,}962341}$$

L – blade length, m.

The mixing time of the components in general will be determined with

$$T_C = -\frac{1}{k} \ln \frac{(1\text{-}\Theta_K)}{(1\text{-}\Theta_H)}$$

k – empirical mixing intensity factor for a particular mixer); Θ_H, Θ_K - initial and final uniformity of the mixture, 0.01% (Chupshev et al., 2018).

According to a number of researchers, mixers have a limitation on the use of the minimum proportion of the control component (Konovalov et al., 2013). The number of mixing steps is determined from the condition:

$$k \geq \log_{(1/d_{kz})}(1/d_{k_{min}})$$

d*kz* - minimum proportion of the control component, less than which the mixer does not ensure the quality of the prepared mixture; $d_{k_{min}}$ is the fraction of the smaller component in the mixture.

RESULTS AND DISCUSSIONS

Analysis of the design of the mixer for step mixing indicates the need to simplify its design in the direction of reducing the number of tanks and increasing the efficiency of their use.

In the conditions of agricultural production in Russia, half of the feed is produced at the feed mill, and half - in the conditions of farms from their own fodder and purchased additives. Either premix (1-2% by weight of feed, i.e. $d_{k_{min1}} = 0.01$ - the proportion of the smaller component 1% in the mixture), or protein-vitamin supplements (10-20% by weight of feed, i.e. $d_{k_{min2}} = 0.1$).

As a result of experimental studies of the influence of the fraction of the controlled component D_k and the duration of mixing T, the calculated values of the coefficient of variation of the content of the control component in the samples are established. The expressions of the non-uniformity of the mixture ν are obtained for the corresponding volume of the mixing tank V_o (Figure 2):- при V_o= 0,5 liters:

$\nu_{0.5}$=–1.469+4.53/Dk+4.18/T–
1.26/Dk·T+1,1·(1–1.8/Dk·T); R=0.921;

V_o= 2,5 liters:
$\nu_{2.5}$=0.185+5.38/Dk+3.124/T–
0.98/Dk·T+0,85·(1+3.08/Dk·T); R=0.92846;

V_o= 9 liters:
ν_9=0.029+6.14/Dk+2.87/T–
1.22/Dk·T+0.64·(1+3.48/Dk·T); R=0.95359;

V_o= 30 liters:
ν_{30}=2.336+4.19/Dk+0.356/T–

3.146/Dk·T+1.69·(1+4.95/Dk·T); R=0.963

R – values of the Pearson correlation coefficient.

When the proportion of the controlled component is more than 1.5%, the mixing time is sufficient T = 1.5 ... 2.0 minutes, while achieving a coefficient of variation of 5 ... 9% (Figure 3). Better mixing quality with lower mixing chamber volumes. When the proportion of the controlled component is 0.5 and 1.0% after T = 15 minutes of mixing, the quality of the mixture for containers is achieved, respectively: V_o = 30 l - ν = 14.1 and 5.9%; V_o = 9 l - ν = 13.8 and 5.0%; V_o = 2.5 l - ν = 11.0, and 5.5% at T = 10 and 5 min; V_o = 0.5 L - ν = 8.6 and 4.8% at T = 4.5 and 1.6 min.

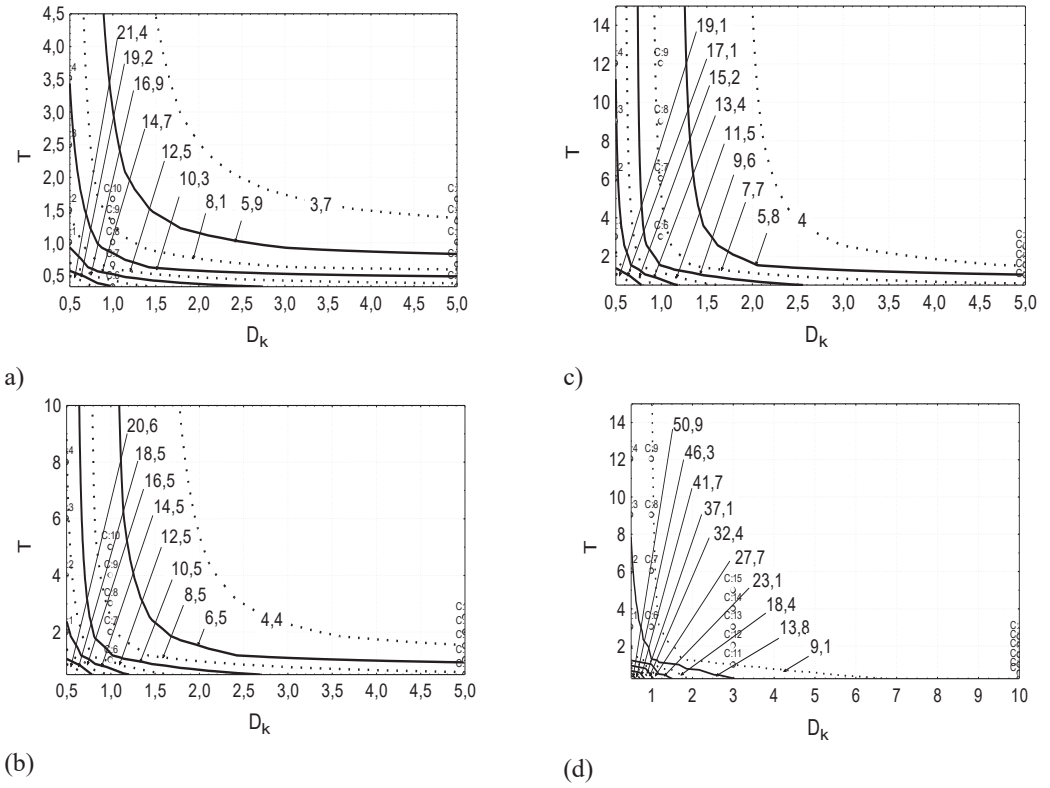

Figure 2. Effect of the duration of the mixed T (min) and accounting for the controlled component $_{Dk}$ (%) in the volume of the mixing tank V_o on the uneven mixture ν (%):
(a) - at V_o = 0.5 L.; (b) - at V_o = 2.5 l; (c) - at V_o = 9 l; (d) - at V_o = 30 l

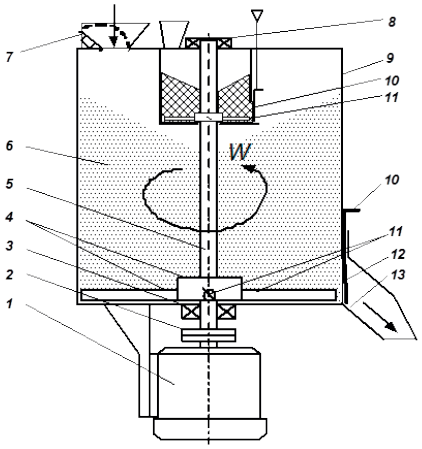

Figure 3. Scheme of a two-staged dry materials mixer: 1– electric motor; 2– coupling; 3– lower bearing support; bunker operational stock of components; 4 - paddle mixer; 5– shaft; 6– mixed material; 7– loading neck; 8– upper bearing support; 9– mixing chamber (capacity); 10– gate; 11– blades; 12– discharge hole; 13– unloading tray

An analysis of the graphs (taking into account real experimental values) shows that when the proportion of the controlled component is more than 1.5-2.0% and the mixture is uneven 10%, the mixing time is T = 1.5-2 min, for ν≤5% - T = 2-5 min. In small containers (2.5 L or less) mixing can be reduced to T = 0.5 ... 1.0 min. When the proportion of the controlled component is 1% and ν≤10%, the mixing time is not less than: for V_o = 0.5 L - 1.0 min; for V_o = 2.5 l - 1.5 min; for V_o = 9.0 l - 2.5 min; for V_o = 30 l - 3.0 min. When the proportion of the controlled component is 1% and ν≤5%, the mixing time is not less than: for V_o = 0.5 L - 1.6 min; for V_o = 2.5 l - 5.0 min; for V_o = 9.0 l - 12.0 min; for V_o = 30 liters - 15 min. If the proportion of the control component is 8%, the mixing time is about 10 minutes (ν≤5%; and 2.2 minutes - ν≤10%). Increasing the mixing time is not economically feasible, therefore, the maximum minimum proportion of the control component, less than which the mixer does not ensure the quality of the prepared mixture: $d_{kz} = 0.08$.

The required number of mixing steps is defined as the logarithm $\left(\frac{1}{d_{kz}}\right) = \frac{1}{0.08} = 12.5$ on the basis of $\left(\frac{1}{d_{k_{min1}}}\right) = \frac{1}{0.01} = 100$, and on the basis of $(1/d_{k_{min2}}) = \frac{1}{0.10} = 10$.

The required number of mixing steps for the proposed type of working body: for preparation from premix - 2 cycles ($k_1 = 1.823 \sim 2$); for the preparation of protein and vitamin supplements - 1 cycle ($k_2 = 0.912 \sim 1$).

Accordingly, the volume of capacities will vary about 10 times.

Thus the minimum number of containers of the mixing unit for the preparation of premix-based mixtures, or when making drugs, consists of two containers of the mixer. The last option is presented in Figure 4. The order of operations: loading the initial components of the mixture in containers; mixing components.

The energy costs of preparing the mixture with two capacitive mixer (J/kg) are determined:

$$E = \frac{[A_1 + A_2]}{\Sigma M_s},$$

$$A_1 = \frac{Tz_s \cdot (P_1 + P`_2)}{2} + \cdot Tc_1 \cdot (P_1 + P`_2) + Tc_2 \cdot (Px_1 + P_2);$$

$$A_2 = Tv_2 \cdot \frac{(Px_1 + P_2)}{2} + Txx_j \cdot (Px_1 + Px_2),$$

$A_1 + A_2$- work spent on all operations in containers, J; ΣM_s - total mass of components of the prepared portion of the mixture, kg; Tz_s, Tv_s - duration of loading of all s-th components and unloading of the finished mixture from the 2nd tank, s; Tc_1, Tc_2 - the duration of mixing of the components in the first (1 - small) and (2 - large) capacity, s; T_{xx} - idle movement of the working body, s; Px_1, Px_2 – power required to drive the working body in the absence of mixture components, W; P_1, P_2 - power required to drive the corresponding working body when

loading all components of the mixture according to the technological process, W; $P`_2$ - power required to drive a large working body when loading all components of the mixture, provided that there are no components from the small capacity of the mixer, W.

CONCLUSIONS

The analysis of mixing devices for the preparation of dry mixtures and technological lines used for this determines the process of formation of mixtures as the interaction of metering and mixing devices. One of the effective options for mixing is step mixing. As a result of the analysis of the process, we clarified the operational sequence of actions for stepwise mixing of material, taking into account the activities of not only the mixer, but already taking into account the entire mixing unit, i.e. during the interaction of the mixer and the multicomponent batcher. The components of the energy intensity of mixture formation are clarified. The operational sequence diagram for the stepwise preparation of dry mixes is analytically determined. It made it possible to establish the dependences of the total work on the preparation of the mixture, carried out by the mixing unit as part of a multicomponent batcher and mixer, mixer performance, the duration of individual cycles and the entire cycle of the mixer.

The required number of mixing steps for the proposed type of working body: for preparation from premix - 2 cycles; for the preparation of a mixture of protein-vitamin supplements - 1 cycle. Mixer tank volumes vary about 10 times.

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TECHNOLOGIES OF THE AGRO FOOD PRODUCTS PROCESSING

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EFFECT OF EXTRACT *TRIBULUS TERRESTRIS* L. AND TECHNOLOGICAL FEATURES OF THE *BOMBYX MORI* L. FED WITH ARTIFICIAL DIET

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Abstract

Cultivation of mulberry silkworm is economically efficient branches of agriculture. It originated in ancient times. The strong dependence on nutritional requirements and especially the seasonality and range of distribution of mulberries are limiting factors for the cultivation of mulberry silk butterfly larvae. Due to its peculiarities, Morus alba develops well and yields high leaf yields at certain seasons of the year in our country. The resulting leaf mass is of high nutritional value. The artificial food for Bombyx mori L. enables cultivation during any season of the year, regardless of the external climatic conditions. Some plant extracts are nutritional stimulants and improve nutritional intake, growth and even disease resistance. The purpose of this study is to test a hybrid I1xBB1xH2xHB2 created in the Scientific Center on Sericulture, Vratsa, Bulgaria on susceptibility to artificial food with added extract of Tribulus Terrestris L. as a stimulant. Tracking the most important biological, reproductive and technological features of silk larvae and butterflies. Artificial food was widely accepted by Bombyx mori L. Higher values were observed in the experimental groups fed with artificial food and added extract of Tribulus terrestris L. We observed the larval growth rate and vitality.

Key words*: Tribulus terrestris L., Bombyx mori L., artificial diet, mulberry silkworm.*

INTRODUCTION

Silk butterfly is one of the most beneficial insects. Nutrition of great importance in sericulture. A very important factor is the quality of the mulberry leaf, on which the normal development and growth of the larvae depends. The species Bombyx mori L. is an insect that receives all the nutrients it needs to develop properly from the leaves of the mulberry (Nasreen, 1999).

Nutrition is the only factor that almost individually enhances the quality and quantity of silk butterfly cocoa production and productivity (Laskar and Datta, 2000)

In their studies, Gobena and Bhaskar (2015) found that larvae fed with mulberry leaves and added plant extracts had better growth and development than the control groups.

Mulberry leaves treated with plant extracts have different effects on growth, development and reproduction.

Aqueous extracts from *Lantana camara*, *Parthenium hysterophorus* and *Tridax procumbens* (Hipparagi et al., 2001), *Tribulus terrestris* (Murugesh and Mahalingam, 2005), *P. hysterophorus* (Rajashekaragouda et al., 1997), *P. hysterophorus* and *Tridax procumbens* (Mahesha et al., 1999b), *Psoralea coryleifolia* and *Phyllanthus niruri* (Shubha, 2005), *Withania somnifera* (Bhaskar et al., 2004) have beneficial effects on *Bombyx mori* L.

Sangamithirai (2014) found that larvae fed with mulberry leaves treated with spirolin extract gave better results. All signs related to the production of cocoons and their quality are significantly influenced.

Bombyx mori L. can also be fed with artificial food. The introduction of technology for the use of artificial mixtures in the practice of breeding, testing and creation of high-performance hybrids suitable for cultivation with artificial food, extends the area and the possibilities for growing and experimenting with laboratory conditions of *Bombyx mori* L.

The use of artificial food has some advantages, such as reducing the care and costs of large mulberry plantations, expanding the range and growing possibilities of Bombyx mori L. regardless of the season, increasing the economic efficiency, reducing the cost of the final product - silk.

The creation and use of semi-synthetic nutrient blends makes it possible to obtain high results in

development, viability and productivity. In many ways, artificial food may be more favorable than natural food. (Ovesenska, 2000)
Feeding larvae during the first age with artificial food is widespread in Japan and South Korea as it provides healthy and viable third-generation larvae and saves labor costs.
In India, artificial food is used to feed larvae during the first ages during seasons when the quality of mulberry leaf is poor.
In Bulgaria, artificial food can be used to grow larvae in the first and second age,
especially in the autumn season, when the quality of the mulberry leaf is lower and then switches to feeding with mulberry leaves until the cocoon is wrapped.
It takes 110 kg of dried artificial food to grow a box until the cocoons are wrapped. Due to the relatively high cost of artificial food, it is not economically justifiable to feed cocoons to produce cocoons throughout the larval period (Tsenov, 2012).
Saviane (2014) works on both larvae feeding patterns and monitors some of the most important indicators and adaptive ability of larvae to artificial food when degrading the properties of the mulberry leaf.
Zhou (2008) proves that artificial nutrition alters the amounts of proteins associated with the immune system, digestion and nutrient uptake, energy metabolism and silk synthesis in poor nutrition and nutrition in silkworms.
Feeding on artificial food results in fewer cocoons, a lower quality of silk thread, a lower survival rate of young larvae and insufficient resistance to specific pathogens in silkworms fed on artificial food.
Murugesh (2007) uses an extract of *Tridax procumbens, Tribulus terrestris* and *Parthenium hysterophorus* and reports significantly greater larval weight and lower mortality than artificial food prepared with distilled water.
Tribulus terrestris L. is widespread in Western Europe, Asia and China, with significant differences in the ratio of active plant substances in different geographical areas. In our country, a number of scientists have worked with Tribulus terrestris L. to test its effect on various experimental animals (pigs, rabbits, chickens). Biologically active substances in the plant, even in minimal quantities, have a significant effect (positive or negative) on the organism of the animals tested. (Valchev, 2008; Dimitrov 1987; Surjiska, 2005)

MATERIALS AND METHODS

The study was conducted at the training and experimental facilities of the Faculty of Agronomy at the University of Forestry, Sofia, in March 2019. The eggs were laid for incubation on March 20, 2019.
The silk larvae feeding experiment used artificial food containing dried mulberry leaf flour provided by Center on Sericulture, Vratsa, Bulgaria. Artificial food is prepared according to the method recommended by the manufacturer. Five days after turning, the cocoon was collected and weighed with the help of an electronic balance and a caliper.
***Tribulus terrestris* L. extract.** B 1 liters of water will begin to sprout / 10 g of Tribulus Terrestris L. herbs in test group 1 and 5 g in test group 2 / After 1 h, distilled water is added to the boil, and then it is left to boil for 24 h. It is roasted through filter paper and stored at low temperature.
Method of preparation of artificial food Distilled water or herb extract is added to the dry substance. 250 g dry substance + 700 ml, 800 ml *Tribulus terrestris* L. extract is homogenized with a mixer and placed in a 2 cm thick box. The resulting mixture was subjected to a heat treatment and cooled. The finished food is stored in a sealed container at 2-5°C until fed. The food can be stored for up to 40 days without losing its nutritional quality. *Tribulus terrestris* L. extract of different concentration was added to the artificial larval food of the experimental group. Control larvae are fed with artificial food without additives, in which the nutrient mixture is prepared only with distilled water. The incubation and cultivation of the larvae was carried out according to the generally accepted methods in our country (Petkov, 1982; Petkov, 1995), which aims to accelerate the development of the embryo in the egg. After reaching stage IV, the eggs were re-stored at 2-5°C. The hatching started 11 days after their incubation bet. The experiment was performed with one control and two experimental groups of 50 booms. Growing and feeding of the larvae was carried out in boxes in a specialized room up to the age of five, including at established temperature and humidity according to Petkov (1980) (Table 1), which is a universal breeding regime.

Table 1. Mode for silkworm rearing with artificial food

Ages	Temperature, ºC	Humidity%
I	27	85
II	26-27	85
III	25-26	80-85
IV	24-25	70-75
V	20-24	65-70

Immediately before each meal, the artificial food is cut into strips of size appropriate to the age of the larvae. The gambling of the food is done after every sleep and after eating. Some of the most important biological, reproductive and technological features of silkworms and butterflies were monitored; Measuring the mass of a silk cocoon, Measuring the mass of a mature beetle.

RESULTS AND DISCUSSIONS

Figures 1 and 2 show higher values of signs under the action of the added extract to the larval feed. The results obtained by us confirm those of Murugesh, (2007), that Tribulus terrestris extract significantly increases larval mass. The controls show slightly lower values.

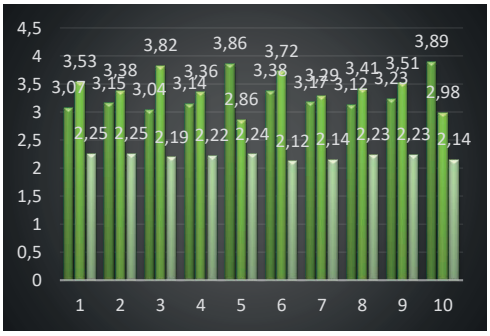

Figure 1. Weight of larva V age (g)

Fresh cocoon weight, mg depends on the mass of the pupa by cocoon shell weight, mg and ekzuvialnata skin of the larva in a V age.
Female pupae are heavier and heavier cocoon shell weight. The mass of the cocoon is determined by the mass of the pupa.
The cocoons in the experimental groups have a higher mass and especially in the experimental group 2, where the effect of the added extract on the food has the greatest influence.

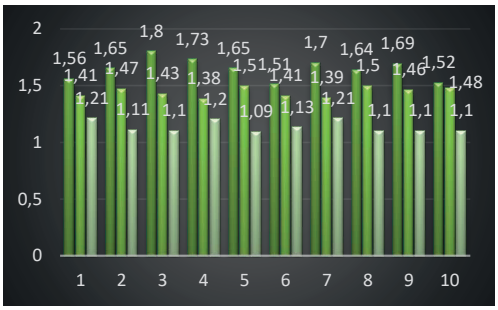

Figure 2. Mass of silk cocoon

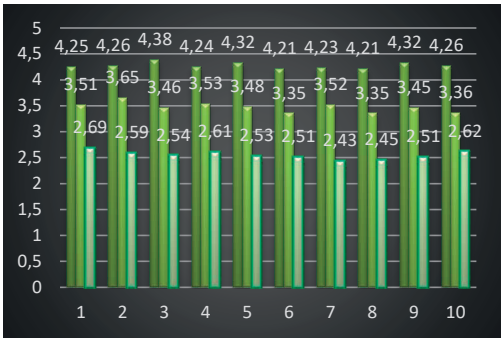

Figure 3 Average length of cocoon

The size of the cocoons depends on the breed or hybrid and the growing conditions. Cocoons with female pupa beans are larger than those with male.
Higher trait values were observed in both experimental groups. In test group 1 the values were from 4.21 to 4.38 and lower in test group 2 from 3.35 to 3.65. These higher values are most likely due to Tribulus terrestris L. added to the diet. The lowest values reported were in the control group from 2.43 to 2.69 mm.

Table 2. Shell ratio (%)

	Silk ratio± SD (%)
Control	22.50 ± 2.02
Experimental Group 1	24.03 ± 1.52
Experimental Group 2	23.97 ± 1.83

Shell ratio is the content of silk, which is determined by the percentage by weight of the silk sheath to the mass of the cocoon. And in these signs, there is a difference in values.
In the control group, shell ratio lower than 22.50% than in the experimental groups. The highest values were reported in test group 1 of

24.03% with higher concentration in the extract and values of 23.97% in group 2.
Shell ratio of cocoons depends on the hybrid used, the sex of the pupa in the cocoon and the growing conditions during the larval period. In cocoons with male pupa, the values of the trait are higher than the cocoons.

CONCLUSIONS

Based on the results obtained from the cultivation of *Bombyx mori* L. with artificial foods and the added extract of *Tribulus terrestris* L., the following conclusions are reached:
Artificial food was perceived by the larvae with great pleasure because of the high percentage of active substances of the herb and well absorbed in the form of an extract. High values were observed in the intensity of growth and vitality of the larvae. They pack cocoons that do not differ from the breed's characteristic features. Due to the stimulating effect of the extract added to the food.
All treated groups, other than the control group, identified a significant increase in larval and cocoon mass, indicating the positive effects of the herbal extract added to the mulberry leaves. for larvae fed with artificial food and plant extract added.
The results obtained confirm that the active substances in the plant, even in minimal quantities have a positive effect on the organism of the investigated larvae.

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DETERMINATION OF FLOUR CHARACTERISTICS FOR KADAYIF PRODUCTION

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Abstract

Kadayıf is a traditional Turkish dessert that is prepared by pouring wheat flour slurry onto a cycling hot plate from a nozzle followed by baking and adding syrup. Recently, demand for specific properties of flours in the bakery industry has increased. In this study, physical, chemical, technological and rheological properties of Kadayıf flours obtained from both Kadayıf producers and flour factories were investigated and a quality profile of flour for Kadayıf production was propounded. It was determined that 43.32 – 59.05% of Kadayıf flours were under 80 µm indicating that flour with fine particles were desired. Ash, protein, sedimentation value, gluten and gluten index changed between 0.53 – 0.70%, 8.10 – 10.77%, 11.2 – 29.2 mL, 19.0 – 27.7% and 50.7 – 97.6% with average values of 0.57%, 9.36%, 19.7mL, 22.1% and 81.1%, respectively. Damaged starch (3.50 – 4.90%) and water absorbtion % (49.5 – 54.3%) were low. As a result, in Kadayıf production special purpose flour with low protein, low gluten content and gluten index, low sedimentation value and water absorbtion capacity is appreciated. Low protein soft wheats with poor technological properties would be appropriate for this purpose.

Key words: Kadayıf, special purpose flour, technological properties, Turkish dessert.

INTRODUCTION

Kadayıf is a semi-finished product obtained by pouring and mixing the liquid dough prepared by adding drinking water to the sifted wheat flour according to its technique and frying it when necessary (TSE, 2010). Kadayıf is presented to the market in two types, as not fried or fried. Kadayıf, which was produced in small enterprises until recently, has been fabricated in large enterprises (Pekak, 2006).

Typically, Kadayıf is produced by pouring water – flour slurry onto a rotating hot metal plate from a couple of nozzles in order to obtain dried Kadayıf filaments. Rotating hot plate ensures sudden evaporation of water from the slurry.

Kadayıf production consists of 4 stages as dough kneading, dough forging, shaping and baking. In the Kadayıf production, flour is mixed into the slurry by kneading with the addition of an average of 80% water, and then the gluten structure is destroyed. This process, called dough forging, is carried out with the help of pallets. It comes to a container with nozzles, which becomes liquified by degrading its gluten structure. The flow rate of the dough from the nozzles is important at this stage. The dough is poured on the sheet rotating at 250 °C. When the metal sheet is turned one full turn, the Kadayıf, which is called raw Kadayıf, is obtained. From here, raw Kadayıf is taken onto a belt conveyor. Cooling takes place as Kadayıf moves on belt conveyor. Raw Kadayıf is rested for about an hour, then placed in the container in which it is shaped and pressed. After pressing, Kadayıf takes its final shape before frying. It is fried at 235 °C (Anon., 2010). It is consumed after syrup is added. The important quality properties of Kadayıf are being not broken up after frying. Kadayıf should not loose brittleness when the syrup is given.

The properties of flour used in Kadayıf production are quite different from other special purpose flour. In the production of Kadayıf, flour with low gluten and gluten index (60%), low protein content is preferred. The slurry should pass through the nozzles without blocking where it will pour onto a hot rotating metal plate (Anon., 2009a). While milling, it is desired that the flour yield in the milling system is high and the semolina yield is as low as possible (Pekak, 2006).

In production of Kadayıf flour, low sedimentation value, low gluten content, low protein soft wheat are preferred (Anon., 2009b). Wheat varieties required for Kadayıf flour are

especially among soft wheats with low protein quality and quantity. Hard wheat with high protein content will result in increased starch damage during milling process. In case of flours with high protein content and high starch damage, more water must be added to obtain the flour - water slurry, and blockages may be observed when flowing through the nozzles due to the excess amount of protein (Pekak, 2006). Evaporation of water is also delayed due to higher amount of water in the slurry. An economically important factor in this situation is that the product stays on the sheet longer for the evaporation of the excess water added and as a result an additional energy cost occurs.

Kadayıf flour production requires a separate mill diagram and great attention. It differs from other special purpose flours in terms of production technique. By using a short diagram and grinding with low tonnage in the mill, using larger mesh size during sieving, by keeping larger gap between rollers in accordance with other special purpose flours, low starch damage is ensured. While 80 micron sieve is used as the smallest sieve in baklava, pie and pastry varieties production, sieves smaller than 132 micron are not used in Kadayıf flour production. In case of using smaller mesh sizes, the flour can become non-sievable by sticking to the sieves due to the low granulation of the Kadayıf flour (Anon., 2009c).

During the interviews with the Kadayıf producers, it was stated that the water absorption of Kadayıf flour, damaged starch ratio, ash and protein content should be low and the particle size of the flour should be also low. In the production of Kadayıf, the paste, which is turned into a slurry with the addition of an average of 80% water, flows from nozzle and is poured on a hot, rotating high temperature sheet, and the cooking process is completed by taking it on the belt conveyor after 1 full turn.

Studies to determine the quality characteristics of Kadayıf flour are very limited. Pekak (2006) studied the effect of 3 different protein ratios (11.1%, 11.6 and 12.2) in 4 different yields (60, 65, 70 and 77%) in a commercial flour mill and he determined physical, chemical and rheological properties of flours and some physical and sensory properties of Kadayıf desserts obtained from these flours. Flours produced from high protein blend had high protein, Zeleny sedimentation and ash values. The highest Kadayıf yield and syrup absorption were obtained from low protein blend flour.

Çakmakçı and Aydın (2001) examined the microbiological and sensory properties of fresh and stored wire Kadayıf produced with whey supplementation. Use of whey at different levels instead of water increased the nutritional value of Kadayıf. The frying time was shortened, and the sense of sugarness increased. Thus, it has been determined that time, electricity and sugar can be saved. As a result of the research findings, it has been determined that 50% whey supplementation provided the best sensory properties Kadayıf dessert.

Özen (2006) utilized Type 550, Type 650 and Kadayıf flour for the production of tulumba dessert in which he studied the effects of different flour type on the quality of tulumba dessert. When the effects of flour type on tulumba dessert quality were examined, it was determined that the desserts produced using Kadayıf flour were harder than other flours and softening was higher than other flours with the increase in time.

In this study, some physical and chemical properties of Kadayıf flour obtained from both flour manufacturers and Kadayıf producers were determined. A range of color profile, protein, ash, gluten quantity, sedimentation value and farinograph quality parameters were propounded for Kadayıf flour.

MATERIALS AND METHODS

Materials

Flour for Kadayıf production was obtained from Ova Flour Factory, On-el Flour Factory (Konya, Turkey), Emek Flour Factory, Ankara Flour Factory (Ankara, Turkey), Tellioğlu Flour Factory, Özdamarlar Flour Factory (Balıkesir, Turkey) Tezcan Flour Factory (İzmir, Turkey) in two replications. 5 kg of Kadayıf flour samples were stored at 15°C in plastic jars and used for analysis.

Methods

100 grams of flour sample was sieved for 5 minutes from 180, 150, 132, 118 and 80 micron sieves. and the percentage of flour remaining on each sieve and under 80 µm sieve was expressed in %. Analysis was carried out in two parallels.

L *, a*, b * and L * - b * values of flour samples were determined according to Ekinci (2001) using Minolta Chromameter CR-310 (Osaka, Japan). Flour samples were filled in petri dishes, their surfaces were smoothed and color values were recorded. Measurements were carried out in 5 parallels.

Moisture content of the samples was determined according to ICC Standard No: 110/1 (ICC, 1982). Ash content was determined according to ICC Standard No: 104/1 (ICC, 1990). Protein content was determined according to ICC Standard No: 105/2 (ICC, 1994). Protein content was calculated by multiplying the total amount of nitrogen by 5.7. The analysis was carried out in three parallels.

Zeleny Sedimentation and modified sedimentation tests were carried out according to ICC Standard No: 116/1 (ICC, 1994) and Pinckney et al. (1957), respectively. Analyzes were carried out in three parallels. gluten and gluten index ratio of the samples were carried out in parallel with ICC Standard No: 155 (ICC, 1994). Falling number of samples were determined according to ICC Standard No: 107/1 (ICC, 1995). The analysis was carried out in three parallels.

Damaged starch percentage of the samples was determined according to AACC Method 76.33.01 based on the principle of iodine absorption using the Chopin SD-Matic device (AACC, 2006). Analysis was carried out in two parallels.

The study was planned as completely randomized design. Data were analyzed by Statistical Analysis System (S.A.S., 2001). Duncan test and one way ANOVA was used to determine differences between results.

RESULTS AND DISCUSSION

Physical Properties of Flours for Kadayıf Production

Particle size distribution of flours for Kadayıf production are given in Table 1. The total flour rate of Kadayıf flour samples under 118 µ was determined as 85.48%. More than 80% of the particle size distribution of flour samples was collected under 118 and 80 µ sieves.

The flour color desired in the production of Kadayıf is white. Therefore, flour is rested for about three weeks in order to allow carotenoid oxidation. As a result, the flour turns creamy white (Elgün, 1995).

Table 1. Particle size distribution of Kadayıf flours

	150 - 132 µ (%)	132 - 118 µ (%)	118 - 80 µ (%)	<80µ (%)
Min.	3.07	4.21	24.30	43.32
Max.	12.83	9.04	44.95	59.05
Mean	4.87	5.76	33.93	51.55
St.D.	2.06	1.10	5.72	4.89
LSD	4.25	2.34	5.85	3.80

Table 2 shows the color profile of flours for Kadayıf production. Average L *, a *, b * and L * - b * values are 95.1, -5.55, 13.31 and 81.78, respectively. For L * value, LSD is 0.83 and samples are statistically different in terms of L * value ($p < 0.01$). The effect of a* on samples is significant while the effect of b * on samples is not statistically significant ($p > 0.05$).

Table 2. L*, a*, b* and L*-b* values of flours for Kadayıf production

	L*	a*	b*	L* - b*
Min.	92.40	-4.90	12.00	73.15
Max.	96.20	-6.00	19.25	84.20
Mean	95.1	-5.55	13.31	81.78
St. D.	0.80	0.29	1.40	2.10
LSD	0.83	0.36	2.64	3.43

Chemical And Technological Properties of Flours for Kadayıf Production

Chemical and technological properties of Kadayıf flour samples were shown in Table 3. Ash content of flour samples ranged between 0.52 – 0.70% with an average of 0.57%. The effect of the ash content on the samples was found significant ($p < 0.01$). Pekak (2006) investigated effect of 3 different protein ratios (11.1%, 11.6 and 12.2) in 4 different yields (60, 65, 70 and 77) in a commercial flour mill and examined the physical, chemical and rheological properties of the Kadayıf flour obtained. In the study, ash content of Kadayıflık flour samples varied between 0.47 and 0.53%. Özen (2006) reported that the ash content of Kadayıf flour was 0.565% (dry matter). Our results were in accordance with Özen (2006) but higher than Pekak (2006).

Production of flour with low yield and low ash content is essential in Kadayıf production. In TSE (10344) Tel Kadayıf Standard (TSE, 2010), the maximum ash content of the Kadayıf is

limited to 0.65%. As a result of interviews with Kadayıf producers, it was determined that the ash content increased by 0.07% during cooking of Kadayıf. Considering the increase in ash content during cooking, it is desired that the average ash content of Kadayıf flour is 0.52% (Anon., 2010). It is known that high ash promotes browning reactions in product color (Pomeranz, 1987). The ash content is desired to be low in Kadayıflık flour. It has been reported that Kadayıf made using flours with an ash content of 0.50 - 0.60% and flour yield of 60 - 70% is appreciated (Pekak, 2006).

Table 3. Some Chemical and Technological Properties of Flours for Kadayıf Production

	Ash (%DM)	Protein (%DM)	Sedimentation (mL)	Modified Sed. (mL)
Min.	0.52	8.11	11.2	9.9
Max.	0.70	10.77	29.2	35.2
Mean	0.57	9.36	19.7	17.8
St.D.	0.05	0.96	5.22	9.15
LSD	0.046	1.40	9.77	13.43

Protein content changed between 8.11 – 10.77% dry matter with an average of 9.36%. Kadayıf flour samples did not change in terms of protein content significantly ($p>0.05$). Pekak (2006) reported that the protein content of Kadayıflık flour samples ranged from 8.11% to 9.41%, while Özen (2006) found that the average protein content of Kadayıflık flour was 9.41%. Our results are consistent with the results of Pekak (2006) and Özen (2006). It is desired that the protein content and quality of the flour should be low in order for the slurry to flow easily from the nozzles onto the sheet and to prevent clogging. High protein content negatively affects the consistency of the dough slurry, causing clogging in the nozzles where the dough is poured onto the sheet, causing problems during Kadayıf production. The average protein content of the flours used in the production of Kadayıf is around 9% (Anon., 2009a).

LSD values for sedimentation and modified sedimentation values were 9.77 and 13.43, respectively, and the effect of both quality parameters on samples was not significant ($p> 0.05$). Low sedimentation and modified sedimentation valus are desirable feature in Kadayıf flour (Anon., 2009b). Similar to the results of the present research, Pekak (2006) stated that the sedimentation values of Kadayıf flour samples ranged between 15.6 - 23.9 mL and modified sedimentation values between 16.5 - 23.3 mL. Özen (2006) reported that Zeleny sedimentation value of Kadayıflık flour was 25 mL. In general, modified sedimentation values decreased compared to Zeleny sedimentation value. This is due to the use of wheat with poor protein and gluten quality in Kadayıf production.

Table 4. shows some technological properties of flours for Kadayıf production. The average gluten content of the samples was 22.1%. The least significant difference between the samples was 3.3 and the effect of gluten on the samples was not statistically significant ($p> 0.05$). Gluten index values ranged from 50.7% to 96.7% with an average of 81.1%. The least significant difference between the samples was 13.4 and the effect of gluten index value on the samples was statistically significant ($p <0.01$).

Table 4. Some Technological Properties of Flours for Kadayıf Production

	Gluten (%)	G.index (%)	Falling Number(sn)	Damaged Starch(%)
Min.	19.0	50.7	280.0	3.50
Max.	27.7	96.7	402.5	4.90
Mean	22.1	81.1	359.7	4.11
St.D.	1.0	15.3	29.8	0.33
LSD	3.3	13.4	71.4	0.79

Pekak (2006) reported that gluten content of Kadayıf flours ranged between 19.8% - 23.6% and gluten index content between 68% - 70%. Özen (2006) reported that the gluten content of Kadayıf flour was 23.6% and the gluten index was 53.0%. During the interviews with flour factories, it has been determined that the gluten index of flours for Kadayıf production was an average of 60% (Anon., 2009a).

The falling number values of Kadayıflık flour samples varied between 280 - 402.5 seconds with an average of 359.7 seconds. The LSD value for the falling number was 71.4. The samples did not differ in terms of falling number ($p> 0.05$). Öztürk et al (2008) reported that the falling number of biscuit flours varied in a wide range such as 350 - 628 seconds.

The LSD value for damaged starch is 0.79 and the effect of the damaged starch ratio on the samples is statistically important ($p <0.01$). Pekak (2006) reported damaged starch ratio of Kadayıf flour samples between 5.8 - 6.3% while

Öztürk et al. (2008) stated that it varied between 4.5 and 6.6%. High starch damage is an undesired feature in Kadayıf flours, since it increases water absorption. As a matter of fact, it has been determined that the damaged starch ratios of Kadayıf flours investigated in the present study were lower than other special purpose flours. Flours with low water absorption is requested for Kadayıf production in order to dry in a short time. Since the damaged starch will absorp more water; it will increase drying time. Therefore, Kadayıf producers prefer flours with a low rate of damaged starch (Pekak, 2006).

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CONCLUSIONS

Kadayıf is a traditional dessert for Turkish people. Determination of its physical, chemical and technological properties may set light to Kadayıf producers and flour factories for appropriate flour properties. As a result, in Kadayıf production special purpose flour with low protein, low gluten content and gluten index, low sedimentation value and water absorbtion capacity is appreciated. Low protein soft wheats with poor technological properties would be appropriate for this purpose

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STUDY ON THE ASSESSMENT OF THE QUALITY OF SOME CHOCOLATE TRUFFLES SOLD IN ROMANIA

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Abstract

The purpose of the study it was represented by the comparative appreciation of the quality of some assortments of chocolate truffles marketed in Romania, following the sensory, chemical (including energy value), and economic characteristics. Three types of truffles (produced in France/Belgium) were taken in the study. Sensory characteristics were analyzed by tasting, using the scoring method; the lipid content was determined by the Soxhlet method, the protein one by the Kjeldahl method, and the moisture and the dry matter by the drying method in the oven (at 105° C); the results were compared with the declared values on the label. In order to determine the economic characteristics, the purchase price for 100 g of product was taken into account. Following the sensory analysis, two products being included in the category of very good products and one was in good quality class, but with a score very close to the very good product. The major chemical content of these products was: sugar between 41-38.7% - on the label), lipids between 45-48.3% on the label vs. 44.93-47.84% determined, proteins between 4.20-4.90% on the label vs. 3.99-4.66% and salt 0.11-0.34% - on the label. The energy value of these products was 599-618 kcal per 100 g.

Key words*: chocolate, truffles, lipids, proteins, energy value.*

INTRODUCTION

Nowadays, the consumption of chocolates has acquired a positive contribution in human nutrition, because despite the lipid and sugar content, cocoa has high concentrations of antioxidant compounds, mainly polyphenols, including flavonoids, such asepicatechins, catechins and, particularly, procyanidins (Efraim et al., 2011; Oracz et al., 2015; Nabavi et al., 2015, Farinazzi-Machado et al., 2018). Due to the presence others compounds, scientific studies have demonstrated that the consumption of chocolates with high cocoa content is associated with the control of risk factors for cardiovascular disease, such as reduced total cholesterol and blood pressure, decreased platelet aggregation, reduced damage to the vascular endothelium and increased plasma levels of high density lipoprotein (HDL-c), in addition to reductions in inflammatory markers (Hooper et al., 2012; Zhang et al., 2013; Colombo et al., 2015; Goya et al., 2016).

After Misnawi et al. (2004) and Heath (2002), flavor is one of the most important characterristics in chocolate products, followed by the perception of the product's texture by the mouth. The aim of the paper was represented by the comparative assessment of the quality of some assortments of chocolate truffles sold in Romania, following their sensory, chemical (including energy value), and economic characteristics.

To carry out this research, chocolate truffles wrapped in cocoa powder were studied. These truffles are made up of a filling in the form of a homogeneous mass of chocolate, over which it was carefully sprayed a layer of cocoa powder, thus creating a special aroma due to the combination of creamy-sweet interior and bitter cocoa powder.

MATERIALS AND METHODS

Three types of truffles, produced in Belgium or France (“Fancy Truffles”, “Belgid` Or” and “Excelcium” - randomly coded A, B and C, five samples for each) were taken in the study. Sensory characteristics were analyzed by tasting, using the scoring method; the lipid content was determined by the Soxhlet method, the protein one by the Kjeldahl method, and the moisture and the dry matter by the drying method in the oven (at 105°C). In order to determine the economic characteristics, the purchase price for 100 g of product was taken into account.

The evaluation of the sensory quality of chocolate truffles was carried out in a sensory analysis laboratory of USAMV Iasi by the participation of a group of twenty-four students in food engineering, each receiving an individual sheet (Table 1), according to standard specifications (Table 2).

Table 1. Individual assessment sheet of the sensory quality of chocolate truffles

Characteristics	Characteristics description	Score
Appearance	Whole pieces with a regular shape and an even distribution of cocoa powder	4
	Whole pieces with regular shape and unevenness in the distribution of cocoa powder	3
	Whole pieces with partially modified shape, with uniform distribution of cocoa powder	2
	Flattened pieces, not covered with cocoa powder	1
Color	Uniform and appropriate on the entire surface of the product	4
	Uneven on a certain region of the product	3
	Uneven on the entire surface of the product	2
	Stains of different shades that are not specific to the product	1
Smell	Pronounced, aromatic, specific	4
	Poorly defined, pleasant	3
	Imperceptible or with a foreign touch	2
	Rancid, sour, inappropriate	1
Consistency	Strong, fine, creamy	4
	Strong, slightly creamy	3
	Soft, slightly creamy	2
	Soft, semi-rough	1
Taste	Sweet more than bitter, very well highlighted and specific to the product	4
	Bitter more than sweet, well highlighted and product specific	3
	Poorly pronounced, but specifically, pleasant	2
	Slightly astringent, non-specific	1
Package	Integral and appropriate packaging	4
	Satisfactory with small imperfectibly imperfections	3
	Unsatisfactory with noticeable imperfections	2
	Deteriorated, endangering the integrity of the constituent chocolate truffles	1

Table 2. Classification of the products in the appropriate quality class according to standards

Total average score	Provided qualifying
22 ÷24	Very good
18÷21.9	Good
11.6÷17.9	Satisfactorily
6÷11.5	Unsatisfactory

RESULTS AND DISCUSSIONS

After the **sensory analysis** the total score determined for two products was between 22 and 24 points (22.4 respectively 23.4), like the total average (22.51), being included in the category of very good products; one product was included in the good quality class, but with a score (21.75) very close to the very good product (Table 3).

Table 3. Total score obtained for the sensory analysis of the chocolate truffle

Products	Total score	Qualifying
A	23.40	Very good
B	21.75	Good
C	22.38	Very good
Average	22.51	Very good

The average score of sensory characteristics determined by tasting highlights differences between products, but not with very high values (Figure 1); thus, for the three analysed products they summed the following score: 10.88 for appearance, 11.13 for color, 11.01 for smell, 11.39 for consistency, 11.26 for taste and 11.88 for package, highlighting close values for all

studied characteristics. On average was obtained: 3.63 for appearance, 3.71 for color, 3.67 for smell, 3.80 for consistency, 3.75 for taste and 3.96 for package.

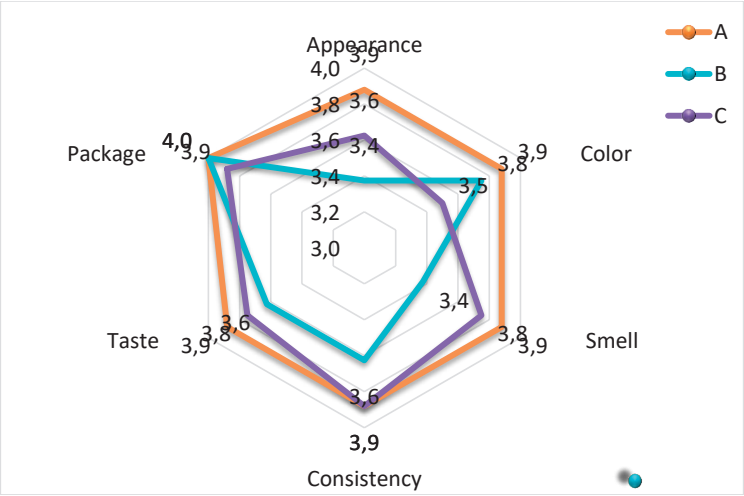

Figure 1. The average score of sensory characteristics determined by tasting

The major chemical content of these products was: total carbs 39.9% (between 41-38.7% on the label), sugar 36.77 (34-39% on the label), total lipids 46.77% / 46.59% (45-48.3% on the label vs. 44.93-47.84% determined), saturated fatty acids (SFA) 38.73 % (35-41.2% on the label), proteins 4.43% / 4.23% (4.20-4.90 on the label vs. 3.99-4.66% determined) and salt 0.23 % (between 0.11-0.34% - on the label) (Table 4 and Table 5).

Table 4. Chemical composition and energy value from the label of the chocolate truffle

Chemical composition	**Product A**	**Product B**	**Product C**	**Average**
Carbohydrates / **sugars** (%)	38.7/ 37.3	41/ 39	40/ 34	39.9 / 36.77
Lipids / SFA (%)	48.3/ 41.2	45/ 40	47/ 35	46.77 / 38.73
Proteins (%)	4.2	4.2	4.9	4.43
Salt (%)	0.34	0.11	0.25	0.23
Energy kcal/100g	618	599	610	609

Table 5. The chemical composition of chocolate truffles studied (values on the label and determined in laboratory)

Content / Product	% Dry Matter, D*	Sugars (%)		Lipids (%)				Proteins (%)				Salt (%)	
		L*	$\bar{x}$ =100	D*	$\bar{x}$ =100	L*	$\bar{x}$ =100	D*	$\bar{x}$ =100	L*	$\bar{x}$ =100	L*	$\bar{x}$ =100
A	98.92	37.3	101.4	48.30	103.3	47.84	102.7	4.20	94.8	4.03	95.3	0.34	147.8
B	99.34	39	106.1	45.00	96.2	44.93	96.4	4.20	94.8	3.99	94.3	0.11	47.8
C	98.80	34	92.5	47.00	100.5	47.00	100.9	4.90	110.6	4.66	110.2	0.25	108.7
	99.02	*36.77*	*100*	*46.77*	*100*	*46.59*	*100*	*4.43*	*100*	*4.23*	*100*	*0.23*	*100*

L - on the Label; D - Determinated in laboratory; x – Average

The energy value of analysed products was on average 609 kcal per 100 g (ranging between 599 - 618 kcal); the highest energy value (618 kcal per 100 g) was observed in the product which also contain the highest amount of lipids. The lipid content of analysed truffles varied with -3.8...+3.3 % versus the average value and in case of protein content the variation was larger, between -5.2...+110.6 %. A large variation was identified in case of sugars content (between -

7.5...+6.1 % versus the average) and the largest variation was observed for the salt content (between - 52.2%...+8.7%

The average content of determined dry matter was 99.02 %, with a very low variation.

The average purchase price for all products studied (Table 6), was 5.37 RON per 100g, ranging between 4.6 and 6.30 RON per 100g) with a relatively large difference of 1.7 RON per 100g (37% of the minimum price).

Table 6. The price of chocolate truffles (RON) compared to their average market price

Stores	A product				B product				C product			
	Unit price	% diff.	Price/100 g	% diff.	Unit price	% diff.	Price/100 g	% diff.	Unit price	% diff.	Price/100 g	% diff.
Auchan	10.60	1.1	5.30	1.1	-	-	-	-	-	-	-	-
Carrefour	10.60	1.1	5.30	1.3	12.05	-4.3	6.03	-4.3	7.4	0.0	4.6	0.0
Cora Romania	10.74	2.4	5.37	2.9	-	-	-	-	-	-	-	-
Mega Image	10.01	-4.5	5.01	-3.6	12.75	0.1	6.38	0.1	-	-	-	-
Mega Market	8.45	-19.4	4.22	-19.3	-	-	-	-	-	-	-	-
Profi	-	-	-	-	12.99	2.0	6.50	2.0	-	-	-	-
Viostel.ro	12.50	19.2	6.25	15.9	-	-	-	-	-	-	-	-
Average	**10.50**	**-**	**5.20**	**-**	**12.60**	**-**	**6.30**	**-**	**7.40**	**-**	**4.60**	**-**

We consider that this price difference is not justified in terms of the ingredients, chemical content or nutritional value of the studied products, being mainly determined by the marketing reasons. It has also been observed that the availability of products in stores varies significantly.

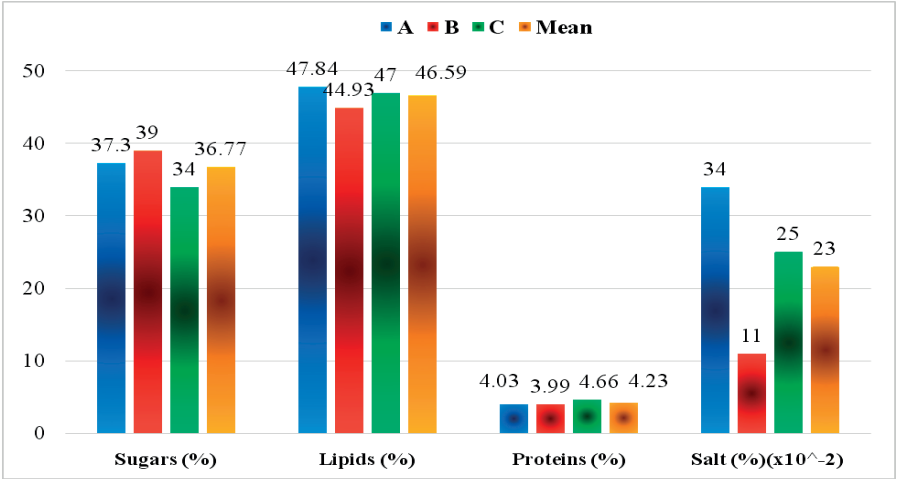

Figure 2. The chemical composition of chocolate truffles (label values)

Other characteristics. The studied chocolate truffles have an appropriate packaging from the point of view of integrity, this aspect materializing following the scores offered for the sensory assessment (on average 3.96 points). All three assortments have appropriate labeling and a resealing mechanism to preserve quality.

Two chocolate truffles (products B and C) are certified according to UTZ "Certified cocoa" (the specific marking of this organism being inscribed on the main face of the packaging) and one product (A) is certified by: BRC, IFS, iTQi and Halal.

CONCLUSIONS

The chocolate truffles studied have on average a very good score on a sensory point of view, rated for six categories of characteristics: two of them obtaining over 22 points and one product being very close to this (21.75 points). Laboratory analysis showed very small differences in the chemical composition compared to the values indicated on the product label, but larger differences could be observed between the studied products.

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BROMELAIN TREATMENTS EFFECT ON PORK LOIN HISTOLOGICAL, TEXTURAL AND TECHNOLOGICAL PROPERTIES

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Abstract

Bromelain is a vegetal originating enzyme known for its effects on meat tenderization. The purpose of this study was to investigate to which extent the treatment with two sources of bromelain affects the histological properties of pork loin (myocytes integrity, proportion of connective and pure muscular tissues) as well the textural ones (shear force) and its technological parameters (drip loss and cooking yield). Forty-five slices of swine Longissimus dorsi muscles (10 mm thickness) were used as biological material, randomly assigned to a control group (CG-no bromelain hydrolytic treatment-15 slices), to B1 group (triturated pineapple, diluted with distilled water 1:1, resulting 50% aqueous extract - 15 slices kept in marinade 12 hours) and to B2 group (bromelain 1% aqueous solution prepared from commercial food additive, bromelain powder of 2000 GDU/g - 15 slices, kept in marinade 12 hours). Tissue square pieces were sampled from the center of the loins and submitted to paraffin impregnation technique, followed by a hematoxylin, eosin, methylene blue staining and studied via photonic microscopy for the histometric assessments. Cubic samples of were also cut from each slice and submitted to a shear force reading cell. Water holding capacity was then measured on the meat provided by half of the remained slice surface, using the grinding-centrifugation technique while cooking yield was calculated after samples weighing and cooking through deep frying at 250°C into sunflower oil, during 15 minutes. The results suggested that bromelain treatments affected the histological integrity of the samples, more than 17.5 % of endomysium being hydrolyzed in experimental groups, compared to control one. This resulted in apparent increase of the surface occupied by muscular tissue (+6.92% B1vs. CG and +13.18% B2 vs. CG) ($P<0.05$; $P<0.01$), versus the one occupied by the connective fibers, as observed and measured within the microscopic field. Bromelain treatments induced better tenderness, suggested by lower instrumentally measured shear force (52.13 N in CG, 48.52 N in B1 and 44.48 N in B2). However, better water holding capacity was measured in CG (WHC=16.78%) due to less tissue disintegration, compared to experimental groups B1 (WHC=14.21%) and B2 (WHC=13.47%) ($P<0.05$). The cooking yield was consequently better in CG (CY=74.35%), due to lower exudation than in enzymatically treated meat (B1, CY=71.82%; B2, CY=70.25%). Therefore, bromelain enzymatic treatment improves the histological and, subsequently, textural features of pork loin, while the technological properties were reduced by the enzymatic proteolysis. It still remains to investigate to which extent the textural improvement is justified by loss of technological properties and by certain taste and flavor expected alterations, due to the known bromelain bittering potential.

Key words*: pork loin, bromelain, connective tissue, shear force, water holding capacity, cooking yield.*

INTRODUCTION

Ultimate gastronomic quality of the meat, given by its nutritional, textural, sensory and technological traits are strongly influenced by pre-cooking treatments and cooking methods (Kharb and Ahlawat, 2010; Haskaraca et al., 2014). One of the ways to enhance sensory properties of meat is to marinate it a few hours prior to cooking, using a mixture of spices, sauces and beverages (Kim et al., 2018). Microbial or vegetal originated enzymes are known to improve meat tenderness, due to the proteolytic effects induced into cell membranes and on the connective stroma of muscles (Sullivan and Calkins, 2010; Chaudary et al., 2015). Many vegetal species are known as sources of such enzymes, that could be used *per-se* as hydro soluble enzymatic complexes or standalone after extraction and purification: pineapple (Nadzirah et al., 2016), *Calotropis procera* (Rawduken et al., 2013), mango (Dhital and Vangnai, 2019), kiwi (Koak et al., 2011), asparagus (Ha et al., 2013), ginger (Naveena and

Mendiratta, 2001), figs (Singh et al., 2019) papaya (Verma et al., 2018), *Averhoa bilimbi* (Ismail et al., 2018), Korean pear (Hao-Liet al., 2009). Besides the proteolytic effects, it is known that such enzymes also have bactericidal or bacteriostatic effect on most foodborne pathogens (Eshamah et al., 2014), therefore a double advantage by using them. Within this state of knowledge, this study aimed to investigate the effect of bromelain, a proteolytic enzyme originated in pineapple on the pork loins sensory, textural and technological traits, using two types of enzymatic treatment solutions (one issued directly from the fruit itself and prepared in dilution with distilled water and the other reconstituted from a commercially available bromelain powder, used as food additive in the meat industry.

MATERIALS AND METHODS

Biological material comprised 45 slices of *Longissimus dorsi* muscles, 10 mm average thickness, cut from adult pigs half carcasses. They were randomly allotted in three groups, in relation with the usage of the experimental factor, bromelain enzyme, as following:

* *CG control* - no bromelain treatment (15 slices)

* *B1 group* (15 slices kept in marinade 12 hours, marinade obtained from triturated pineapple pulp, diluted with distilled water 1:1, resulting 50% aqueous extract)

* *B2 group* (15 slices, kept in marinade 12 hours, marinade consisted in bromelain 1% aqueous solution prepared from commercial food additive, bromelain powder of 2000 GDU/g).

Square pieces of tissue were sampled from the center of the loins and submitted to paraffin impregnation technique, followed by a hematoxylin, eosin, methylene blue staining and studied via photonic microscopy for the histometric assessments. General protocol applied for parrafination and staining was adapted from the methods proposed by Mobini and Asaid Khoshoii, 2013, using a using a spin tissue impregnation processor - THERMOSCIENTIFIC STP-120-2, a rotary automatic microtome - histology line - THERMOSCIENTIFIC HM355S, an automatic tissue stainer - histology line - Varistain Gemini AS – THERMOSCIENTIFIC.

The smears were analyzed by microscopic measurements (Motic M230 with camera, endorsed with Motic Image 3+ software) to assess myocytes and 1st order muscular fascicles cross section areas (sqμm). By difference from the whole area of fascicles and the areas of cross-sectioned muscle cells, contained by each 1st order fascicle, it was found the part occupied by the connective stroma in muscle architecture. Knowing these dimensional and morphostructural details, myocytes density (number of muscle cells per sqmm of muscle) and proportion of main tissue categories (% pure muscular tissue and % connective tissue) were also calculated, following a method applied in similar investigations in the past, by some of the authors in the present paper (Radu-Rusu et al., 2019). One hundred readings on muscle cells and eight readings on 1st order musce fascicle were carried on per each smear (one microscopic blade elected from each slice of loin).

Perten Instruments TVT 7600 texture analyzer equipped with straight Warner Bratzler blade and with the appropriate rig - heavy duty stand – was used to test the cutting strength in a single cycle compression mode, following a methodology published by Bratcher et al., 2006. The maximum peak force (Newtons) was considered as the shear force necessary to cut the meat sample. Two samples were tested per slice; therefore 30 repetitions were run in order to acquire cutting strength data in each treatment group.

Water holding capacity has been tested in accordance with the method proposed by Rawduken et al., 2013: 20 g of grinded, homogenized samples, transferred into glass tubes with 30 ml NaCl 6%, stirred for 1 minute and cooled 4℃, for 15 minutes then were centrifuged 25 minutes, at 3000 rpm. After centrifugation, the moist precipitate remained in the tube after supernatant removal (% of the initial measured volume) was considered as the individual water holding capacity for each sample.

Cooking yield was measured through to 180ºC oil frying for 10 minutes, to simulate the conditions existing in a frying pan. Using gravimetric differences and relative ratios, the cooking yield was obtained (%) (Wyrwisz et al., 2012).

Acquired data were statistically processed using MsExcel Data Analysis toolpack, in order to obtain the descriptors (mean, standard deviation, coefficient of variation - CV%) and to run comparisons between treatments (single factor ANOVA) (Wu et al., 2016).

RESULTS AND DISCUSSIONS

Under the action of bromelain solutions, the pork loin slices modified certain of histological properties, thus the apparent proportion of pure muscular tissue increased and the one of connective tissue decreased (table 1). Of course, this phenomena, observed within the microscopic field is in fact due to the destruction of sarcolemma and to the certain level of liquefaction of connective tissue between the myocytes, leading to the increase occupied by the muscle cell content within the total area of a 1st order muscle fascicle. Thus, in control group (normal muscle) the diameter of myocytes was measured at 56.38±4.65µm and the action of proteolytic enzyme bromelain obtained straight from pineapple pulp triturate induced significant enlargement of cells apparent thickness, by 13.85% (P<0.05), till 64.19±6.30 µm.

Table 1. Histological and textural properties of pork loins, as influenced by bromelain proteolysis

Trait	Group	Mean	±StDev	CV%	± % vs. CG
Muscle cells thickness (µm)	CG	56.38^{a}	4.65	8.25	100%
	B1	64.19^{b}	6.30	9.81	+13.85
	B2	68.79^{c}	7.13	10.37	+22.01
% Pure muscular tissue (%)	CG	71.34^{a}	4.49	6.29	100%
	B1	76.28^{b}	6.83	8.95	+6.92
	B2	81.23^{c}	9.29	11.44	+13.86
Connective tissue in muscle (%)	CG	28.66^{c}	1.80	6.29	100%
	B1	23.72^{b}	2.12	8.95	-17.24
	B2	18.77^{a}	2.15	11.44	-34.51
Shear force (Newtons)	CG	52.13^{c}	5.35	10.27	100%
	B1	48.52^{b}	5.74	11.83	-6.93
	B2	44.48^{a}	5.64	12.69	-14.67

ANOVAS, on the means from the same trait:
$^{ab, bc}$ – significant differences for *P<0.05*
^{ac} – *distinguished significant differences for P<0.01*

Usage of the bromelain reconstituted solution of 1% concentration from commercially available food additive powder induced more severe degradation of connective tissue surrounding muscle cells, thus an increase of diameter by 22.01%, till an average measured value of 68.79±7.13µm (P<0.01).

Data in table 1 and figure 1 also reveal the consequence of apparent cell inflating on the tissue constituent categories of *L. dorsi* analyzed slices. Thus, pure muscular tissue increased from 71.34% in control group (not treated samples) to 76.28% in B1 group (+6.92%), respectively to 81.23% in B2 group (+13.86%), while the connective tissue conversely decreased from 28.66% till 23.72% and 18.77%. Each stage of treatment induced significant and distinguished significant differences.

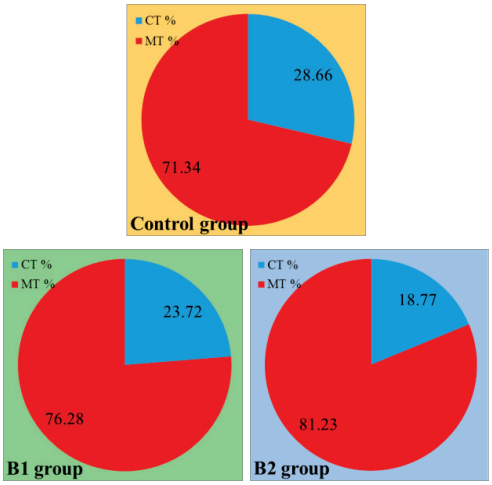

Figure 1. Transformation of tissue structure of pork loin muscles, under the influence of bromelain treatments

Of course, these findings observed in the microscopic field must not be interpreted like a literal change of muscle structure, in terms of hypertrophy of myocytes and atrophy of connective fibers, but more likely like a wiping of membrane limits and fiber delimitation, leading to such an apparent aspect and to the subsequent dynamics of the two categories of tissues, due to photonic microscopy observations.

As expected, due to reduction of connective like tissue proportions, the shear forces necessary to cross-cut the samples (in an imitative test for the consumers' teeth bite force) gradually decreased from 52.13 N in untreated samples to 48.52 N in B1 group (bromelaine from pineapple extract), respectively to 44.48N in B2 group (bromelaine food additive), resulting in an instrumentally measured tenderization of meat by 6.93 to 14.67%, due to proteolysis (figure 2).

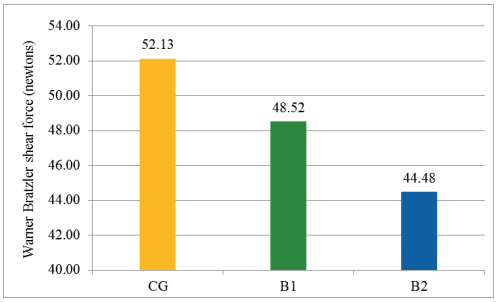

Figure 2 – Dynamics of Warner-Bratzler shear force (N) of pork loin muscles, under the influence of bromelain treatments

These alterations in meat ultrastructure, observed at the microscopic level also affected the technological properties of the meat, such as water holding capacity and cooking yield (table 2 and figure 3).

Table 2. Technological properties of pork loins, as influenced by bromelain proteolysis

Trait	Group	Mean	±StDev	CV%	± % vs. CG
Water Holding Capacity (%)	CG	16.78[a]	1.38	8.25	
	B1	14.21[b]	1.39	9.81	-15.32
	B2	13.47[b]	1.40	10.37	-19.73
Cooking yield (%)	CG	74.35[a]	4.68	6.29	
	B1	71.82[b]	6.43	8.95	-3.40
	B2	70.25[b]	8.04	11.44	-5.51

ANOVAS, on the means from the same trait:
[ab] – significant differences for *P<0.05*

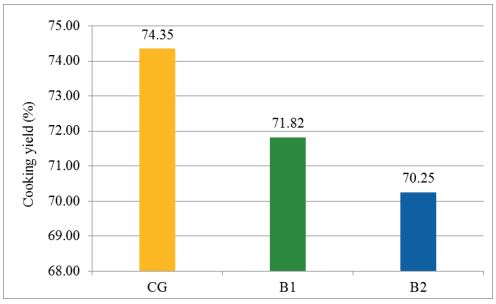

Figure 3. Dynamics of Cooking yield (%) of pork loin muscles, under the influence of bromelain treatments

Water holding capacity significantly decreased from 16.78 till 14.21% and 13.47% in B1 and B2 groups, compared to CG group, suggesting more water drip or loss due to degradation of the connective stroma and to the breakage of cell membranes.

This dynamics also affected cooking yield and, in the case of the raw fried muscle slices, the mass reduced to 74.35% after hot oil frying, while in the samples treated with pineapple extract, the decrease was more pronounced, with 3.41%, in comparison with the control group and reached 71.82% (P<0.05).

Bromelain solution reconstituted from food additive powder induced lower cooking yields, by 5.51%, compared to the raw pork loin.

CONCLUSIONS

Basing on the original findings in our study, it could be stated that the enzymatic treatment seems to beneficially affect the sensory quality of the meat, because the tenderization would attract more the consumers. However, looking at the technological features that decreased, bromelain usage should be better reasoned, because tenderness could increase at the cost of juiciness and at the cost of commercial efficiency, especially when the meat is used for processing or when is cooked for public food services, where gravimetric loss are straightly linked to economic effects.

However, enzymatic proteolysis treatments could be successfully used in order to improve the quality of spent meats, such as those issued from aged animals or of those meats with different unconformities, like pale-soft exudative and dry-firm-dark defects.

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STUDY REGARDING THE APPLICATION OF THE FMEA (FAILURE MODES AND EFFECTS ANALYSIS) METHOD TO IMPROVE FOOD SAFETY IN FOOD SERVICES

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Abstract

The aim of this study was the application of the latest FMEA (Failure Modes and Effects Analysis) method to improve food safety (for soup production) in food services. One of the major changes with the new FMEA method is that the Risk Priority Number (RPN) has been replaced by the Action Priority (AP) process. The working methodology consisted in the collection and processing of information based on practical experience provided by food industry and food services specialists, as well as those from the literature related to similar studies. Among the steps and activities required to apply the FMEA method is distinguished, as specificity, the calculation of AP depending on the severity (S) of consequences of manifestation of nonconformities to the consumer, on the probability of occurrence (O) of a potential hazard for food safety and on the probability of its detection (D). The AP was determined for each category of identified potential hazards (physical, chemical and biological) for all ingredients and for all stages of the technological flow. Through AP, a quantitative assessment can be made of the potential food safety problems in a system, and respectively a prioritization of implementation of preventive, corrective actions and the improve of prevention or detection controls. Based on AP, the identified potential nonconformities can be classified now in the Low priority category even if the old considered RPN value is higher than 100 (125, for the row material storage, at the level of physical hazards). On the other hand, at values lower than 100 of the RPN, the AP can be in the Medium priority category (96, for preparation of ingredients, at the level of chemical hazards), the value of S being the decisive element for classification of potential hazards and nonconformities.

Key words: *failure modes and effects analysis, food safety, soups.*

INTRODUCTION

Quality is defined as the group of those product characteristics that satisfy explicit and implicit customer requirements. Thanks to the adoption of predefined standards, Quality Assurance means to give to the customer the warranty that the company works on the basis of these requirements. In Food Industry two different aspects of product quality can be identified: on one hand, food safety and sanitary integrity, compulsory requirements for selling a food; on the other hand all those components, such as exterior aspect, functionality, nutritional characteristics, etc., that attract the customer (Scipioni et al., 2002) FMEA (Failure Modes and Effects Analysis) it is a predictive and preventive method specific to non-compliance and risk management. The FMEA is a modern tool used in the purpose of identifying potential failure modes (of appearance of nonconformmities), the causes and effects of each failure (nonconformities) on a system, subsystem, or component part.

FMEA has been applied in areas such as the automotive industry, the plastics industry, food service, software, healthcare (Chen, et al., 2020), etc. Traditionally, FMEA has been conducted according to manufacturer perspective until recently the customer perspective has been incorporated in FMEA (Shahin, 2004; Koomsap and Charoenchokdilok 2016). The concept of customer-oriented FMEA arises from the observation that customers are the ones who are directly affected by the occurrence of failure and how they perceive the effects of failure may differ from the manufacturer or provider, leading to different prioritization with and without customer involvement; therefore, their viewpoint must be considered in the FMEA process (Labajan and Koomsap, 2019). The standard BS EN IEC 60812:2018, Failure modes and effects analysis (FMEA and FMECA-failure modes effects and

criticality analysis) includes significant technical changes with respect to the previous edition, transposed in automotive industry in special. One of the major changes with the new AIAG-VAD (*Automotive Industry Action Group-Verband der Automobilindustrie*) FMEA manual, is that the Risk Priority Number (RPN) has been replaced by the AP process. Where RPN considers occurrence, severity and detection rankings equally (OxSxD) now which correlates to the new AP system considers first the severity then the occurrence values and so on.

The AP tables included in the new manual take all 1000 variations of S, O and D into consideration. The tables assign one of three suggested rankings for each action based upon the S, O and D values. The AP rankings are as follows:

H - Highest priority. The FMEA team "Needs" to identify an appropriate action or improve the prevention or detection controls.

M – Medium priority. The FMEA team "should" identify an appropriate action or improve the prevention or detection controls.

L–Low priority. The FMEA team "could" improve upon the prevention and detection rankings. Although the team is not prevented from taking action at any level.

The "Could, Should and Needs" descriptive terms communicate the urgency for the team to address the associated design or process risk.

The FMEA is very similar to HACCP (Hazard Analysis and Critical Control Points) booth being concerned with customer safety and requirements set by legislation. The FMEA goes further in examining in detail every aspect of customer requirements /satisfaction. HACCP drives excellence in every aspect of food safety and is used to guarantee the safety of the food produced by identify and eliminate biological (B), chemical (C) and physical (P) hazards in a food production process. Hazards that if left uncontrolled could result in illness or even death of consumers. The hazards specific to each stage of the technological flow, are assessesd according to the probability (frequency) of occurrence and the severity of the effects of the manifestation on the consumer, establishing risk classes and later stages of the process which constitute critical control points (CCPs). The FMEA method extends this hazard assessment by introducing a new parameter, namely, probability of detection of hazards (Pop C., et al., 2019). These three elements (severity of the effect, hazard occurring probability, and probability of detection) are used to calculate action priority (AP).

Using AP in the decisional process of establishing a CCP, bring a plus of precision and trust in the functioning, control and evaluation of food quality and safety management systems specific to the food products. There has been a steady increase in the number of ethnic restaurants all over the world. International soups are linked to different nations or cultures. For example, Borscht is a beet soup originally from Russia. It is not uncommon to find authentic Indian and Thai soups offered as specialties. Soup is almost always offered on both lunch and dinner menus in restaurants. The soups use ingredients that are associated with a culture's cuisine, and in North-East of Romania the Rădăuțean soup is one of the most consumed/requested by customers, being present in the majority of restaurants. The aim of this study was the application of FMEA (Failure Modes and Effects Analysis) method to improve food safety (for Rădăuțean soup) in food services.

MATERIALS AND METHODS

The working methodology consisted in collecting and processing information based on practical experience and provided by specialists from food industry domain, as well of those related to similar studies provided by the literature.

The activities required to apply the FMEA method in a food safety management for manufacturing of an assortment of soup have been phased, realizing the setting of the technological flow stages (flow diagram, identification, for each step in the flow, of potential hazards (nonconformities, physical, chemical and biological), identifying the causes that led to the emergence of dangers, determining the probability of occurrence of each hazard category (O), determining the severity (seriousness) of the occurrence of the hazard to the consumer (S), establishing the probability of detection of hazards (D),

calculating the AP, setting critical control points (CCP), establishing the HACCP plan.
At the same time, after calculating AP, corrective actions were identified for each category of hazards specific to the different stages of the flow chart.
The corrective actions application led, in all cases, to considerable diminution of AP value.
The results obtained have led to the formulation of some conclusions and recommendations for improving and expanding the FMEA application within food safety management systems.

RESULTS AND DISCUSSIONS

Technological steps specific to the preparation of Rădăuțean soup are schematically presented in Figure 1, through a flow chart diagram, which uses standardized international symbols.
For the technological flow was identified the three categories of potential hazards, P, C and B, as well as the generators causes of their occurrence. The new process FMEA uses specific colors for warning the team depending on the AP ranking: red „must", yellow „should", green „could" (Table 1 and Table 2).

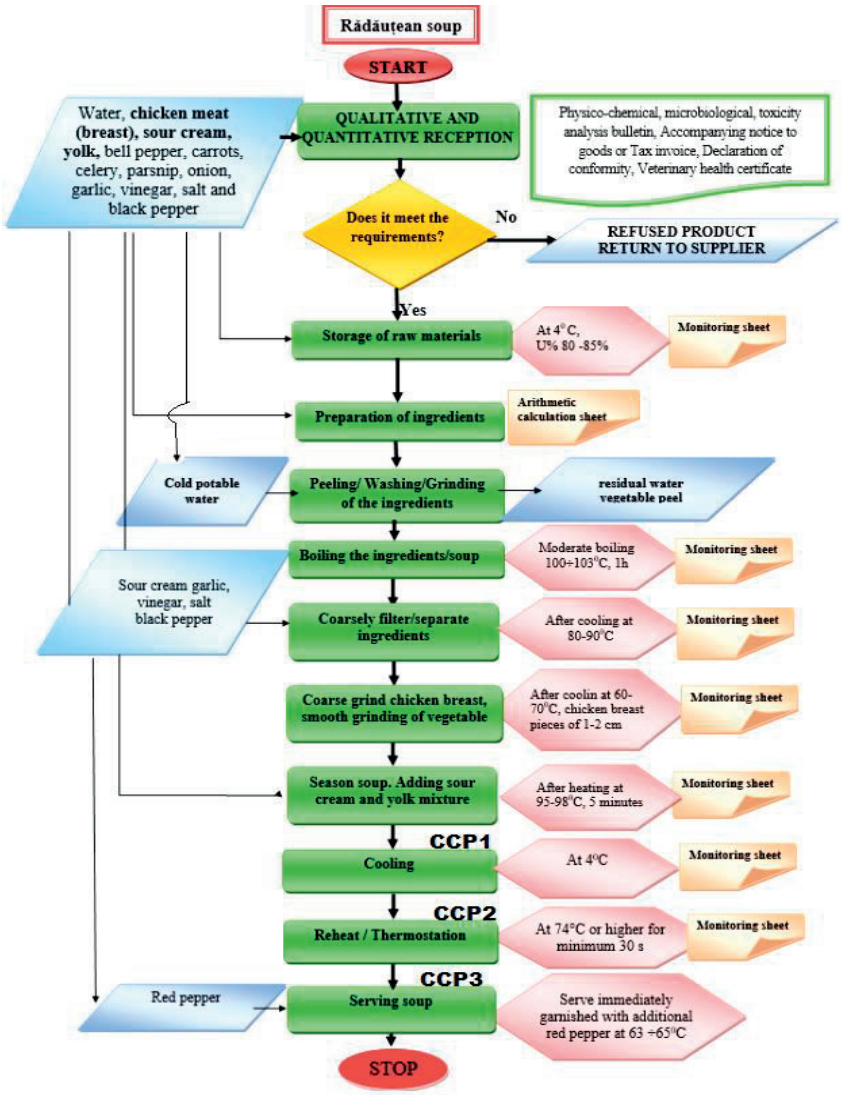

Figure 1. The technological steps of Rădăuțean soup

Table 1. Application of the FMEA methodology for Rădăuțean soup

Risk analysis → Risk evaluation → Risk mitigation

Risk / Ingredients		Nonconformity	CAUSE	Nonconformity effects	S	O	D	RPN	AP	CORECTIVE ACTIONS (CA)	S	O	D	RPN	*AP after CA
Water	P	Impurities, foreign bodies, sediments	Unauthorized supplier, negligence of cooks	Consumer dissatisfaction, loss of customers.	5	5	3	75	L	Supplier selection; checking the analysis bulletins periodically Personal training	5	2	3	42	L
	C	Pesticide residues, heavy metals (Pb, Cu, Hg, etc)	Unauthorized supplier	Affecting the health of consumers	7	3	7	147	M	Supplier selection; checking the analysis bulletins periodically Personal training	7	1	1	7	L
	B	Contamination with microorganisms.	Unauthorized supplier; missing analysis bulletins	Customer /consumer illness.	9	5	5	225	H	Supplier selection; checking the analysis bulletins periodically Personal training	9	1	3	27	L
Chicken meat	P	Impurities from evisceration, handling etc.	Unauthorized supplier, negligence of cooks	Consumer dissatisfaction, loss of customers.	5	5	3	75	L	Supplier selection; checking the analysis bulletins periodically Personal training	5	2	3	30	L
	C	Antibiotics, pesticide residues, heavy metals (Pb, Cu, Hg, Zn), detergents.	Unselected/ Unauthorized supplier. Personal negligence	Affecting the health of consumers	8	5	3	120	M	Provider evaluation. Documents control from supplier. Batch rejection.	8	1	1	8	L
	B	Contamination with pathogenic microorganisms (*Salmonella, E. coli*)	Unauthorized supplier, negligence of chefs, lack of analysis bulletins and veterinary certificates.	Serious illness of consumers. Dangerous product. Expenses for hospitalization of clients.	9	5	5	225	H	Supplier selection; checking the analysis bulletins periodically (for each batch). Personal training	9	2	2	36	L
Vegetables	P	Impurities: sand, earth, straw, leaves etc	Unauthorized supplier, negligence of cooks (non-compliance with the stages/parameters of the technological flow)	Consumer dissatisfaction, loss of customers.	5	5	3	75	L	Supplier selection; checking the analysis bulletins periodically Personal training	5	2	3	30	L
	C	Pesticide residues, heavy metals	Unselected supplier. Personal negligence	Affecting the health of consumers	8	5	3	120	M	Provider evaluation. Documents control from supplier. Batch rejection.	8	1	1	8	L
	B	Diseases, microbial contamination, insects, larvae etc.	Unauthorized supplier, negligence of chefs, lack of analysis bulletins	Notable consumer dissatisfaction, possible digestive disorders, additional costs .	9	5	3	135	H	Supplier selection; checking the analysis bulletins periodically (for each batch). Personal training	9	1	1	9	L

Ingredients \ Risk		Nonconformity	CAUSE	Nonconformity effects	S	O	D	RPN	AP	CORECTIVE ACTIONS (CA)	S	O	D	RPN	*AP after CA
Sour cream	P	Impurities: hair, sand, straw etc.	Unauthorized supplier, negligence of cooks	Consumer dissatisfaction, loss of customers.	5	3	3	45	L	Supplier selection; checking the analysis bulletins periodically Personal training	5	2	3	30	L
	C	Antibiotics, pesticide residues, heavy metals (Pb, Cu, Hg, Zn), detergents.	Unselected supplier. Personal negligence	Affecting the health of consumers	8	5	3	120	M	Provider evaluation. Documents control from supplier. Batch rejection.	8	1	1	8	L
	B	Contamination with pathogenic microorganisms: *E. coli, Enterobacteriaceae, Shigella dysenteriae*.	Unauthorized supplier; negligence cooks (), lack of analysis bulletins.	Serious illness of consumers. Dangerous product. Expenses for hospitalization of clients.	9	5	4	180	H	Supplier selection; checking the analysis bulletins periodically (for each batch). Personal training	9	2	3	54	L
Eggs	P	Impurities: manure etc.	Unauthorized supplier, negligence/untrained staff.	Inconvenients for kitchen staff. Supplementary costs	5	3	3	45	L	Supplier selection; quality control from reception. Personal training	5	1	1	5	L
	C	Antibiotics, pesticide residues, heavy metals	Unauthorized supplier. Personal negligence	Affecting the health of consumers	8	5	3	120	M	Provider evaluation. Documents control from supplier. Batch rejection.	8	1	4	32	L
	B	Contamination with pathogenic microorganisms: (*Salmonella, E. coli*)	Unauthorized supplier; negligence cooks, lack of analysis bulletins.	Serious illness of consumers. Dangerous product. Expenses for hospitalization of clients.	9	5	4	180	H	Supplier selection; checking the analysis bulletins periodically (for each batch). Personal training	9	1	2	18	L
Vinegar	P	Foreign bodies, sediments.	Unauthorized supplier; the chef's negligence.	Consumer dissatisfaction, loss of customers.	5	2	2	20	L	Supplier selection; checking the analysis bulletins periodically (for each batch). Personal training	5	1	1	5	L
	C	Pesticide residues, heavy metals (Pb, Cu, Hg, etc)	Unauthorized supplier. Personal negligence	Affecting the health of consumers	8	5	3	120	M	Provider evaluation. Documents control from supplier. Batch rejection.	8	1	1	8	L
Salt and pepper	P	Impurities, sand, foreign bodies.	Unauthorized supplier; the chef's negligence.	Consumer dissatisfaction, loss of customers.	4	3	2	24	L	Supplier selection; checking the analysis bulletins periodically (for each batch). Personal training	4	1	1	4	L
	C	Impurity salts ($CaSO_4$, $CaCl_2$, $MgSO_4$, $MgCl_2$), heavy metals (Pb, Cu, Hg, etc)	Unauthorized supplier	Possible damage of the health of consumers	8	5	3	120	M	Supplier selection; checking the analysis bulletins periodically (for each batch). Personal training	8	1	1	8	L

*AP =AP after CA

Table 2. Application of the FMEA methodology for technological steps of Rădăuțean soup

No	Flow stages	Noncompliance/Hazard		Causes	S	O	D	RPN	AP	Corrective Actions (CA)	S	O	D	RPN	*AP
1.	Quantitative and qualitative raw material reception	P	Foreign bodies, hair, insects	Non-compliant handling. Untrained personal. Unselected supplier	4	6	5	120	H	Provider evaluation Batch rejection. Personal training	4	1	1	4	L
		C	Antibiotics, mycotoxins, pesticide residues, heavy metals (Pb, Cu, Hg, Zn), detergents.	Unselected supplier. Personal negligence	8	5	3	120	H	Provider evaluation. Documents control from supplier. Batch rejection.	8	1	1	8	L
		B	Pathogenic microorganisms: *Escherichia coli, Salmonella sp, Staphylococcus aureus, Listeria monocitogenes*	Unsanitary manipulation. Unselected supplier. Inadequate temperature and transport conditions.	9	7	3	**189**	**H**	Provider evaluation. Batch rejection. Personal training. Checking analysis bulletins, sanitary veterinary certificates. Checking transport conditions and thermograms. The temperature of raw materials control and recording.	9	1	1	9	L
2	Raw material storage	P	Foreign bodies, hair, insects, personal objects, etc.	Improper handling. Unselected supplier	5	5	5	125	L	Personal training. Respecting hygiene procedures. PRP	5	1	1	5	L
		C	Traces of detergents, disinfectants	Improper rinsing of machinery and equipment.	8	5	4	160	H	Personal training. Respecting hygiene procedures.	8	1	1	8	L
		B	Pathogenic microorganisms: *Escherichia coli, Salmonella, Staphylococcus aureus*	Unhygienic handling. Inappropriate temperature and conditions of storage	9	5	4	180	H	Personal training. Enhance raw material temperature monitoring, control and recording.	9	1	1	9	L
3.	Preparation of ingredients	P	Bone fragments, hair, insects, personal objects, etc.	Improper handling. Personal negligence	5	5	5	125	M	Personal training. Compliance with procedures, metrological verification plan	5	1	1	5	L
		C	Traces of detergents, disinfectants	Improper rinsing of equipment. Personal negligence	8	4	3	96	M	Personal training. Respecting hygiene procedures	8	1	1	8	L
		B	Pathogenic microorganisms: *Escherichia coli, Salmonella, Staphylococcus aureus*	Unhygienic handling. Inappropriate temperature and conditions of preparation	9	6	3	162	H	Checking staff hygiene, machinery, utensils, equipment, work environment by performing sanitation tests.	9	1	1	9	L
4.	Peeling/ Washing/ Grinding of the ingredients	P	Hair, insects, personal objects, etc.	Improper handling. Untrained staff	5	5	2	50	L	Personal training	5	1	1	5	L
		C	Traces of detergents, disinfectants	Unselected supplier. Personal negligence - faulty rinsing (machines, utensils, equipment)	8	4	3	96	M	Personal training. Respecting hygiene procedures	8	1	1	8	L
		B	Pathogenic microorganisms: *Escherichia coli, Salmonella etc.*	Unsanitary manipulation. Contaminated water. Inadequate environmental conditions	9	6	3	162	H	Checking the staff, utensils, equipment, work environment hygiene by performing sanitation tests. Keeping maintenance plan. Checking analysis bulletins	9	1	1	9	L
5.	Boiling the ingredients/soup	P	Hair, insects, personal objects, etc.	Improper handling. Untrained staff	5	4	2	40	L	Personal training	5	1	1	5	L
		C	Traces of detergents, disinfectants	Faulty rinsing (utensils, equipment)	8	3	2	48	L	Personal training.	8	1	1	8	L
		B	Pathogenic microorganisms: *Escherichia coli, Salmonella, Staphylococcus aureus*	Unsanitary manipulation. Inadequate temperature and environmental conditions	9	5	3	135	H	Personal training. Checking the staff, machinery, utensils, equipment, work environment state of hygiene by sanitation tests.	9	3	1	27	L
6.	Coarsely filter/ separate	P	Foreign bodies, hair, insects, personal objects, etc.	Noncompliance manipulation. Untrained personal. Personal negligence	5	3	2	30	L	Personal training	5	1	1	5	L

No	Flow stages	Noncompliance/Hazard		Causes	S	O	D	RPN	AP	Corrective Actions (CA)	S	O	D	RPN	*AP
	ingredients	C	Traces of detergents, disinfectants	Untrained personal. Improper rinsing of equipment	8	3	2	48	L	Personal training	8	1	1	8	L
		B	Pathogenic microorganisms: *Escherichia coli, Salmonella, Staphylococcus aureus*	Unsanitary manipulation. Inadequate temperature conditions	9	7	3	189	H	Performing periodic sanitation tests. Personal training. Ventilation control	9	2	1	18	L
7.	Coarse grind chicken breast, smooth grinding of vegetable	P	Foreign bodies, hair, insects, personal objects, metallic fragments etc.	Noncompliance manipulation. Untrained personal. Personal negligence. Lack of maintenance plan	5	2	2	20	L	Personal training	5	1	1	5	L
		C	Traces of detergents, disinfectants	Improper rinsing of equipment.	8	2	1	16	L	Personal training	8	1	1	8	L
		B	Pathogenic microorganisms: *Escherichia coli, Salmonella, Staphylococcus aureus*	Unsanitary manipulation. Inadequate temperature and environmental conditions	9	4	3	108	H	Checking the staff, utensils, equipment, work environment state of hygiene by performing sanitation tests. Personal training	9	1	1	9	L
8.	Season soup. Adding sour cream and yolk mixture	P	Foreign bodies, hair, insects.	Improper handling. Uninstructed staff. Personal negligence	5	5	4	100	L	Personal training	5	1	1	5	L
		C	Traces of detergents, disinfectants.	Personal negligence. Outdated of smoking cells	8	4	3	96	M	Personal training	8	1	1	8	L
		B	Pathogenic microorganisms: *Escherichia coli, Salmonella, Staphylococcus aureus*	Unsanitary manipulation. Inadequate temperature and environmental conditions	9	5	2	90	H	Monitoring of smoking specific parameters. Sanitation of smoking cells. Performing sanitation tests. Personal training	9	1	1	9	L
9.	Cooling soup	P	Foreign bodies, hair.	Improper handling. Uninstructed staff. Personal negligence	5	3	2	30	L	Personal training	5	1	1	5	L
		C	Development of other types of molds with mycotoxin production	Failure to comply technological parameters. Inappropriate hygiene	9	3	2	54	L	Personal training	9	1	1	9	L
		B	Pathogenic microorganisms: *Escherichia coli, Salmonella, Staphylococcus aureus*	Inappropriate hygiene of storage areas. Personal negligence	9	3	1	27	L	Performing periodic sanitation tests. Personal training	9	1	1	8	L
10.	Reheat / Thermostation	P	Foreign bodies.	Improper handling. Uninstructed staff. Personal negligence.	5	2	1	10	L	Personal training. Maintaining and compliance with the DDD plan	5	1	1	5	L
		C	Traces of detergents, disinfectants	Faulty rinsing equipment.	8	3	1	24	L	Personal training	8	1	1	8	L
		B	Pathogenic microorganisms: *Escherichia coli, Salmonella, Staphylococcus aureus*	Inappropriate hygiene of storage areas. Personal negligence	9	2	2	36	L	Personal training	9	1	1	9	L
11	Serving soup	P	Foreign bodies, hair, insects.	Improper handling. Uninstructed staff. Personal negligence	5	5	4	100	L	Personal training	5	1	1	5	L
		C	Traces of detergents, disinfectants.	Personal negligence. Outdated of smoking cells	8	2	2	32	L	Personal training	8	1	1	8	L
		B	Non-compliance with the stages / parameters of the technological flow. Pathogenic microorganisms: *Escherichia coli, Salmonella, Staphylococcus aureus*	Unsanitary manipulation. Inadequate environmental conditions	9	3	5	135	M	Personal training	5	1	1	9	L

*AP =AP after CA

There are high differences compared to the old FMEA methodology which based on the RPN value automatically applies corrective actions (at values higher than 100 resulting in serious quality problems).

At present, after the application of the new methodology (AIAG/VDA, 2019) different values of RPN are observed, but based on AP, the identified potential nonconformities can be classified in category L even if the RPN value is higher than 100 (125, for the row material storage, step two of technological flow, Table 2). On the other hand, at values lower than 100 of the RPN, the AP can be in M category (96, for preparation of ingredients, at the level of C hazards, table 2), the value of S being the decisive element for classification of potential hazards and nonconformities.

The 2019 AIAG/VDA FMEA handbook replace the improvement prioritization through RPN threshold to AP risk matrix that determine the level of risk based on combination of S, O and D ranking (Edly and Hood, 2020). This provide solution to ambiguous recommendation from previous AIAG method that required to prioritized based on highest S first, the O second and the D third. This method has make no sense in determining the action, for example the severity score is 10, while occurrence is 2 and detection is 2. It is because the O and D are already considered as low as practicable. The S being so high, it does not matter if the possibility of O and D is low, even if the danger seriously affects the health of a single consumer (and not of a group), the consequences of non-compliance are just as serious.

The possibility of diminishing the risks signalled by the FMEA methodology, through preventive and corrective interventions, was also reported in other similar studies conducted for food safety specific to the different categories of food products in Greece (Arvanitoyannis and Savelides, 2007 for chocolate production, Arvanitoyannis and Varzakas, 2007a/b for manufacturing of strudel and potato chips, Arvanitoyannis and Varzakas, 2008a/b for industrial processing of salmon and common octopus and Varzakas and Arvanitoyannis, 2008 for processing of ready to eat vegetables) and in Turkey (Ozilgen, 2012 for Turkish delight and Ozilgen, et all., 2013 for red pepper spice). Other approaches in Italy (Shirani, 2015 for milk), Poland (Trafialek, 2014 for the audit process) and China (Wang, 2015 for the meat supply chain) further demonstrates the utility application of FMEA.

In order to obtain a quality product, the raw materials and the technological process are very important, along with the training of the human resource involved, in the field of quality management and food safety.

Following the analysis performed for the most potential biological hazards, the AP from category H was identified (for the stages before boiling the soup, but also for the water and basic ingredients used, especially those of animal origin).

For the potential chemical hazards, the ranking of the AP in category M was observed, and for the physical ones, the classification was made mainly in category L (Table 1 and Table 2).

The compliance and monitoring of critical limits (the parameters related to each technological stage) ensure the conformity of the obtained product.

After the application of heat treatments (the boiling of soup) the biological hazards are reduced, if the necessary time are respected. Also the stages after boiling must be carried out in strict hygienic conditions, the staff being healthy, responsible and previously trained, to satisfy the requirements of consumers and to ensure the quality and safety of the food produced.

In the same time the storage of ingredients are very important Rațu et al., 2014, Rațu et al., 2015, Murariu et al., 2019, especial of hen eggs, chicken meat (breast) and sour cream.

Another key element is the reception stage, which is usually a very important control point, preceded by the conclusion that is taken after the decision-making process based on the fulfilment of the standard requirements. If the raw or auxiliary materials do not meet the requirements are rejected, so the system is not loaded with possible non-conformities.

CONCLUSIONS

In the new FMEA method, based on AP, the identified potential nonconformities can be classified now in the L category even if the old considered RPN value is higher than 100 (125,

for the row material storage, at the level of physical hazards). On the other hand, at values lower than 100 of the RPN, the AP can be in the M category (96, for preparation of ingredients, at the level of chemical hazards), the value of S being the decisive element for classification of potential hazards and nonconformities.

FMEA method combined with HACCP principles provides a solid basis for identifying food hazard and CCPs specific to the manufacturing process of soups, offering the possibility of applying control measures, monitoring and corrective intervention.

The greatest efficiency in FMEA application in a food safety management system is achieved at the design stage because it emphasizes the preventive nature of the method, essential aspect in order to achieve food products safe for consumption.

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RESEARCHES ON THE QUALITY ASSESSMENT OF MOZZARELLA CHEESE FOR THE SAFETY OF USE

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Abstract

In this paper we intended to perform the qualitative analysis of an assortment of cheese with semi-hardened paste, namely the Mozzarella cheese obtained in a milk processing unit in Iasi County, as well as the qualitative analysis of the raw milk. The organoleptic, physico-chemical and microbiological parameters were analyzed, their results being compared with the company's quality standards. All milk of quality indicators were compliant, so the raw material was classified as safe in the manufacturing process. From the physicochemical point of view, the calculated mean for the fat content was 21.51%, the protein content was 19.17%, 3.17% higher than the minimum stipulated in the standard and the sodium chloride content averaged 2.88%. We determined Salmonella / 25 g, coliform bacteria (ufc / g), Escherichia coli (ufc / g) and coagulase-positive staphylococci (ufc / g). As a result of the microbiological examination, all 4 parameters analyzed were below the maximum admissible limit. The final conclusion was that Mozzarella cheese meets all the physico-chemical and microbiological characteristics but instead was classified as "good" only because of organoleptic deficiencies, especially taste.

Key words: *milk, Mozzarella, parameters, quality, safety.*

INTRODUCTION

Together with the vegetable sector, the zootechnical sector in our country represents one of the main branches of the economy, which provides the necessary raw material for the food industry, but also food for the population. The sector occupied by milk and dairy products is one of the most important sectors of Romanian agriculture, accounting for over 25% of total agricultural production and 10% of animal production. Over 95% of the total milk production is obtained from the private sector (Adamov, 2010). The European Union is the main factor on the world market for milk and dairy products, being the most important exporter for many dairy products, and especially for many cheese assortments (Țibulcă and Jimborean, 2008). At European level, the milk sector ranks first in European agricultural production, accounting for approximately 14% of its total value. Milk is a primary product or a raw material used to make derivative dairy products. In Romania, the main source of milk is made up of cattle kept in private livestock units, which provide over 95% of total milk production (Noje, 2011). Worldwide, cheese is considered a major agricultural product. According to FAO, over 20 million tonnes of cheese are produced worldwide annually (FAO, 2016). Cheeses are considered foods with a high nutritional value, due to their high content in proteins, lipids, mineral salts and vitamins, nutrients that have a very good quality and a high bioavailability (Costin et al., 2003), and dairy products are not associated with increased risk of cardiovascular disease (Givens, 2018). Regardless of the assortment manufactured, cheeses continue to be among the foods preferred by consumers, both for their nutritional value and for the numerous benefits brought by their consumption (Jimborean and Țibulcă, 2006). Despite the fact that dairy products are healthy nutritional foods, there are

increasingly obvious discussions about their quality and safety. The health and standard of living of people are in full agreement with the quality and safety of food, and from a social point of view, risk assessment has a special significance (Han, 2019). It is important to be aware of the dangers associated with the consumption of unsafe foods, which do not correspond qualitatively due to non-conformities occurring in the production chain, traceability being extremely important. Proper hygiene of work equipment, presence of healthy personnel, water quality, quality of raw materials, appropriate cooling, storage temperature are some of the measures aimed at the safety of the finished product (Fox and Cogan, 2004). Therefore, in the present paper we set out to carry out a qualitative analysis of the raw material milk, of the Mozzarella cheese, obtained within a milk processing unit in Iasi county. The quality of Mozzarella cheese is given by all the organoleptic, physico-chemical and microbiological properties, but also by the consumers' perception of the product as a whole, a perception that concerns the satisfaction of the implicit needs, but also of the expressed needs. Mozzarella cheese is a dairy product highly appreciated by most consumers, it is considered one of the most natural foods, the ingredients used to obtain it prove this fact.

MATERIALS AND METHODS

The reception of the raw material milk represents an extremely important stage of the technological flow of Mozzarella cheese and especially the qualitative reception, considering that these parameters can influence the quality of the finished product.

In order to assess the quality of raw milk and Mozzarella cheese produced within the unit, a series of organoleptic, physico-chemical and microbiological determinations were made. The organoleptic examination focused on the main sensory characteristics of the studied products (external appearance, shape, color, consistency, smell, taste and aroma). The chemical analyzes performed on Mozzarella cheese took into account the quantitative values of their main constituents (water%, dry matter%, fat%, proteins%, salt%). In the case of milk raw material was followed the state of freshness: its acidity (°T), its density (g/cm3), but also its fat content (%) and protein (%). Regarding the microbiological examination of milk were determined the total number of germs (NTG ufc / ml) and the number of somatic cells (NCS ufc / ml), and in the case of Mozzarella cheese were determined the presence of bacteria of the genus Salmonella, the number of bacteria coliforms, Escherichia coli bacteria and coagulase-positive staphylococci.

The organoleptic analysis of the raw material milk was performed by a team of 5 people, who also carried out the organoleptic analysis of Mozzarella cheese and this was aimed at tasting and appreciating the organoleptic properties of the milk used to obtain Mozzarella cheese, by awarding points, with the help of a base of sensory appreciation. The organoleptic analysis was performed by the scoring method.

The determination of milk fat was done by the acid-butyrometric method, using the Gerber butyrometer. The principle of the method consists in the separation of milk fat by centrifugation, after the protein substances have been dissolved in the presence of sulfuric acid and isoamyl alcohol (STAS 6352 / 2-87).

The determination of the acidity of the milk is made in order to assess its freshness. In this case, the acidity was determined by the Thörner method, which consists of neutralizing the acids by titration with a 0.1n NaOH solution. The acidity of the milk is expressed in ° T / 100 ml milk. The formula used is as follows: A = 10 x V, where: V = volume of 0.1 n NaOH used in the titration (ml) (STAS 6353-85).

The determination of milk protein substances was performed by the protein titration method. The calculation formula is as follows: Protein titer = V / 2, where: V = volume of 0.143 n sodium hydroxide solution used in the second titration (ml) (Usturoi, 2012). The estimation of the density of the raw material milk was made at the temperature of + 15 ... + 20°C, by the areometric method, the density being expressed in g/cm^3 (Usturoi, 2007). The total number of aerobic mesophilic germs in milk was determined using the Koch method or the dilution method and the somatic cell count in milk was calculated using the fluoro-opto-electronic method (Usturoi, 2012). The physical-chemical examination of Mozzarella cheese aimed to determine the water content

(%), by the drying method in the oven (STAS 6344-88), a method that consists in drying the Mozzarella cheese samples until constant weights are reached, and then by difference calculates the water content (%) (Jimborean and Ţibulcă, 2006). The amount of dry matter from the Mozzarella cheese samples was determined by difference, using the formula: S.U. (%) = 100 - water, fat determination was performed using the acid-butyrometric method, using a Van-Gulik butyrometer, and Kjeldahl method was used to determine the proteins in the Mozzarella cheese samples.

To determine the sodium chloride in cheese, the method used to precipitate the chlorides with the help of silver nitrate was used, respectively of the potassium chromate as an indicator. The calculation formula used to determine sodium chloride in cheese samples is: sodium chloride (%) = V / m, where: V = volume of silver nitrate 2.906% used for titration (cm^3), m = amount of Mozzarella cheese used in analysis (g) (STAS 6354-84). The working method for determining the presence of Salmonella bacteria is composed of 4 successive stages, namely: pre-enrichment in a liquid culture medium, non-selective; enrichment using a liquid, selective environment; isolation of Salmonella bacteria, by passing on two selective and differential solid culture media; recognition of colonies of bacteria of the genus Salmonella, which have developed on selective media, by highlighting the biochemical and serological properties (Usturoi, 2012). Determination of the number of coliform bacteria (NBC) was performed using the presumptive test and the confirmatory test. It is known that coliform bacteria ferment lactose from a lactose environment, resulting in gas release. The presence of coliform bacteria is confirmed by the appearance of characteristic colonies, which have a dark blue-green color and have a metallic luster, as well as some atypical, opaque, pink colonies and having a dark center.

Also, the number of coagulase-positive staphylococci / g in Mozzarella cheese samples was calculated by determining the product between the presumptive number of staphylococci / g produced and the number of coagulase-positive colonies, the result being based on the total number of colonies that were checked (Garlic, 2007).

RESULTS AND DISCUSSIONS

The data obtained from the sensory analysis of the raw material milk revealed that it obtained a total score of 15.2 points, a score considered good for the raw material milk used in the manufacture of Mozzarella cheese (table 1).

Table 1 Organoleptic characteristics of raw material milk

Product name analyzed: raw material milk **Manufacturing factory:**_____ **Date:**_____			
Tasters	**Organoleptic characteristics**		
	Appearance, texture, color	**Smell**	**Taste**
Taster 1	4	3	3
Taster 2	4	5	4
Taster 3	4	4	3
Taster 4	4	4	5
Taster 5	4	4	3
Average unweighted score (Pmnp)	4	4	3.6
Weighted average score (Pmp)	4	4	7.2
Total weighted score	15.2		

The most appreciated organoleptic characteristics in the case of raw material milk were the appearance, the consistency, the color and the smell that received an average score of 4 points, and the least appreciated was the taste, which obtained an average score of 3.6 points. In order to carry out this work, the following physico-chemical parameters of milk were taken into account: fat (%), acidity (°T), protein (%) and density (g/cm^3). The fat content of milk raw material registered an average value of 3.73± 0.03%, the minimum being 3.60% and the maximum value being 3.80%. Regarding the studied character, it presented a very good homogeneity, the value of the coefficient of variation being 2.19% (Table 2).

Table 2 Physical-chemical properties of raw material milk analyzed

Specification	Company standard	Statistical estimators				
		N	$\bar{X} \pm s_{\bar{X}}$	CV %	Min.	Max.
Fat (%)	min. 3	6	3.73±0.03	2.19	3.60	3.80
Acidity (°T)	15-19	6	16.17±0.31	4.66	15.00	17.00
Protein (%)	3,30-4,20	6	3.75±0.05	3.51	3.55	3.91
Density (g/cm3)	min. 1,029	6	1.029±0.00	0.08	1.029	1.031

In order to properly assess the data obtained by us, it was considered necessary to compare them with the company's Quality Standard. For the fat content (%) the average obtained by us was higher by 0.73% than the minimum imposed by it. Milk acidity is a very important parameter that indicates the degree of freshness of raw milk. The average values obtained in this case were within the limits mentioned in the company standard, so the average value obtained was 16.17±0.31°T, with a minimum of 15°T and a maximum of 17°T. The coefficient of variation obtained was 4.66%, which indicates a very good homogeneity within the analyzed group. For this parameter, the company standard imposes values between 15 and 19°T, the average obtained by us being higher by 1.17°T compared to the maximum allowed by the standard. The milk protein content recorded values between 3.55% and 3.91%, the average value obtained in this case being 3.75±0.05%. The coefficient of variation (V%) was 3.51%, a value that indicates a very good homogeneity of the samples within the analyzed group. For the protein content the standard imposes a minimum value of 3.30% and a maximum of 4.20%. The average obtained by us from the determinations was 0.45% higher than the minimum allowed and 1.55% lower than the maximum indicated in the standard.

Regarding the density of raw milk, the average value obtained was 1.29±0.00 g / cm3, the minimum being 1.029 g/cm3 and the maximum 1.031 g/cm3. The coefficient of variation that was obtained this time as well was a very small one, of 0.08%, which indicates a very good homogeneity within the analyzed group. The company standard indicates for this qualitative parameter a minimum value of 1.029 g/cm^3, the average obtained by us falling within these limits.

Milk samples subjected to microbiological examination were taken immediately after the quantitative reception of the milk, according to current standards, after which they were subjected to analysis. For raw milk, the number of somatic cells (NCS) and the total number of germs (NTG) for each sample were determined (Table 3).

Table 3 Microbiological load of raw material milk analyzed

Specification	Standard value	Obtained results				
		n	$\bar{X} \pm s_X$ (ufc/ml)	CV%	Min.	Max.
NCS	max. 400000	6	158333.33±14586.90	22.57	110000	215000
NTG	max. 100000	6	44500.00±6716.89	36.97	18000	64000

The mean value obtained for the number of somatic cells (NCS) in the raw material milk analyzed was 158333,33±14586.90 cfu/ml, lower by 241666.67 cfu/ml than the maximum permitted limit. The minimum determined in the case of milk samples subjected to analysis was 110,000 cfu/ml, and the maximum was 215,000 cfu/ml, values that gave the group a very heterogeneous character, the value of the coefficient of variation being 22.57%.

Regarding the total number of germs (NTG), it was found that the average value obtained was 44500.00±6716.89 cfu/ml, lower by 55,500 cfu/ml than the maximum allowed by the standard.The value of the coefficient of variation registered in this case was 36.97%, a value that indicates a very low homogeneity within the group studied.

From an organoleptic point of view, we can see that the most important characteristics of

Mozzarella cheese were the consistency, appearance and color that obtained the score of 5.00 and 4.00 points, respectively. At the opposite pole was the smell and taste, which obtained an average score of 3.5 and 3.3 points compared to the maximum score of these characteristics which was 5 points.

Mozzarella cheese subjected to organoleptic examination obtained an average total score of 15.5 points, a score considered good for this range of cheese (Table 4).

Table 4 Organoleptic characteristics of Mozzarella cheese

Product name analyzed: Mozzarella cheese **Manufacturing factory:**_____ **Date:**_____						
Tasters	**Organoleptic characteristics**					
	Exterior appearance	**Appearance in section**	**Consistency**	**Color**	**Smell**	**Taste**
Taster 1	4	5	5	4	4	4
Taster 2	5	4	5	5	3	3
Taster 3	5	4	5	5	5	4
Taster 4	5	5	5	5	4	5
Taster 5	5	5	5	5	5	4
Average unweighted score (Pmnp)	4	3.8	5	4	3.5	3.3
Weighted average score (Pmp)	1.60	3.00	4.00	1.60	1.40	3.90
Total weighted score	15.5					

For all the physico-chemical parameters analyzed, a report was made of the values obtained to the values imposed by the company standard. Regarding the amount of water found in Mozzarella cheese, we can specify that the average value obtained was 49.17 ± 1.28%. The firm standard imposes a maximum value of 52.00%, and the coefficient of variation calculated in this case showed a small value (V=6.36%), which indicates a good homogeneity within the studied group (Table 5).

Table 5 Physico-chemical indicators calculated for Mozzarella cheese

Specification	**Company standard**	**n**	**Statistical estimators**			
			$\bar{X} \pm s_{\bar{X}}$	**CV%**	**Min.**	**Max.**
Water (%)	max. 52	6	49.17±1.28	6.36	44.00	52.00
Dry substance (%)	min. 48	6	50.83±1.28	6.15	48.00	56.00
Protein (%)	min. 16	6	19.17±0.60	7.68	17.00	21.00
Fat / dry matter (%)	17-23	6	21.51±1.56	7.27	18.80	22.86
NaCl (%)	max. 3,5	6	2.88±0.17	14.78	2.40	3.50

For the dry matter content, the average value obtained was 50.83±1.28%, 2.83% higher than the minimum required by the standard. The calculated coefficient of variation was 6.15%, which indicates a good homogeneity. The values obtained for the amount of protein in Mozzarella cheese ranged from 17.00 to 21.00%, the average value calculated for this parameter being 19.17±0.60%. The homogeneity obtained in the case of the amount of protein is a good one, fact demonstrated by the calculated coefficient of variation, which has a value of 7.68%. Regarding the fat content of Mozzarella cheese, the company standard imposes values between 17.00% and 23.00%, the average calculated by us falling within these limits. As a result of determining the sodium chloride content of Mozzarella cheese, an average value of 2.88±0.17% was obtained, the minimum value being 2.40% and the maximum value 3.50%. Considering the fact that the differences obtained between the minimum and maximum value were quite large, the studied group presented an average homogeneity, the value of the coefficient of variation being 14.78%. The microbiological examination showed that Salmonella was not found to be present in any of the samples of Mozzarella cheese analyzed (Table 6).

Table 6 Microbiological characteristics of Mozzarella cheese

Sample	Salmonella/25g		Coliform bacteria ufc/g		Escherichia coli ufc/g		Coagulase-positive staphylococci ufc/g	
	Values obtained	Permitted limits	Values obtained	Permitted limits	Values obtained	Permitted limits	Values obtained	Permitted limits
1	absent	absent	750	10000	absent	1000	<10	100
2	absent		240		<10		<10	
3	absent		750		absent		<10	
4	absent		800		<10		<10	
5	absent		800		<10		<10	

Regarding the coliform bacteria, Escherichia coli, but also the coagulase-positive staphylococci, the average values obtained as a result of performing the microbiological examination were within the limits established by the legislation in force. At the end of the evaluation of the quality of Mozzarella cheese, from a microbiological point of view, it was found that this product corresponds in terms of quality, so all 4 analyzed microbiological parameters were below the maximum allowed limit.

CONCLUSIONS

During this research on the quality parameters of Mozzarella cheese, we can formulate some conclusions, namely: from an organoleptic point of view the raw milk used in the manufacture of Mozzarella cheese was evaluated with a good rating, obtaining an average total score of 15.2 points, with some minor defects.

Regarding the physico-chemical quality parameters analyzed for the raw material milk, small variations were registered, but the calculated averages were within the limits provided by the quality standard of the company.

The values obtained for the number of somatic cells and the total number of germs in the case of the analyzed milk samples were lower than the maximum values allowed by the legislation in force, which denotes a microbiologically appropriate milk. As a result of the organoleptic examination on Mozzarella cheese, the total average score obtained was 15.5 points, a score that categorizes this product as a good one. Thus, the organoleptic examination identified a homogeneous, fine, white-yellow paste with a pleasant smell and taste, characteristic of this variety of cheese.

All values obtained in the case of the chemical properties of Mozzarella cheese were within the limits imposed by the unit standard.

At the end of the evaluation of the quality of Mozzarella cheese, from a microbiological point of view, we can say that this product corresponds from a qualitative point of view, so all the analyzed microbiological parameters were below the maximum allowed limit.

The final conclusion of our study was that Mozzarella cheese meets all quality physicochemical and microbiological parameters, but instead was classified only as "good" against the background of organoleptic deficiencies, especially taste.

It is vehemently stated that both the raw milk and the Mozzarella cheese correspond from a sanitary-veterinary point of view, the food safety parameters being respected.

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THE IMPACT OF THE USE OF CANDIED LINGONBERRIES ON THE PHYSICAL-CHEMICAL, MICROBIOLOGICAL CHARACTERISTICS AND ANTIOXIDANT PROPERTIES OF CHEESE CREAM

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Abstract

Cheese creamis a category of dairy product highly appreciated by people of all ages, due both to its sensory characteristics and very pleasant texture, as well as its nutritional properties.Also, the lingonberries, both fresh and candied are considered very valuable because of their antioxidant and nutritional properties. The aim of this studyis to assess the impact of addition of candied lingonberries, in different proportions (10%, 15%, 20%, 25%),on the physical-chemical, microbiological characteristics and antioxidant properties of cheese cream. The experimental results of this research showed a decrease in themoister content and titratable acidity of the cream cheeseswith increasing of the proportion of fruit addeddue to the replacement of fresh cream cheese with candied fruit, whose acidity is lower. Further the total soluble solids content of the samples increased with the amount of added fruit. The total antioxidant activity and total polyphenol content of the cheese cream have been significantly improvedby using the candied lingonberries in the cheese cream formula.In the same time, was found that the microbial load of cheese creams with candied lingonberries decreased with increasing the amount of fruit added.Thus, the addition of candied lingonberries in the cream cheese formula resulted in a change of all the analyzed parameters and could be recommended as natural antioxidants in this type of dairy product.

Key words*: cheese cream, candied lingonberries, total antioxidant activity, total polyphenol content.*

INTRODUCTION

Dairy products represent on average 25-30% of a person's daily food intake (Richmond, 2007). Both milk and dairy products obtained in production units which are in accordance with food safety regulations,represent foods rich in nutrients such as: oleic acid, conjugated linoleic acid, omega-3 fatty acids, vitamins, micro and macro elements and not least in antioxidants (Savu et al., 2002, Mitrea et al., 2003; Saxelin et al., 2003; Petcu, 2006; Oprea et al., 2019). Antioxidants are a class of chemicals considered responsible for neutralizing and eliminating free radicals produced in the human body (Yazdanparast and Ardestani, 2007). Consumer preference for packaged food containing natural antioxidants has grown significantly globally (Vișoescu et al, 2015). For this, obtaining food products with the addition of natural antioxidants has become an important concern of researchers and manufacturers in the field (Santillo, 2009; Predescu et al., 2016). Antioxidant properties of milk and dairy products is attributed to their content in cysteine, vitamins A, E, carotenoids, catalase and glutathione peroxidase, superoxide dismutase, enzyme systems and equol, which it is a polyphenolic metabolite of daidzein (Usta and Yilmaz-Ersan, 2013; Mustonen et al., 2009). There are several researches which present the use of fruits in dairy products in order to

improve their nutritional value and antioxidant capacity. It was demonstrated that the use of wine grape pomace it is useful to enhance the antioxidant properties of yogurt and ice cream (Tseng and Zhao, 2013; Hwang, 2009). In another study, the use of grape pomace powder in fortifying semi-hard cheese was reported (Marchiani et al., 2015). In view of the above, we have proposed in this study to use candied lingonberries as a potential source of improving the nutritional properties of cream cheese. This is a soft cheese, characterized by a slightly acidic tasting and diacetyl flavour. It is processed by the coagulation of milk mixed with cream and acidified by the addition of starter cultures. Lingonberries (*Vaccinumvitis-idaea*) are one of the most valuable fruits, distinguished by their pleasant sensory properties, antibacterial and antifungal properties, but also by the remarkable content in bioactive compounds such as folate, potassium and soluble fiber. The antioxidant properties of lingonberries are attributed their content in phenolic compounds, vitamin C (Samad et al., 2014; Paredes-López et al., 2010; Vyas et al, 2013). In some studies conducted before, it was reported that 28 phenolic compounds were identified, among which anthocyanidins, catechins together with their glycosides, flavonols, and different caffeoyl and ferulic acid conjugates, respectively (Ek et al., 2006; Hajazimi et al., 2016; Tian et al., 2017; Antolak et al., 2017). Along with polyphenols, the *Vaccinumvitis-idaea*fruits also contain antioxidant compounds such as organic acids and vitamins (A, B1, B2, B3 and C) (Dróżdż et al., 2018).The aim of the present research was to evaluate the impact of candied lingonberries on physical-chemical, microbiological and antioxidant properties of cream cheese.The moisture content, titratable acidity,total phenolic compounds, total antioxidant activity and microbiological properties werecarried out for each cream cheese with candied lingonberries sample.

MATERIALS AND METHODS

The cheese cream was prepared using the classical method described in introduction. Then the cream cheese was divided into fiveequal portions, in four of them wereadded candied lingonberries (purchased from a local supermarket) in following proportion: 10% (CCrL10), 15% (CCrL15), 20% (CCrL20), 25%(CCrL25). After preparationall cheese cream samples, were packed in closed glass containers and kept in the refrigerator,at 2^0Cuntil the experimental analyzes were performed.All samples were analyzedin terms of physical-chemical characteristics, microbiological properties, -total polyphenols content and total antioxidant activity. All determined parameters of the cream cheese with lingonberries were analyzed by comparison with those of simple cheese cream (CCr) and candied lingonberries (CL)used as a control.

Moisture fraction was performed by drying in the oven using the reference method for the determination of the total solids content of cheese and processed cheese ISO 5534|IDF 4:2004. Determination of cheese samples titratable acidity (expressed in °Thorner) was conducted according whit SR ISO 6092:2008. Titratable acidity (% malic acid) of lingonberries was performed according with AOAC Official Method 942.15. Total soluble solids content (°Brix) were measured with an ABBE refractometer (ORT 1RS, KERN & SOHN GmbH, Germany) - in an aqueous extract(1:1, m/V) - (ISO 2173: 2003).Bacteriological analysis - was performed according to national standards ISO 4831-92/2006 - for coliform bacteria, SR ISO 7251-96/2005, for *E. coli*, SR EN ISO 6579-2003, *Salmonella*, SR EN ISO 6888-2002, for *Staphylococcus aureus* and SR ISO 7954-2001, for yeasts and molds (thermoscientific.com/microbiology, 2013).The evaluation of total polyphenolic content was performed by Folin-Ciocalten method (Folin and Ciocâlteu, 1927; Singleton et al., 1999). The method is based on the reducing properties of polyphenols compared to hexavalent molybdenum in polyphosphomolybdate contained in Folin-Ciocâlteu reagent. Two g of cheese cream were mixed with 20 mL of 70% methanol solution. After two hours,0.5mL from each prepared solution was mixed with 2.5mL of Folin-Ciocâlteu reagent diluted 1:10 and 2 mL of a 7.5% sodium carbonate solution.The mixtures were incubated atdark at room temperature for 30 minutes and then the absorbance of the reaction mixture was read at

750nmwavelengths using a UV–VIS spectrophotometer (SPECORD 205, Analytic Jena). The concentration in polyphenols wasexpressed as mg gallic acid equivalents per 100 g of cheese cream sample (mg GAE/100 g sample). In order to evaluate the total antioxidant capacity of the samples, the CUPRAC method was used (Özyürek et al. 2011). The method is based on reduction of the copper-neocuproine complex in the presence of ammonium acetate with the formation of the copper-neocuproine complex [Cu(Nc)2]+, which is a yellow compound, with a maximum absorption at 450 nm wavelengths. As reference substance was used TROLOX (6-hydroxy-2,5,7,8-tetramethylchromate-2-carboxylic acid). To perform the analysis mix 1mL of copper solution with 1mL of alcoholic ligand solution, 1mL of acetate buffer and 1.1mL of sample and shake well. After 30 minutes at rest in the dark, the absorbance of the blank at 450nm is determined. The molar absorbance coefficient for TROLOX in the CUPRAC method is $\varepsilon = 1.67$ x104 L x mol-1x cm^{-1}. The results can be expressed in mmol Trolox / 100 g sample. All the results are presented as mean of four determinations ± standard deviation (OriginPro 8.5).

RESULTS AND DISCUSSIONS

The physical-chemical characteristics as moisture content (Moisture), titratable acidity (TA) and total soluble solids content (TSS) of samples experimentally analyzed are presented in Table 1.

Table 1 Physical-chemical characteristics of samples determined experimentally

Sample	Moisture[%]	TA	TSS[Brix]
CCr	60,81±0.174	169±0.256[°T]	3±0.348
CL	8,62±0.151	1,5±0.238[%]	60±0.389
CCrL10	54,30±0.161	158±0.238[°T]	6,4±0.419
CCrL15	51,14±0.132	146±0.238[°T]	8,6±0.411
CCrL20	47,66±0.143	131±0.238[°T]	11±0.259
CCrL25	44,84±0.133	121±0.226[°T]	12,8±0.419

The moisture content of cheese cream with lingonberries was in the range of 54,30% (CCrL10) and 44,84% (CCrL25) and decreased with increasing concentration of added fruit as a result of the replacement of cheese cream with candied lingonberries. The highest moisture was registered in case of simple cheese cream (CCr) and the smallest level in candied lingonberries (CL). Taking into account the mentioned results, it can be stated that the addition of candied fruits in cream cheese can be considered responsible for the lower water content of samples with fruits.

As expected, the *titratable acidity of the cream cheeses with candied fruits*was lower than of simple cheese cream and is due to the candied lingonberries from their composition. The candied lingonberries used in cream cheese formula are obtained by osmotic dehydration of fresh fruit in a concentrated sugar solution. During the osmosis process some of the fresh fruit acidsare removed together with water causing a decrease in the acidity of the candied fruits (Chavan, 2012). The highest titratable acidity of the cheese samples with fruits was determined for CCr10 (158^0T) and the lowest value for CCrL25 (121^0T).The experimental results obtained in the case of humidity and titratable acidity for all the analyzed samples are in accordance with those reported in the literature (Perveenet al., 2011; Phadungath C., 2005) and regulated in the national and international standards regarding cream cheese.

Contrariwise the presence of candied fruits in the composition of cheese creams led to an increased–of total soluble solidscontent of the samples directly proportional to the amount of candied lingonberries added from 6.4 Brix in CCrL 10 sample to 12.8 Brix in CCrL 25 sample.The increase of the sugar content in the cheese cream with candied fruits, it can be considered that also benefited from an improvement of their sensory properties.

Bacteriological analysis of cheese cream with candied lingonberries

The values determined experimentally for each of the isolated germs are presented in Table 2.

Table 2 Microbiological load of the analyzed product

Germs	CCr	CL	CCr L10	CCr L15	CCr L20	CCr L25
Coliforms/g	89	0	83	79	72	67
E. coli/g	10	0	8	7	5	3
Salmonella/g	0	0	0	0	0	0
S. aureus/ g	9	0	7	6	6	4
Yeasts and molds/g	81	21	79	75	71	64

The number of coliform bacteria determined experimentally was below the maximum limit imposed by the legislation, for all samples analyzed. The same observation was made for *Escherichia coli*. As expected, the absence of these microorganisms in candied lingonberries led to the reduction of coliforms number in the cream cheese samples in which they were used. This decrease was more advanced as the amount of CCr substituted with CL was higher and varied in the range (7-25%), the lowest value being registered in CCrL25. *Salmonella* was absent in 25 grams of product in all samples analyzed.

The experimentally determined values for the number of positive coagulase staphylococci indicate a compliance with the maximum limit allowed by law for this parameter in the case of all samples of cream cheese and their absence in the candied fruit. Similar to the other microbiological parameters analyzed, the increase in the proportion of fruit added to the composition of the cream cheese had the effect of decreasing the number of staphylococci in these samples. In terms of yeasts and molds number, the obtained results revealsa similarity to those obtained in case of other microorganisms analyzed. Simple cheese cream registered the highest fungal load, and candied lingonberries the lowest load. The decrease in the number of yeasts and molds in the cheese cream with candied lingonberriescould be attributed tothe increase in the percentage of added fruit.This can be explained by the replacement of cream cheese with candied fruit whose load in yeasts and molds is lower than that determined in fresh cream cheese.

Total antioxidant capacity and total polyphenol content

Nowadays there are not many studies regarding the antioxidant capacity of cheeses but in the last period has registered an increase in interest of researchers and producers in this field(Revilla et al., 2016). It was demonstrated that total antioxidant capacity is significantly influenced by the technological process by which the cheese is made(Lucas et al., 2006b). Alsoit is mainly attributed to the fat-soluble vitamins of cheese (Lucas et al., 2006b, 2008b)

Total antioxidant capacity and the total polyphenol content of the samples of cream cheese and candied lingonberries are presented in Table 3.

Table 3. Total antioxidant capacity and the total polyphenol content of the samples

Sample	Total antioxidant capacity (mM TE/g)	Total polyphenol content(mg GAE/g)
CCr	4,399±0.033	n.d.
CL	11,933±0.045	254,704±0.012
CCrL10	4,906±0.033	92,382±0.041
CCrL15	5,749±0.055	116,703±0.112
CCrL20	6,056±0.140	143,232 ±0.032
CCrL25	7,635±0.051	178,411 ±0.037

n.d.- undetectable

Total antioxidant capacity

The experimental values obtained in the case of candied lingonberries were similar with those reported in literature (Dróżdż et al., 2018) and significantly higher than those determined in the case of than all other samples, due to the much higher content in other bioactive compounds with antioxidant activity. The lowest antioxidant activity was determined in the case of CCr (4,399 mM TE/g) and is correlated with literature data (Revilla et al., 2016). The highest antioxidant capacity was registered for CL (11,933mM TE/g) and was 63% higher than that of CCr. Also, the values obtained for antioxidant activity of lingonberries are in accordance with those obtained by other researchers (Dróżdż et al., 2018). As expected this led to increase of this parameter incream cheeses with candied lingonberries up to 27% in the case of CCrL25, Increasing the antioxidant capacity of cheese creams with added fruit was on average 17%.

Total polyphenol content

Cheeses are poor in phenolic compounds and have a low antioxidant activity (Han et al., 2011b). This is due to the fact that the interaction of phenolic compounds and proteins is often influenced by the pH, molar ratio, and molecular properties of the polyphenols (Gad and El-Salam, 2010).

The polyphenol content of the samples determined experimental, reveals that the highest values was obtained in case of candied lingonberries (11,933 mg GAE/g) in according with those from literature (Ozola and Kampuse, 2018)and registered a dynamic similar to those recorded for antioxidant activity. The results obtained for the cream cheese samples were on

average 2 times lower than that of the candied lingonberries. The highest content in polyphenols of cheese cream with candied fruits was determined in CCrL25 (178,411mg GAE/g). It must to be noted that in the case of CCr the polyphenol content was not detected. The addition of candied fruit to the cheese-making recipe resulted in significant changes in total polyphenols of these samples. The increase rate of this parameter was on average 18% and can be attributed to the increase of the amount of CL added.

CONCLUSIONS

Results obtained in the present research reveal that the addition of the candied lingonberries to cheese creamincreases significantly the antioxidant properties and total polyphenol content of this. The moisture content, titratable acidity and total soluble solids content are affected by the addition of candied lingonberries in cheese cream formula. The microbiological load of samples was within the limits of the legislation in force. Overall results suggested that the use of candied lingonberries in cream cheese formula can be an effective option for improving the antioxidants properties and to enhance the bioactive compounds of dairy products.

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MEAT CHARACTERISTICS OF WILD PHEASANT VERSUS FARMED PHEASANT

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Abstract

The pheasant is a widespread bird in most European countries, especially raised to be hunted. The pheasant can be raised in captivity and released before hunting. Nutritionists recommend an increased consumption of pheasant meat due to its nutritional qualities. This meat contains small amounts of fat; it is rich in vitamins, minerals, calcium and has a low percentage of cholesterol and carbohydrates. The pheasant meat also contains B vitamins, iron, zinc, copper, magnesium, phosphorus. It is a good aliment for people who suffer from thyroid disease, due to the large amount of iodine. It is considered that the pheasant reaches culinary maturity at the age of 6 months, at this age reaching a weight of 1.5 kg. According to studies, the slaughter yield of pheasants is 60.9% to 67.7%. Regarding body sizes, in the case of wild pheasant, the size of chest was bigger compared with the thighs. In terms of meat quality, depending on rearing conditions, were reported slightly higher values for the amount of fat in meat in farmed pheasants compared to those in the wild. Compared to broilers, the amount of protein in the dry matter of meat extracted from the breast was much higher, as it follows: 5.16% in females and 9.95% in males.

Key words*: carcass, meat quality, pheasant*.

INTRODUCTION

Pheasant is widespread in most European countries, where birds hunting represent a tradition. It is still practiced today, taking many forms and having a high economic and social effect on rural areas (Lecocq, 2004; Pogurschi et al., 2018). Following the intensification of agriculture, in recent decades the number of pheasants raised in captivity has increased and they are released before hunting, this being a common practice in European countries (Draycott et al., 2002). In Romania pheasants are divided into two large classes: game pheasants and pet pheasants (Dronca et al., 2008). Pheasants are part of Galliformes Order, and Phasianidae Family.

The pheasant have origins in Asia and was introduced to Romania in order to be raised in freedom for hunting or to be kept in aviaries as a species of decoration. Later, the pheasant began to be raised in captivity.

In the fauna of the Asian continent, starting from the eastern shore of the Black Sea to the Far East, from a taxonomic point of view, there are 42 subspecies, but only three subspecies are important for our country:

- Common pheasant – *Phasianus colchicus colchicus* Linnaeus, 1758;
- Mongolian pheasant – *Phasianus colchicus mongolicus* von Brandt, 1844;
- Collared pheasant – *Phasianus colchicus torquatus* Gmelin, JF, 1789 (https://avibase.bsc-eoc.org/).

The oldest and best known in Europe is *Phasianus colchicus colchicus.* The male is called the pheasant rooster, and the female pheasant or pheasant hen. The colour of the male is more intense and more attractive than that of the female. It is native to the Caucasus region, from where it was brought to Europe by Greeks and Romans. It has a smaller body than the collared and Mongolian pheasant and unlike them it does not have a white collar. The general colour is darker than the two mentioned above; metallic dark green with a shade of blue on the head and neck, while the whole body is dark red, with greenish brown wings, and a tail with closed transverse stripes. It is less prolific than the others; instead it has a greater resistance,

requiring less care from people, to stay in the field (Cotta et al., 2008).

The pheasant is an active, diurnal bird, looking for its food in the environment. Its main food source are vegetables (various seeds of wild plants, cereal crops, with small fruits or their seeds, leaves, stems, tubers), as well as animals (larvae, caterpillars, butterflies, Colorado potato beetle, field mice and other small rodents that fall into their claws), a ratio that changes depending on weather and season conditions.

Pheasants are polygamous birds. Males choose a high place in their territory and begin to sing until they attract between 4-6 females who begin to build their nest. The females lay eggs, depending on the climatic conditions of the area, from the end of April to the beginning of May. They produce 7 to 18 eggs, which they hatch for 23-27 days. The average size of the egg is 4.5 x 3.6 cm. The chicks become independent 12-14 days after hatching. At the age of 9 months, pheasants reach sexual maturity. Body weight in females is 600 – 900 g, and in males between 1 – 1.5 kg. The pheasant carcass has a triangular shape, the chest and waist are narrow, and the back and legs are thin, being different from the carcasses of other birds (Figure 1).

Figure 1. The pheasant carcass aspect (www.wildmeat.co.uk)

The colour of the meat is reddish with a mixture of blue. It is considered that the pheasant reaches culinary maturity at the age of 6 months, at this age reaching a weight of 1.5 kg.

Pheasant meat is tender, very juicy, tasty and nutritious. It is considered to be dietary and has low cholesterol content. The energy value is relatively low, 253.9 kcal per 100 g because it contains a small amount of fat (https://ro.blabto.com).

The presence of large amounts of B vitamins and the balance between protein and fat makes this meat beneficial for health and it has a wide range of minerals that fully meet the needs of the body. With such properties, pheasant meat is highly valued and is considered a delicacy.

MATERIALS AND METHODS

This article presents a review of several relevant literatures on pheasant species, biology, and carcass structure, chemical and physical characteristic of pheasant meat. The study can represent a base for further studies about pheasant meat characteristics, useful for human nutrition, health, food processing and safety.

RESULTS AND DISCUSSIONS

In the literature, pheasant meat is presented as having a high content of proteins and a low fat content, which makes it nutritionally superior to chicken. Also, the chest muscles have a higher nutritional value than the haunch muscles (Straková et al., 2012). It was observed that there are differences in carcass structure and chemical composition of muscle tissues (Tucak et al., 2008).

The chemical composition of pheasant meat and chicken meat is presented in Table 1.

Table 1. Chemical composition of chicken and pheasant meat

Chemical Composition	Species	
	Pheasan	Chicken
Water	72.05%	72-74%
Protein	22.15%	18-22%
Lipid	0.98%	3%
Mineral salts	1.16%	0.9-1.2%

According to Franco et al. (2013), the dry protein content was much higher compared to meat from broiler chickens. The breast of pheasant females has a 5.16% higher protein content than the broiler chicken breast, and the pheasants meal can exceed the protein content compared to the broiler chickens by 9.95%.

Following the studies performed by Tucak et al. (2008), he made a comparison between hunting pheasants and breeding pheasants, by sex, analyzing both the physical aspect of pheasants and the chemical composition of the chest and leg muscles.

Table 2 shows the weight and the main parts of the pheasant's body, presented as a percentage. The study was performed on a number of 10 pheasants in each group. The male hunter pheasant weighed the most. The differences between these group are significant, the largest haunch and the largest chest being also recorded in the male hunting pheasant.

Table 2. The weight and main parts of the pheasant (Tucak et al., 2008)

Specification	Farmed pheasant		Wild pheasant	
	Male	Female	Male	Female
Weight (g)	1144.20±197.58	969.80±157.42	1232.4±147.36	918.80±89.88
Weight without feathers (%)	95.30±1.09	95.61±2.37	95.08±2.93	95.68±1.69
Breasts (%)	26.74±3.23	26.88±2.84	29.89±3.14	31.41±1.38
Backs (%)	19.96±2.79	19.43±1.72	15.55±2.47	16.70±2.28
Tights (%)	20.08±1.37	20.30±1.35	22.52±1.95	21.51±1.00
Wings (%)	8.15±0.56	7.83±057	8.78±1.14	8.77±0.76
Liver and heart (%)	3.14±0.57	3.44±0.60	2.77±0.24	2.72±0.73
Head and legs (%)	7.46±0.88	6.25±0.73	6.51±0.60	6.10±0.30
Subcutaneous skin and fatty tissue (%)	6.54±1.66	13.98±5.02	4.58±1.13	5.35±1.47
Bones (%)	12.05±3.54	95.61±2.37	15.50±5.62	17.20±3.89

As with other hunting birds, female pheasants have a body weight with 15-40% smaller than male pheasants, of the same age and growing conditions (Richter et al., 1992; Tucak et al., 2008; Golze, 2010). Also, race, age and diet, can have a significant influence on body weight (Tucak et al., 2008; Golze, 2010).

After sacrificing the birds, values of 875.4 ± 86.6 g and 555.3 ± 88.8 g were obtained for the eviscerated carcasses of male and female pheasants from hunting, which represents 65.5 ± 1.8 %, respectively 60.9 ± 3.7 % of the mass of whole birds. Other studies have shown better carcass yield results, such as 67.7 % and 64.5 % according to Richter et al. (1992), and 66 - 68 % according others (Golze, 2010).

For the male pheasant, the edible viscera (the lower part of the lower stomach, heart, liver), chest and haunch are 75.0 ± 9.9 g, 270.8 ± 27.7 g, 264.0 ± 22.4 g, and for the female pheasant it is 70.4 ± 8.5 g, 169.2± 28.7 g and 166.8 ± 23.9 g (Tucak et al., 2008; Richter et al., 1992). Also, the chest and thigh muscles represent 61.2 ± 2.8 % for females versus 61.5 ± 2.5 % for males in the mass of the eviscerated carcass. A number of studies indicate that gender does not influence the percentage of carcass muscle weight (Kuzniacka et al. 2007; Tucak et al. 2008; Golze 2010), while the free range versus pheasantry system will influence the ratio between the thighs and chest (Golze, 2010).

Table 3. Chemical composition of chest and thights muscles (Tucak et al., 2008)

Specification	Farmed pheasants				Wild pheasants			
	Male		Female		Male		Female	
	Breast	Tights	Breast	Tights	Breast	Tights	Breast	Tights
Water (%)	76.61	71.58	71.77	71.42	72.33	74.50	72.43	73.65
Lipides (%)	1.15	6.62	1.69	6.81	0.96	2.11	1.14	2.92
Proteins (%)	25.11	20.71	25.38	20.63	25.57	22.22	25.53	22.32
Mineral salts (%)	1.16	1.09	1.15	1.06	1.14	1.15	1.12	1.11
Ca (%)	0.019	0.021	0.018	0.020	0.032	0.039	0.029	0.039
P (%)	0.219	0.205	0.230	0.197	0.239	0.209	0.228	0.208
Energy value KJ/100g	485.66	621.91	512.23	629.20	487.02	472.92	489.45	506.01

In the experiment made by Tucak et al., in 2008, it was reported higher lipid values in farmed pheasants compared to hunting pheasants, the highest amount of lipids being recorded in the leg muscles in farmed pheasant (Table 3). The protein content, the energy value of the meat and

the mineral salts content having similar values in both groups of birds studied.

On the other hand, according to the study by Straková et al. (2011), protein and fat levels were 93.72% and 2.95%, respectively, compared to the dry matter of the pheasant breast, while in the haunch there were found concentrations of 78.18% for protein and 16.36% for fat.

Also the same parameters were analyzed in fresh meat where there were found the following proportions: for the breast 20.73% protein and 0.13% fat, while in the meat from the haunch were found 25.66% for protein and 3.9% for fat (Severin et al., 2006; Hofbauer et al., 2010; Franco & Lorenzo, 2013).

Nuernberg et al. (2011), noted that both the wild pheasant and the farmed pheasant accumulate more fat in the thigh muscles than in the chest muscles. It also should be noted that the chemical composition of poultry meat in general, and especially of pheasant, is not a constant attribute (Ciobanu et al., 2019). It is affected by season (Smankó et al., 2007), gender (Piaskowska et al., 2015; Purchas et al., 2010), age (Dannenberger et al., 2013) and muscle type (Razmaité et al., 2015).

There are not many information in the literature on the physical characteristics of pheasant meat, such as colour, pH, water retention capacity (Fletcher, 1999; Bendall, 1988; Mach et al., 2008).

Pheasant meat has a slightly acidic pH, between 5.66 - 6.03 values and the water retention capacity varies between 1% and 3% (Hofbauer et al., 2010).

In Table 4 are presented, according to Hofbauer et al. (2010), the variation of pH and water-holding capacity (drip loss) in both chest and thigh muscles for hunting pheasants and farmed pheasants. Water-holding capacity has implications in fresh meat shelflife and in meat processing technology. It is influenced by meat pH.

Table 4. Physical characteristics of the chest and thigh muscles (Hofbauer et al., 2010)

Specification	Farmed pheasant		Wild pheasant	
	Breast	Tights	Breast	Tights
pH	5.66±0.08	6.03±0.20	5.55±0.16	5.93±0.25
Water-holding capacity (%)	3.03±0.57	1.49±0.36	2.19±1.37	1.00±0.56

In both studied groups of birds, the pH has a significant value, 0.4 units in the thigh muscles, similar to previous studies conducted by Richter et al., 1992; Kuzniacka et al., 2007; Paulsen et al., 2008.

In muscle composition, the pheasant breast is predominantly made of muscle fibres with rapid contraction or glycolytic metabolism (>70 %), while the thigh muscles are composed of muscle fibres with glycolytic-oxidative metabolism or other types of fibres with oxidative metabolism (Kissling, 1977). This is specific to all poultry, being an advantage of fast take-off in flight at the expense of long-haul flights (Pyörnilä et al., 1998).

Also, stress in the *prerigor mortis* period can greatly influence the structure of muscles rich in type II B fibres but, given the cited studies on pheasants, it is not clear how to assess the stress caused by hunting.

Water-holding capacity was higher in muscles with a lower pH (Hofmann, 2004).

In the farmed pheasant, the quality of the meat is influenced, as in the other birds, by species, breed, gender, age, food, maintenance condition and slaughter conditions (Marin et al., 2015).

The quality of wild pheasant meat, according to Cristescu (2004), can be influenced by a number of factors that can sometimes determine its total or partial elimination from consumption. The factors that influence the quality of game pheasant meat are: how the pheasant is shot, the removal of internal organs, the cooling of the body, transport and storage, as well as the diseases it suffers from before harvesting.

The wound must be protected from dirt and moisture, as it can become infected and develop microbial flora. At the same time, the wound must be protected from flies because from their eggs become worms, which can penetrate into the muscle tissues.

The quality of the meat is also influenced by the place where the bullet enters in the body of the pheasant. For example, the penetration of the bullet into the abdomen, produces in most cases,

the overflow of the intestinal contents in which various microbes are found that influence the quality of the meat and give it an unpleasant odour (Nesterov, 1969).
The cooling of the venison after the shooting has a significant importance for the quality of the meat, its preservation and use in human food. The time required to cool the pheasant body differs from ambient and seasonal temperatures. The cooling time in autumn is 4 - 5 h and in winter 3 - 4 h. It is very important to respect the cooling time because it can lead to burning of the meat, biochemical process of decomposition of substances in the muscles, damaging the meat, printing them an unpleasant, repulsive, sour smell.
To prevent the depreciation of pheasant meat, it is necessary to remove the intestines because the intestinal contents decompose easily, producing gases and bad-smelling substances, which are transmitted to the meat.
Transporting shot pheasants is the most difficult part. During transport, overlapping, crowded storage, wounding of the wound must be avoided. Packing in plastic bags should be avoided as there is a danger of quickly alteration due to the fact that complete ventilation and cooling cannot be ensured.
All these factors contribute to the quality of pheasant meat. Food quality is a concept associated to products requirements to comply with standards, specifications, and consumer expectations (Nicolae et al., 2016).

CONCLUSIONS

As shown above, pheasant meat is suitable for consumption because it can be considered dietary due to its low fat content and is extremely rich in protein, B vitamins and high mineral content with increased bioavailability and bioactivity. In terms of economic efficiency, the pheasant contributes to the development of areas where hunting is practiced.
The studies show a significant difference between farmed pheasant and wild pheasant, in term of lipid content. It has been found that the farmed pheasant contains a higher amount of fat in the thigh muscles than the wild pheasant. Instead, the content of protein and minerals shows similar values for both rearing systems.
Regarding the weight related aspects, the highest weight recorded in the body, breasts and tights muscles was in the male wild pheasant.

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ARSENIC CONTENT IN THE PARASITE-HOST SYSTEMS: *POMPHORHYNCHUS LAEVIS-ABRAMIS BRAMA* AND *ACANTHOCEPHALUS LUCII-ABRAMIS BRAMA*

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Abstract

During the ecological study of 31 specimens of freshwater bream (Abramis brama (Linnaeus, 1758)) from Danube River, by applying standard techniques for parasites, an infestation was found with two acanthocephalan species – Pomphorhynchus laevis and Acanthocephalus lucii. The content of arsenic in water, sediments, parasites, tissues and organs of Abramis brama (infected and uninfected) were establishedin the present study. P. laevis showed 42-170 times higher content of arsenic than its host tissues and organs. The content of arsenic in A. lucii was 37-205 times higher than the content of arsenic in its host tissues and organs. The highest concentration of arsenic in uninfected specimens of A. brama was found in liver (1.35±1.29 mg.kg^{-1}). The highest concentration of arsenic for the infected with P. laevis specimens of freshwater bream was established for muscles (0.68±0.08mg.kg^{-1}), while for the infected with A. lucii specimens of fish, the highest concentration of arsenic was established for skin (1.75±1.32 mg.kg^{-1}). A highly significant correlation ($p<0.01$) was fixed for the relationship between $C_{As/P.\ laevis}$–$C_{As/Muscles}$.

Key words*:Abramis brama;Acanthocephalus lucii; arsenic;Danube River;Pomphorhynchus laevis.*

INTRODUCTION

The concentration of heavy metals in fish tissues and their parasites was studied by many authors (Turčekova et al., 2002; Kirin et al., 2014; Brázová et al., 2015; etc.). It was established that certain parasites had high accumulation possibilities and thus they could be used as bioindicators of heavy metals (Sures et al., 1997; Tenora et al., 2000; Thielen et al., 2004). This study aims to present the results of examinations of arsenic contents in water, sediments, *Pomphorhynchus laevis*, *Acanthocephalus lucii*, skin, muscles and liver of infected and uninfectedwith acanthocephalans specimens of freshwater bream from the Bulgarian part of the Danube River.

MATERIALS AND METHODS

In 2016, water, sediments, fish and fish parasites werecollected and examined from the Lower Danube River (village of Vetren, Bulgaria; 44^0133'N, 27^0033'E, Figure 1).

A total of 16 uninfected and 15 infected with acantocephalan specimens of freshwater bream (*Abramis brama* (Linnaeus, 1758)) were collected and examined.

The fish samples were examined immediately after their capture for gastrointestinal parasites using standard techniques.

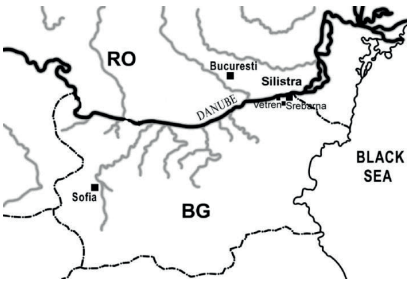

Figure 1. Danube River

The established acanthocephalan species were examined as temporary slides in ethanol-glycerin and identified (Petrochenko,1956; Bykhovskaya-Pavlovskaya, 1985). The ecological terms wereused and calculated (prevalence, mean intensity (MI) and mean abundance (MA), based on Bush et al. (1997). The dominant structure of the component helminth communities was determined based on

the prevalence (P%) as: accidental (P% < 10), component (P% < 20) and core (P% >20) species according to the criteria proposed by Kennedy (1993).

The freshwater bream (*Abramis brama* (Linnaeus, 1758)) specimens chosen for the examination of arsenic (As) content in this study were weighed (total weight of the fish ranging from 24to 291 g) and measured (total length ranging from 13 to 26.5 cm). Samples of muscles, skin and liver were collected from all specimens of *A. brama*. The freshwater bream is freshwater, brackish, benthopelagic, potamodromous fish species that inhabit a wide variety of lakes and large to medium-sized rivers (Fröse and Pauly, 2020). It is estimated as least concern species (LC=Least Concern; IUCN Red List Status) and is not included in the Red Data Book of the Republic of Bulgaria (Golemanski (Ed.), 2011). The samples of water, sediment, fish tissues, organs and parasites were analyzed for content of arsenic (As) by the Inductively Coupled Plasma (ICP) Spectrometry.

In order to determine the relative accumulation capability of the fish tissues and parasites in comparison with water and sediments, bioconcentrationfactors (BCF=[C host/parasites tissues]/[C water/sediments] were calculated (Sures et al., 1999). The bioconcentration factors were used for the estimation of trace metal pollution in the freshwater ecosystem through the examined fish and their parasites. The differences in concentration factors are discussed with respect to the bioavailability of arsenic from water and sediments. In order to determine the relative accumulation capability of the parasites and the host tissues, bioaccumulation factors (BAF=[C parasite]/[C host tissues]) were calculated. A linear correlation coefficient (Spearman's rank correlation coefficient, r_s) was determined to test the association between parasites and their host tissues and organs.

RESULTS AND DISCUSSIONS

A total of 31 specimens of freshwater bream (*Abramis brama* (Linnaeus, 1758)) were collected and examined for parasites from the Danube River. Helminth parasites were recorded in 15 specimens of *A. brama* (48.39 %). Five specimens from the examined freshwater breams were infected with the acantocephalan *Pomphorhynchus laevis* (Table 1). *P. laevis* develops with the participation of an intermediate host – *Gamarus pulex* (Amphipoda), (Petrochenko, 1956).

Ten specimens from the examined *A. brama* were infected with the acantocephalan *Acanthocephalus lucii* (Table 1).

The intermediate host of *A. lucii* is *Asellus aquaticus,* and definitive hosts are fish species of different families as Cyprinidae, Salmonidae, Percidae, Anguillidae and others(Kakacheva-Avramova, 1983). Both acantocephalan species are autogenic, matured in fish.

The component community of freshwater bream from Danube River is presented only of acanthocephalan specimens. In the component community of *Abramis brama* from the Danube River, *A. lucii* (P%=32.26) is core species, and *P. laevis* (P%=16.13) is component parasite species for the helminth communities of *A. brama* (Table 1).

Table 1. Ecological indices of the helminth parasites of *A. brama* from the Danube River (N – number of examined fish specimens, n – number of infected hosts, p – number of parasites, P% – prevalence, MA – mean abundance, MI – mean intensity)

Helminth species	N	n	p	P%	MA±SD	MI±SD	Range
Pomphorhynchus laevis (Zoega in Müller, 1776)	31	5	14	16.13	0.45±1.13	2.8±1.17	1-4
Acanhocephalus lucii (Müller, 1776)	31	10	19	32.26	0.61±1.04	1.9±0.94	1-4

Species richness in infracommunity of freshwater bream ranges from 0 to 1 species. Fifteen fishes (48.39%) were infected with one helminth species. The largest number of helminth specimens established in a single host specimen is 4. The average species richness (mean number of species for fish specimen) in the infracommunity of freshwater bream is 0.48±0.5 species.

The content of arsenic in samples of sediments was much higher than the content of arsenic in samples of water from the examined freshwater

ecosystem – Biotope Vetren on the Danube River. From the tissues and organs of uninfected specimens of fish, the highest content of arsenic was determined in samples of liver, followed by those of skin and muscles. The highest bioconcentration factor (BCF) was in the liver, followed by the one for the skin and muscles (Table 2).

Table 2. Content of arsenic (mg.kg^{-1}) and bioconcentration factor (BCF) determined for the content of arsenic in tissues and organs of uninfected *A. brama* and water and sediments

A.brama	Mean±SD	Relationships	BCF	Relationships	BCF
Liver	1.35±1.29	C_{Liver}/C_{water}	103.85	$C_{Liver}/C_{Sediments}$	0.235
Muscles	0.41±0.17	$C_{Muscles}/C_{water}$	31.54	$C_{Muscles}/C_{Sediments}$	0.071
Skin	0.46±0.16	C_{Skin}/C_{water}	35.38	$C_{Skin}/C_{Sediments}$	0.080
River Danube		Water (mg.l^{-1})	0.013	Sediments (mg.kg^{-1})	5.74

Data are present as mean±standard deviation (SD)

From the tissues and organs of freshwater breams infected with *P. laevis* the highest content of arsenic was determined in samples of muscles, followed by those of liver and skin. The acanthocephalan *P. laevis* showed higher content of arsenic than its host *A. brama*. The highest bioconcentration factor (BCF) was in *P. laevis,* followed by the one in the muscles, liver and skin (Table 3).

Table 3. Content of arsenic (mg.kg^{-1}) and bioconcentration factor (BCF) determined for the content of arsenic in tissues, organs of *A. brama*and its parasite *P. laevis* and water and sediments

A. brama/P. laevis	Mean±SD	Relationships	BCF	Relationships	BCF
Liver	0.39±0.10	C_{Liver}/C_{water}	30.00	$C_{Liver}/C_{Sediments}$	0.068
Muscles	0.68±0.08	$C_{Muscles}/C_{water}$	52.31	$C_{Muscles}/C_{Sediments}$	0.12
Skin	0.17±0.002	C_{Skin}/C_{water}	13.08	$C_{Skin}/C_{Sediments}$	0.03
P. laevis	29.01±0.97	$C_{P.\ laevis}/C_{water}$	2231.54	$C_{P.\ laevis}/C_{Sediments}$	5.06
River Danube		Water (mg.l^{-1})	0.013	Sediments (mg.kg^{-1})	5.74

Data are present as mean±standard deviation (SD)

From the tissues and organs of freshwater breams infected with *A. lucii* the highest content of arsenic was determined in the samples of skin, followed by those of liver and muscles. The acanthocephalan *A. lucii* showed significantly higher content of arsenic than its host *A. brama*. The highest bioconcentration factor (BCF) was for *A. lucii*, followed by the one in the skin, liver and muscles (Table 4). The highest content of arsenic in the liver was determined in samples of uninfected *A. brama* ($C_{As/Liver}$=1.35±1.29), followed by those of liver of *A. brama* infected with *A. lucii* ($C_{As/Liver}$=0.57±0.22) and infected with *P. laevis* ($C_{As/Liver}$=0.39±0.10). The highest content of arsenic in muscles was determined in samples of *A. brama* infected with *P. laevis* ($C_{As/Muscles}$=0.68±0.08), followed by those of muscles of uninfected *A. brama* ($C_{As/Muscles}$=0.41±0.17) and infected with *A. lucii* ($C_{As/Muscles}$=0.32±0.20). The highest content of arsenic in the skin was determined in samples of *A. brama* infected with *A. lucii* ($C_{As/Skin}$=1.75±1.32), followed by those of skin of uninfected *A. brama* ($C_{As/Skin}$=0.46±0.16) and infected with *P. laevis* ($C_{As/Skin}$=0.17±0.02).

Table 4. Content of arsenic (mg.kg^{-1}) and bioconcentration factor (BCF) determined for the content of arsenic in tissues, organs of *A. brama* and its parasite, *A. lucii*, and water and sediments

A. brama/ A. lucii	Mean±SD	Relationships	BCF	Relationships	BCF
Liver	0.57±0.22	C_{Liver}/C_{water}	43.85	$C_{Liver}/C_{Sediments}$	0.099
Muscles	0.32±0.20	$C_{Muscles}/C_{water}$	24.62	$C_{Muscles}/C_{Sediments}$	0.056
Skin	1.75±1.32	C_{Skin}/C_{water}	134.62	$C_{Skin}/C_{Sediments}$	0.305
A. lucii	65.78±0.97	$C_{A.\ lucii}/C_{water}$	5060.0	$C_{A.\ lucii}/C_{Sediments}$	11.46
River Danube		Water (mg.l^{-1})	0.013	Sediments (mg.kg^{-1})	5.74

Data are present as mean±standard deviation (SD)

The highest bioaccumulation factor (BAF) established for *P. laevis* was found inthe skin, followed by the one in the liver and muscles.

Regarding *A. lucii,* the highest bioaccumulation factor (BAF) was in the muscles, followed by the one in the liver and skin (Table 5).

Table 5. Bioaccumulation factors (BAF= [Cparasite]/[C host tissues]) of *P. laevis* and *A. lucii*

P. laevis/*A. brama*	BAF	*A. lucii* /*A. brama*	BAF
$C_{P.\ laevis}/C_{Liver}$	74.38	$C_{A.\ lucii}/C_{Liver}$	150.40
$C_{P.\ laevis}/C_{Muscles}$	42.61	$C_{A.\ lucii}/C_{Muscles}$	205.56
$C_{P.\ laevis}/C_{Skin}$	170.65	$C_{A.\ lucii}/C_{Skin}$	37.59

A linear correlation coefficient (Spearman's rank correlation coefficient, r_s) was determined to test the association between *P. laevis* and fish tissues and organs, and between *A. lucii* and fish tissues and organs. A highly significant correlation ($p<0.01$) was fixed for the relationship between $C_{As/P.\ laevis}$– $C_{As/Muscles}$.

In general, the content of arsenic in tissues and organs of infected and uninfected specimens of *A. brama* decreased in a different order. For the uninfected specimens of *A. brama,* the order is: $C_{As/Liver}>C_{As/Skin}>C_{As/Muscles}$, for the specimens infected with *P. laevis* the order is $C_{As/Muscles}>C_{As/Liver}>C_{As/Skin}$, and for the ones infected with *A. lucii* it is: $C_{As/Skin}>C_{As/Liver}>C_{As/Muscles}$. The values of the bioaccumulation factor for *P. laevis* were the highest for skin and ranged as follows: $BAF_{Skin}>BAF_{Liver}>BAF_{Muscles}$. The values of the bioaccumulation factor for *A. lucii* were the highest for muscles and ranged as follows: $BAF_{Muscles}>BAF_{Liver}>BAF_{Skin}$.

In the scientific papers, there are a relatively small number of researches regarding the arsenic content in *A. brama* from the Danube River.

For example, Jovičić et al. (2015) studied the concentrations of 11 elements in tissues and organs of four commercial fish species from the Danube River (Serbia). They reported that the arsenic concentrations were in muscles– 0.325±0.145 μg g^{-1} dry weight and in the liver – 7.522±4.008μg g^{-1} dry weight of *A. brama*. Zrnčić et al. (2013) studied the heavy metal contamination of fish in the Croatian part of the Danube River. In their study, the concentration of arsenic in muscles of *A. brama* was 0.035±0.024 microgram per dry weight.

Kirin et al. (2014) studied the concentration of Pb, Zn and Cu in muscles, liver, intestine and bones of *A. brama* and its parasite *Pomphorhynchus tereticollis* from the Danube River, Bulgaria. In their study, highly significant correlations were determined for *P. tereticollis* as a sensitive indicator of Pb, Cu and Zn.

The ability of *Acanthocephalus lucii* to accumulate heavy metals was studied mostly as a parasite of *Perca fluviatilis* (Sures et al., 1994a; Sures et al., 1997a; Turčeková et al., 2002; Jankovská et al., 2011; Jankovská et al., 2012).

In the scientific papers, there are studies on how two helminth species from the same fish host accumulate heavy metals and impact their concentrations in host tissues, or they compare to the concentrations in host tissues. Some of them refer to acanthocephalans and cestodes (Turčekova et al., 2002; Brázová et al., 2015) and some to acanthocephalans and nematodes (Sures et al., 1994b; Nachev et al. 2013). Our research did not reveal studies for two acanthocephalan species from the same fish host. The studies mentioned above were based on parasite species that were either in different localization in the host body or were in different development stages. In this study, the investigated species (*A. lucii* and *P. laevis*) were in the same localization and development stage, which is why it is not possible to make a comparison with them. The obtained results for the differences in accumulation of arsenic for *P. laevis* and *A. lucii* from the same fish host (*A. brama*) might be explained with the differences in biology and selective capabilities to accumulate heavy metals between those two acanthocephalan species.

CONCLUSIONS

The highly significant correlation ($p<0.01$) fixed for the relationship between $C_{As/P.\ laevis}$– $C_{As/Muscles}$, determines *P. laevis* as a sensitive

indicator for arsenic. The obtained results allow the two acanthocephalan species (*A. lucii* and *P. laevis*) to be proposed as useful for the evaluation of arsenic exposure in freshwater ecosystems. However, this should be confirmed with additional studies.

The results in the present study revealed that attention should be drawn not only to differences in bioaccumulation potential between the different parasite classes but also between the different species of the same parasite class (class *Acanthocephala*).

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NEW DATA ON PARASITES AND PARASITE COMMUNITIES OF *ALBURNUS ALBURNUS* (LINNAEUS, 1758) FROM THE DANUBE RIVER

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Abstract

In the spring, summer and autumn of 2019, ecoparasitological studies were conducted on 165 specimens of Alburnus alburnus (Linnaeus, 1758) from the Danube River (Koshava, Kudelin and Novo selo villages, Vidin region). The species diversity of parasites of the bleak was determined. Seven species of parasites were identified: 4 species of Trematoda (Nicolla skrjabini (Iwanitzky, 1928), Allocreadium isoporum (Looss, 1894), Posthodiplostomum cuticola (von Nordmann, 1832), Sphaerostomum bramae (Müller, 1776)), one species of Cestoda (Neogryporhynchus cheilancristrotus (Wedl, 1855), larvae), one species of Acanthocephala (Pomphorhynchus laevis (Müller, 1776)) and one species of Nematoda (Contracaecum sp., larvae). In the spring the dominant species was the trematode A. isoporum, detected in 9 specimens of A. alburnus from the Koshava biotope. The dominant parasite species in summer and autumn was the trematode P. cuticola. A. alburnus is a new host for S. bramae and N. cheilancristrotus, from the studied river ecosystem. The structure of the parasite communities of the bleak from the investigated section of the freshwater ecosystem of the Danube River was presented.

Key words*: Alburnus alburnus, Bulgaria, Danube River, parasites, parasite communities.*

INTRODUCTION

The Danube River crosses Europe in a west-east direction (Keckeis and Schiemer, 2002) for 2,857 km. The river reaches a maximum width of 1.5 km (https://www.icpdr.org). The Danube basin covers the territory of nineteen European countries and can be divided into three parts: Upper, Middle and Lower Danube (Hock and Kovdcs, 1987). The Bulgarian section of the Danube River covers 470 km from the Lower Danube (extending between 845 and 375 km of the river). The Lower Danube, together with the Danube Delta, is distinguished by the highest diversity of fish (Polačik et al., 2008). The ichthyofauna of the Danube River includes fish species of different families like Cyprinidae, Siluridae, Esocidae, Percidae, Acipenseridae, Salmonidae (Juhásová et al., 2019). According to Polacik et al. (2008) in Bulgarian territory of the Danube River with the highest prevalence (more than 88 %) are the following three fish species: *Neogobius fluviatilis*, *Alburnus alburnus* and *Neogobius kessleri*. With the highest abundances are *N. fluviatilis* and *A. alburnus*, as well as *Neogobius melanostomus*. The high species richness of fish in the Danube River is the reason for the great abundance of fish parasites (Juhásová et al., 2019).

Parasitism is the most common way of life. Each organism is a host of at least one type of parasite. Parasites can be established in different trophic levels in different food webs. In this way, they provide information on the location of their hosts in the food web and changes in the ecosystems. Parasites, as well as free-living organisms, respond to environmental changes and can be used as indicators of the state of ecosystems (Marcogliese, 2004; 2005).

Parasites are the subject of increasing research on their bioindicator role and their ability to show changes occurring in the environment as a result of various impacts (Sures, 2001). Investigations on parasites of fish from the Danube River are carried out by multiple authors (Cojocaru, 2003; Cojocaru 2007; Cakić et al., 2008; Cojocaru 2009; Nachev and Sures, 2009; Đikanović et al., 2013; Kirin et al., 2013; Kvach et al., 2013; Kirin et al., 2014; Đikanović et al., 2015; Chunchukova et al., 2016; Kvach et al., 2016; Kvach et al., 2017; Chunchukova and Kirin, 2018; Chunchukova et al., 2018; Đikanović et al., 2018; Juhásová et al., 2019; Radačovská et al., 2019). Few authors provide

information on parasites of *Alburnus alburnus* (Linnaeus, 1758) from the Danube River (Cojocaru, 2007; 2009; 2010; Đikanovic et al., 2011; Kirin et al., 2013; Chunchukova et al., 2018).

The purpose of this study is to provide information on the species composition and structure of the parasite communities of *A. alburnus* from the upper Danube River in the Bulgarian section.

MATERIALS AND METHODS

During the spring, summer and autumn of 2019, fishes were collected and examined from the Danube River (biotopes near Koshava village, Kudelin village and Novo selo village, Vidin region) for endoparasites (Figure 1).

Figure 1. Danube River (Kudelin, Novo selo and Koshava villages)

The village of Kudelin (44°11′30″N, 22°40′5″E) is the first settlement in the Bulgarian section of the Danube River (844 river km) in the country. The village of Novo selo (44°10′0″N, 22°47′0″E) is located on the bank of the river (833 river km) and borders it for 3 km. The village of Koshava (44°4′0″N, 23°2′0″E) is located along the Danube River (807 river km). During the year, a total of 165 specimens of *A. alburnus* were collected, of which 63 in the spring, 64 in the summer and 38 in the autumn (Table 1).

Table 1. Number of *Alburnus alburnus* specimens studied by biotopes from the Danube River

Season (N = 165)	Kudelin	Novo selo	Koshava
Spring (N = 63)	32	–	31
Summer (N = 64)	30	34	–
Autumn (N = 38)	22	16	–

The fish were caught under a fishing permit for scientific research by the Executive Agency for Fisheries and Aquaculture, the Ministry of Agriculture, Food and Forests in Bulgaria. Species belonging to the studied fish specimens were determined by Karapetkova and Jivkov (2006); Kottelat and Freyhof (2007).

The scientific name of the species is represented by Froese and Pauly (2019). Metric data (weight (g) in grams, maximum body length (L) in centimetres and maximum body width (H) in centimetres) for all examined specimens *A. alburnus* were determined (Table 2).

Table 2. Metric data (L, H and g) of the examined specimens *A. alburnus* by biotopes from the Danube River

Alburnus alburnus	Spring		Summer		Autumn	
	Kudelin	Koshava	Kudelin	Novo selo	Kudelin	Novo selo
L Average ± SD	10.54 ± 1.04	12.98 ± 0.58	11.18 ± 2.13	12.65 ± 1.46	11.26 ± 0.81	11.34 ± 1.09
H Average ± SD	2.20 ± 0.26	2.82 ± 0.19	2.25 ± 0.44	2.40 ± 0.37	2.16 ± 0.18	2.12 ± 0.23
g Average ± SD	6.56 ± 2.08	16.42 ± 2.01	9.73 ± 6.89	14.28 ± 5.04	8.73 ± 1.93	8.63 ± 3.61

The collected specimens of bleak from the three biotopes were examined for helminths. Helminthological investigations were performed according to Petrochenko (1956); Zashev and Margaritov (1966); Kakacheva-Avramova (1983); Bauer (Ed.) (1987); Moravec (2013). The isolated endoparasites were fixed and stored in 70% ethyl alcohol. Permanent microscope preparations were prepared by the representatives of the class Trematoda and class Cestoda according to the method of Georgiev et al. (1986) and Scholz and Hanzelova (1998), and temporary microscope preparations were prepared by the representatives of class Nematoda and class Acanthocephala (Zashev and Margaritov, 1966; Moravec, 2013).

Prevalence (P %), mean intensity (MI) and mean abundance (MA) were determined for each parasite species. The structure of the component parasite communities was determined according to the criteria proposed by Kennedy (1993); Bush et al. (1997). Based on prevalence (P%), species are divided into accidental (P%<10), component (10<P%<20), and core (P%>20). The infracommunities are analyzed based on indicators: total number of species, the mean number of endoparasites, the Brillouin's diversity index (HB) (Magurran, 1998). The calculations were performed with MS Excel (Microsoft 2010) and Statistica 10 (StatSoft Inc., 2011).

RESULTS AND DISCUSSIONS

Ecoparasitological studies were conducted on a total of 165 specimens of bleak *Alburnus alburnus* (Linnaeus, 1758) from the Danube River. Bleak is a freshwater fish that is widely distributed throughout Europe. It is also found in the waters of the Danube River. It belongs to the family Cyprinidae. The bleak is a fish with small size – it reaches a maximum length of 17-19 cm and a maximum weight of 80-100 g. The fish prefers the upper water layers, where it feeds mostly on insects (Karapetkova and Jivkov, 2006).

Helminth community structure

Parasites were found in 41 specimens (24.85%) out of all 165 examined specimens *A. alburnus* from the Danube River. In spring, 18 specimens (28.57%) of 63 specimens bleak were infected, in summer 14 specimens (21.88%) of 64 specimens bleak were infected, and 9 specimens (23.68%) of 38 specimens bleak were infected in the autumn. In all 165 studied specimens *A. alburnus*, seven parasite species were established: 4 species of the class Trematoda (*Nicolla skrjabini* (Iwanitzky, 1928), *Allocreadium isoporum* (Looss, 1894), *Posthodiplostomum cuticola* (von Nordmann, 1832), *Sphaerostomum bramae* (Müller, 1776)); 1 species of the class Cestoda (*Neogryporhynchus cheilancristrotus* (Wedl, 1855), larvae); 1 species from the class Acanthocephala (*Pomphorhynchus laevis* (Müller, 1776)); 1 species from the class Nematoda (*Contracaecum* sp., larvae) (Table 3).

Table 3. Species diversity of *Alburnus alburnus* parasites by seasons and biotopes from the Danube River

Parasite species	Spring		Summer		Autumn	
	Koshava	Kudelin	Kudelin	Novo selo	Kudelin	Novo selo
Nicolla skrjabini (Iwanitzky, 1928)	•					•
Allocreadium isoporum (Looss, 1894)	•					
Posthodiplostomum cuticola (von Nordmann, 1832)			•		•	•
Sphaerostomum bramae (Müller, 1776)			•	•	•	•
Neogryporhynchus cheilancristrotus (Wedl, 1855), larvae	•	•		•		
Pomphorhynchus laevis (Müller, 1776)		•	•			
Contracaecum sp., larvae		•			•	

Component community

In the component community of *A. alburnus* from the Danube River (Koshava, Novo selo and Kudelin biotopes), the trematodes (4 species with > 971 specimens) are represented with the largest number of specimens, followed by the nematodes (1 species with 7 specimens), the cestodes (1 species with 4 specimens) and the acanthocephalans (1 species with 2 specimens). Three species of parasites were found in bleak from Koshava biotope: *Nicolla skrjabini*, *Allocreadium isoporum* and *Neogryporhynchus cheilancristrotus*, larvae. The trematode *A. isoporum* (P % = 29.03) is a core, the trematode *N. skrjabini* (P % = 16.13) is a component, and

the cestode *N. cheilancristrotus* (P % = 3.23) is an accidental parasite species in the parasite communities of bleak from Koshava biotope. The highest mean intensity (MI) and the highest mean abundance (MA) has *A. isoporum* (MI = 4.11; MA = 1.19) (Table 4).

Table 4. Species diversity and main ecological terms of parasites and parasite communities of *Alburnus alburnus* from the Danube River, Koshava biotope

Parasite species	Koshava N = 31					
	n	p	MI	MA	P %	Range
Nicolla skrjabini	5	12	2.40	0.39	16.13	1–4
Allocreadium isoporum	9	37	4.11	1.19	29.03	1–12
Neogryporhynchus cheilancristrotus, larvae	1	1	1.00	0.03	3.23	1

N – number of studied fish hosts, n – number of infected fish hosts, p – number of fish parasites, MI – mean intensity, MA – mean abundance, P % – prevalence.

Four species of parasites were found in bleak from Novo selo biotope: *Nicolla skrjabini*, *Sphaerostomum bramae*, *Posthodiplostomum cuticola* and *Neogryporhynchus cheilancristrotus*, larvae. The trematode *S. bramae* (P % = 10.00) is a component parasite species, while *N. skrjabini* (P % = 2.00), *P. cuticola* (P % = 2.00) and *N. cheilancristrotus* (P % = 2.00) are accidental parasite species in the parasite communities of bleak from Novo selo biotope. *P. cuticola* has the highest mean intensity (MI = 100.00) and the highest mean abundance (MA = 2.00) (Table 5).

Table 5. Species diversity and main ecological terms of parasites and parasite communities of *Alburnus alburnus* from the Danube River, Novo selo biotope

Parasite species	Novo selo N = 50					
	n	p	MI	MA	P %	Range
Nicolla skrjabini	1	1	1.00	0.02	2.00	1
Sphaerostomum bramae	5	11	2.20	0.22	10.00	1–5
Posthodiplostomum cuticola	1	> 100	100.00	2.00	2.00	> 100
Neogryporhynchus cheilancristrotus, larvae	1	1	1.00	0.02	2.00	1

N – number of studied fish hosts, n – number of infected fish hosts, p – number of fish parasites, MI – mean intensity, MA – mean abundance, P % – prevalence.

Five species of parasites were found in bleak from Kudelin biotope: *Sphaerostomum bramae*, *Posthodiplostomum cuticola*, *Neogryporhynchus cheilancristrotus*, larvae, *Pomphorhynchus laevis* and *Contracaecum* sp., larvae. All identified parasites, *P. cuticola* (P % = 9.52), *Contracaecum* sp., larvae (P % = 4.76), *S. bramae* (P % = 3.57), *P. laevis* (P % = 2.38) and *N. cheilancristrotus*, larvae (P % = 1.19) are accidental parasite species in the parasite communities of bleak from Kudelin biotope. The highest mean intensity (MI) and the highest mean abundance (MA) has *P. cuticola* (MI = 100.00; MA = 9.52) (Table 6).

Table 6. Species diversity and main ecological terms of parasites and parasite communities of *Alburnus alburnus* from the Danube River, Kudelin biotope

Parasite species	Kudelin N = 84					
	n	p	MI	MA	P %	Range
Sphaerostomum bramae	3	10	3.33	0.12	3.57	1–6
Posthodiplostomum cuticola	8	> 800	100.00	9.52	9.52	>100
Neogryporhynchus cheilancristrotus, larvae	1	2	2.00	0.02	1.19	2
Pomphorhynchus laevis	2	2	1.00	0.02	2.38	1
Contracaecum sp., larvae	4	7	1.75	0.08	4.76	1–4

N – number of studied fish hosts, n – number of infected fish hosts, p – number of fish parasites, MI – mean intensity, MA – mean abundance, P % – prevalence.

Only from Kudelin biotope were taken samples during the three seasons (spring, summer and autumn). During all studied period, parasite species on *A. alburnus* from the Danube River were detected in this biotope. *N. cheilancristrotus* was found in one specimen of *A. alburnus* only in the spring. *P. laevis* was found in one specimen *A. alburnus* in spring and summer. *Contracaecum* sp. occurred in spring and autumn, in 1 and 3 specimens *A. alburnus*, respectively. *S. bramae* and *P. cuticola* were found in summer and autumn. In the component community of bleak from Kudelin biotope, *P. cuticola* has the highest number of specimens (> 700) in summer, the highest mean intensity (MI) in summer (MI = 100.00) and autumn (MI = 100.00), and the highest mean abundance (MA) again in summer (MA = 23.33). In Kudelin biotope, in all three seasons, an equal number of parasite species (3 species) were detected. The highest number of parasite specimens (> 707) were identified during the summer. The dominant parasite species in the summer and autumn is the trematode *P. cuticola* (Table 7).

Table 7. Seasonal differences in invasion indices of *Alburnus alburnus* from the Danube River, Kudelin biotope

Season	Spring (N = 32)				Summer (N = 30)				Autumn (N = 22)			
Parasite species	n/p	MI	MA	P % (Range)	n/p	MI	MA	P % (Range)	n/p	MI	MA	P % (Range)
S. bramae	–	–	–	–	1/6	6.00	0.20	3.33 (6)	2/4	2.00	0.18	9.09 (1–3)
P. cuticola	–	–	–	–	7/>700	100.00	23.33	23.33 (>100)	1/>100	100.00	4.55	4.55 (>100)
***N. cheilancristrotus* larvae**	1/2	2.00	0.06	3.13 (2)	–	–	–	–	–	–	–	–
P. laevis	1/1	1.00	0.03	3.13 (1)	1/1	1.00	0.03	3.33 (1)	–	–	–	–
***Contracaecum* sp., larvae**	1/1	1.00	0.03	3.13 (1)	–	–	–	–	3/6	2.00	0.27	13.64 (1–4)

N – number of studied fish hosts, n – number of infected fish hosts, p – number of fish parasites, MI – mean intensity, MA – mean abundance, P % – prevalence.

Infracommunity

Of all 165 examined specimens *A. alburnus* from the Danube River (Koshava, Novo selo and Kudelin biotopes) – 124 specimens (75.15%) were not infected, and 41 specimens (24.85%) were infected with only one parasite species (Table 8).

Table 8. Infracommunity of *Alburnus alburnus* from the Danube River

Number of specimens *Alburnus alburnus*	Number of parasite species	
	0	**1**
	124	41
Total number of species (Mean number of species ± SD)	7 (0.25 ± 0.43)	
Total number of specimens (Mean number of specimens ± SD)	984 (82.0 ± 227.86)	
Brillouin's diversity index (HB), Mean ± SD	0.60 ± 0.19	

In the infracommunities of *A. alburnus* from the Danube River, Koshava biotope, the number of parasite specimens ranged from 1 to 12. Whereas in infracommunities of *A. alburnus* from the Danube River, Novo Selo and Kudelin biotopes, the number of parasite specimens ranged from 1 to > 100. A total of 984 helminth specimens were studied. The Brillouin's diversity index for the studied sample of the three biotopes is 0.60, ranging from 0.82 to 0.46 for the spring and summer seasons, with an average value of 0.53 for the autumn season (Table 8).

CONCLUSIONS

As a result of the conducted study of 165 specimens *A. alburnus* caught from the Danube River, 7 parasite species were identified: *N. skrjabini*, *A. isoporum*, *P. cuticola*, *S. bramae*, *N. cheilancristrotus*, larvae, *P. laevis* and *Contracaecum* sp., larvae. *A. alburnus* is a new host for *S. bramae* and *N. cheilancristrotus*, from the studied river ecosystem. With the highest parasite species diversity (5 species) are the studied specimens *A. alburnus* from Kudelin biotope. The highest mean intensity has *P. cuticola* (MI = 100.00), established as a parasite of *A. alburnus* from Novo Selo and Kudelin biotopes. With the highest mean abundance is distinguished the trematode *P. cuticola* (MA = 9.52), a parasite of *A. alburnus* from Kudelin biotope. The trematode *A. isoporum* (P % = 29.03) is a core parasite species in the component community of *A. alburnus* from the Danube River, Koshava biotope. In Kudelin biotope, samples were taken in all three seasons. During each season, three parasite species were detected. The highest mean intensity has *P. cuticola* (MI = 100.00) established in *A. alburnus* in the summer and autumn seasons, and the highest mean abundance has *P. cuticola* (MA = 23.33) established in the summer season.

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A CONTRIBUTION TO THE STUDIES ON THE CONTENT OF CU, CD AND AS IN *ALBURNUS ALBURNUS* (LINNAEUS, 1758) FROM THE DANUBE RIVER

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Abstract

The study aimed to present the results of investigations on the content of heavy metals/metalloids (Cu, Cd and As) in tissues and organs (liver, skin and muscles) of Alburnus alburnus (Linnaeus, 1758), and in waters and sediments of the Danube River (Kudelin, Vidin), Bulgaria. The highest Cd contents were found in liver samples (0.80 ± 0.56 mg.kg^{-1}), followed by contents in skin samples (0.21 ± 0.15 mg.kg^{-1}) and muscle (0.08 ± 0.07 mg.kg^{-1}). The highest As contents were found in liver (28.14 ± 10.98 mg.kg^{-1}) and skin (14.36 ± 11.45 mg.kg^{-1}), and the lowest in muscle (2.14 ± 0.64 mg.kg^{-1}). The highest content of Cu was established in liver samples (7.32 ± 2.39 mg.kg^{-1}), followed by this in the skin (3.78 ± 2.24 mg.kg^{-1}) and muscles (1.29 ± 1.03 mg.kg^{-1}). The highest values for As contents (0.07 ± 0.05 mg.l^{-1}) were determined in surface waters, and the highest values for Cu contents were determined in sediments (204.09 ± 121.05 mg.kg^{-1}). The results of studies on the circulation of the studied elements in the water-sediment-tissue and organs of A. alburnus system were analysed.

Key words*: Alburnus alburnus, arsenic, Bulgaria, cadmium, copper, Danube River.*

INTRODUCTION

The Danube River is the only river in Europe which course from west to east. In this way, the river is a connection between the countries of Western, Central and Eastern Europe (Schiemer et al., 2004). The Danube River Basin occupies ten per cent of Europe's surface. It covers the territory of 19 countries. The Danube River is adversely affected by the presence of numerous sources of pollution throughout its course. The river collects pollutants of various nature and takes them to its delta into the Black Sea. The upper reaches of the rivers are less polluted than the lower ones (Gasparotti, 2014). The Bulgarian territory of the Danube River falls into the lower reaches of the river (Polačik et al., 2008). Northwestern Bulgaria is a region with a long history of ore mining. The larger mines are situated near Chiprovtsi town and Martinovo village. They are a source of contamination with heavy metals (arsenic, copper, lead, zinc, etc.) (Dimitrova et al., 2016). Various authors have conducted studies on the heavy metals pollution in the mining area near Chiprovtsi and the Ogosta and Chiprovska rivers (Cholakova et al., 2006; Dimitrova et al., 2007; Mladenova et al., 2010; Mladenova et al., 2011; Kotsev and Zhelezov, 2014; Dimitrova et al., 2016; Benderev et al., 2017; Gerginov et al., 2017). A significant proportion of heavy metals that enter the aquatic environment pass into the sediments. They remain there for an extended period and continue to be a source of pollution and a threat to living organisms. Heavy metals have different toxicity. Some of them are dangerous to organisms even in small quantities (e.g. cadmium, lead, mercury), while others (e.g. copper, zinc, iron) are required in small amounts and dangerous in large numbers (Ionescu et al., 2014; Ionescu et al., 2015). Heavy metals and metalloids can be established in various tissues and organs of fish. At significantly higher concentrations, they can be found in the liver, kidneys and gills of fish (Javed and Usmani, 2017). Different authors have investigated the concentrations of heavy metals in tissues and organs of fish from the Danube River (Diaconescu et al., 2008; Nachev et al., 2010; Urdeş et al., 2010; Lenhardt et al., 2012; Kirin et al., 2014; Subotić et al., 2014; Milanovet al., 2016; Milošković et al., 2016; Burada et al., 2017; Chunchukova and Kirin, 2017; Chunchukova and Kuzmanova, 2017;

Chunchukova, Kirin, Shukerova, et al., 2017; Chunchukova, Kirin, Kuzmanova, et al., 2017; Jovanović et al., 2017; Kirin and Chunchukova, 2017; Shukerova et al., 2017, etc.). Studies are also carried out on the content of heavy metals in waters and sediments from the Bulgarian territory (Kirin et al., 2014; Chunchukova and Kirin, 2017; Chunchukova and Kuzmanova, 2017; Kirin and Chunchukova, 2017; Shukerova et al., 2017, etc.); from the Serbian territory (Antonijević et al., 2014; Ćirić et al., 2016; Milanov et al., 2016, etc.), from the Romanian area (Urdeş et al., 2010; Radu et al., 2017; Begy et al., 2018, etc.) of the Danube River.

This study aimed to determine the concentrations of heavy metals and metalloids (Cu, Cd, and As) in the tissues and organs (liver, skin, and muscles) of *Alburnus alburnus* (Linnaeus, 1758) as well as in waters and sediments of the Danube River; to analyze the circulation of the studied elements in the water-sediment-tissue and organs of *A. alburnus* system.

MATERIALS AND METHODS

During 2019, fish, water and sediments samples were collected from the Bulgarian territory of the Danube River (Kudelin village, designated as Kudelin biotope). The Kudelin village (44 ° 11 ′ 30 ″ N, 22 ° 40 ′ 5 ″ E) is located along the Danube River in northwestern Bulgaria.

Thirty specimens of *Alburnus alburnus* (L., 1758) were captured and investigated. Five water samples and four sediment samples were also collected. The fish are collected in accordance with the scientific fishing requirements of the Executive Agency for Fisheries and Aquaculture in Bulgaria. The species of fish were determined by Karapetkova and Jivkov (2006); Kottelat and Freyhof (2007). The scientific name of fish is written according to FishBase (Froese and Pauly, 2019). Maximum body length (L) in centimeters, maximum body width (H) in centimeters, and weight (g) in grams were recorded for all specimens of *A. alburnus* (Table 1). According to standard methods, mean representative samples of organs (liver) and tissues (skin and muscle) of the collected specimens of bleak were prepared. The chemical analysis of the samples was carried out by ICP “Optima 7000” Perkin-Elmerin an accredited laboratory for atomic absorption spectrophotometry of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia.

Table 1. L, H and g of the specimens *A. alburnus* from the Danube River (Kudelin biotope)

Alburnus alburnus	Min – max	Average ± SD
L	8.5 – 12.8	10.59 ± 1.05
H	1.8 – 3	2.21 ± 0.27
g	4 – 13	6.60 ± 2.14

The concentrations of the examined heavy metals (Cu, Cd) and metalloids (As) are presented in $mg.kg^{-1}$ wet weight and $mg.kg^{-1}$ dry weight (for samples of liver, skin and muscle of *A. alburnus*), in $mg.l^{-1}$ (for samples of water) and $mg.kg^{-1}$ dry weight (for samples of sediment). The bioconcentration factor for water/sediments ($BCF = [C_{host\ tissues}]/[C_{water/sediments}]$) was determined in this study. The linear correlation coefficient of Spearman (r_s) also was determined. Data processing was performed with MS Excel (Microsoft, 2010), BioDiversity Pro (McAleece, 1997) and Statistica 10 (StatSoft Inc., 2011).

RESULTS AND DISCUSSIONS

Thirty specimens of bleak (*Alburnus alburnus* Linnaeus, 1758) were collected and investigated for the content of copper, cadmium and arsenic from the Danube River (Kudelin biotope). *Alburnus alburnus* is a freshwater fish of the Cyprinidae family. Bleak is a brackish fish that inhabits lakes and larger rivers, is found in the upper water layers, and feed on insects and invertebrates (Kottelat and Freyhof, 2007).

The study provides information on the concentrations of heavy metals and metalloids (copper, cadmium and arsenic) in tissues and organs of 30 specimens *A. alburnus*, as well as in five samples of water and four samples of sediment from the Danube River (Kudelin biotope).

The highest concentrations of the three elements (Cu, Cd and As) were found in the liver of *A. alburnus* ($C_{Cu} = 7.32 \pm 2.39$ $mg.kg^{-1}$ wet weight; $C_{Cd} = 0.80 \pm 0.56$ $mg.kg^{-1}$ wet weight and $C_{As} = 28.14 \pm 10.98$ $mg.kg^{-1}$ wet weight); followed by

those in the skin (C_{Cu} = 3.78 ± 2.24 mg.kg^{-1} wet weight; C_{Cd} = 0.21 ± 0.15 mg.kg^{-1} wet weight and C_{As} = 14.36 ± 11.45 mg.kg^{-1} wet weight). The lowest concentrations of Cu, Cd and As were reported in the muscle of *A. alburnus* (C_{Cu} = 1.29 ± 1.03 mg.kg^{-1} wet weight; C_{Cd} = 0.08 ± 0.07 mg.kg^{-1} wet weight and C_{As} = 2.14 ± 0.64 mg.kg^{-1} wet weight). The content of the three elements in tissues and organs of bleak reduced as follows: liver > skin > muscles. The concentrations of the studied elements in the water samples were established to decrease in the order: As>Cu>Cd (respectively C_{As} = 0.07 ± 0.05 mg.l^{-1}; C_{Cu} = 0.04 ± 0.03 mg.l^{-1} and C_{Cd} = 0.001 ± 0.001 mg.l^{-1}). While the concentrations of the investigated elements in the sediment samples decreased in the order: Cu>As>Cd (respectively C_{Cu} = 204.09 ± 121.05 mg.kg^{-1} dry weight, C_{As} = 19.52 ± 9.76 mg.kg^{-1} dry weight and C_{Cd} = 1.54 ± 0.35 mg.kg^{-1} dry weight) (Table 2 and Table 3).

Table 2. Cu, Cd and As (mg.kg^{-1} wet weight) in liver, skin and muscle of *A. alburnus* and water (mg.l^{-1}) from the Danube River (Kudelin biotope)

Alburnus alburnus		Cu	Cd	As
Liver	Min – max	5.25 – 10.26	0.11 – 1.49	17.03 – 39.66
	Mean ± SD	7.32 ± 2.39	0.80 ± 0.56	28.14 ± 10.98
Skin	Min – max	1.26 – 5.68	0.04 – 0.39	3.73 – 30.46
	Mean ± SD	3.78 ± 2.24	0.21 ± 0.15	14.36 ± 11.45
Muscle	Min – max	0.41 – 2.47	0.01 – 0.16	1.63 – 3.04
	Mean ± SD	1.29 ± 1.03	0.08 ± 0.07	2.14 ± 0.64
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. Cu, Cd and As (mg.kg^{-1} dry weight) in liver, skin and muscle of *A. alburnus* and sediments (mg.kg^{-1} dry weight) from the Danube River (Kudelin biotope)

Alburnus alburnus		Cu	Cd	As
Liver	Min – max	10.42 – 27.24	0.33 – 2.20	41.95 – 93.45
	Mean ± SD	19.25 ± 7.31	1.46 ± 0.91	63.48 ± 21.76
Skin	Min – max	3.33 – 9.41	0.11 – 1.34	12.23 – 45.10
	Mean ± SD	6.36 ± 2.81	0.67 ± 0.61	25.56 ± 14.21
Muscle	Min – max	1.65 – 6.48	0.05 – 0.60	6.07 – 7.96
	Mean ± SD	3.77 ± 2.43	0.23 ± 0.26	6.90 ± 0.80
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 reported concentrations of copper, cadmium and arsenic in liver, skin and muscle samples of *A. alburnus* were compared to norms in national (Ordinance No. 31 of 2004 on the maximum levels of contaminants in foodstuffs) and international (WHO and FAO) documents. The norms for Cu, Cd and As determined in Ordinance No. 31 of 2004 are 10 mg/kg; 0.05 mg/kg and 1 mg/kg, respectively. The norm for Cu indicated by WHO is 20 mg/kg. The norms for Cu and Cd reported by FAO are 30 mg/kg and 0.2 mg/kg, respectively. It was found that the Cu content in the liver, skin and muscles of *A. alburnus* did not exceed the norms specified in Ordinance No. 31, WHO and FAO. It was found that the Cd content in the liver, skin and muscles of *A. alburnus* exceeded the norms specified in Ordinance No. 31, respectively by 16.08, 4.24 and 1.52 times. The Cd content in liver and skin also exceeded the standards defined by FAO by 4.02 and 1.06 times, respectively. It was established that the As

content in liver, skin and muscles of *Alburnus alburnus* exceeded the norms specified in Ordinance No. 31, respectively by 28.14, 14.36 and 2.14 times (Figure 1).

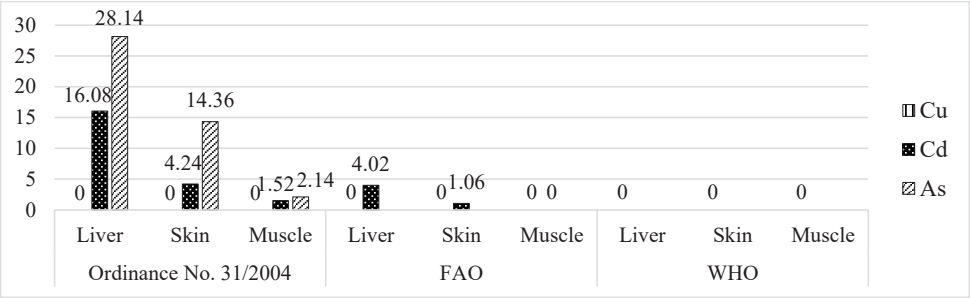

Figure 1. Exceedances of Cu, Cd and As in tissues and organs of *Alburnus alburnus* from Danube River (Kudelin biotope) according to national and international documents

The concentrations of copper, cadmium and arsenic in water samples were compared to norms in national documents (Ordinance No. 18 of 2009 on the quality of water for irrigation of crops; Ordinance No. H-4 of 2012 on the characterization of surface water and Ordinance on environmental quality standards for priority substances and certain other pollutants of 2010). The norms for Cu, Cd and As determined in Ordinance No. 18 of 2009 are 0.2 mg/dm^3; 0.01 mg/dm^3 and 0.1 mg/dm^3, respectively. The norm for As recorded in Ordinance No. H-4 of 2012 is 0.025 mg/l. The norm for Cd recorded in Ordinance on environmental quality standards is 0.0009 mg/l. Exceedances of Cd and As were found. The cadmium content exceeded the norms shown in Ordinance on environmental quality standards by 1.11 times, while the arsenic content exceeded the norms shown in Ordinance No. H-4 by 2.96 times (Figure 2).

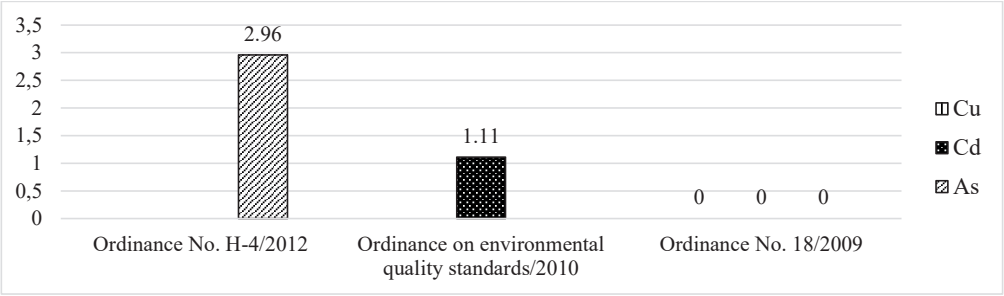

Figure 2. Exceedances of Cu, Cd and As in surface waters of the Danube River (Kudelin biotope) according to national documents

The reported concentrations of copper, cadmium and arsenic in sediment samples were compared to the norms in national (Ordinance No. 3 on the norms for permissible content of harmful substances in soils) and international (Dutch target values) documents. The maximum permissible concentrations (MPC) for Cu, Cd and As determined in Ordinance No. 3 are 150 mg/kg; 2 mg/kg at pH = 7.4 and 25 mg/kg, respectively. The Dutch target values for Cu, Cd and As are 36 mg/kg; 0.8 mg/kg and 29 mg/kg, respectively. Exceedances of Cu and Cd were found. The copper content exceeded 1.36 times the MPC in Ordinance No. 3. The copper and cadmium content exceeded the Dutch target values by 5.67 and 1.92 times, respectively (Figure 3).

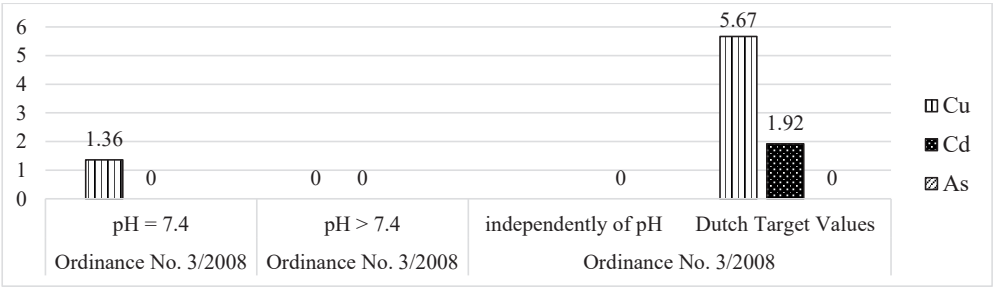

Figure 3. Exceedances of Cu, Cd and As in sediments from the Danube River (Kudelin biotope) relative to national and international documents

High bioconcentration of water/sediments was found for all three studied elements in the liver of bleak. In the muscles of bleak, the bioconcentration was the lowest. The study showed that the liver of *A. alburnus* bioaccumulated 4.09 times more Cd from the water compared to the accumulation of Cu and 2.11 times more Cd from the water compared to the accumulation of As. The liver of *A. alburnus* accumulated 1.92 times more As from the water, compared to that of Cu. The liver of *A. alburnus* bioaccumulated 36.12 times more As from the sediments compared to the accumulation of Cu and 10.56 times more Cd from sediments compared to that of Cu (Table 4–5).

Table 4. Bioconcentration factor BCF = [$C_{host\ tissues}$]/[C_{water}]

Alburnus alburnus/Water	BCF_{Cu}	BCF_{Cd}	BCF_{As}
C_{liver}/C_{water}	197.84	804	380.20
C_{skin}/C_{water}	102.27	212	193.98
C_{muscle}/C_{water}	34.81	76	28.85

Table 5. Bioconcentration factor BCF = [$C_{host\ tissues}$]/[$C_{sediments}$]

Alburnus alburnus/Sediments	BCF_{Cu}	BCF_{Cd}	BCF_{As}
$C_{liver}/C_{sediments}$	0.09	0.95	3.25
$C_{skin}/C_{sediments}$	0.03	0.44	1.31
$C_{muscle}/C_{sediments}$	0.02	0.15	0.35

Positive linear correlations were established between the Cu, Cd and As content in the investigated tissues and organs of the bleak and those in the water and sediments of the Danube River (r_s = 0.86 – 0.99 relative to water content; $p<0.05$ and r_s = 0.96 – 0.99; $p<0.05$). The positive linear correlations showed the direct impact of the water and the sediments on the content of the three studied elements in the liver, skin and muscles of the bleak. In this study, it was established a very high correlation and significance of correlation dependencies.

There are studies for heavy metals in bleak, but from the lower sections of the Danube River in Bulgaria. These are the first studies of the border sections of Danube River in North-Western Bulgaria. Few such studies, however, exist in other countries. Chunchukova and Kuzmanova (2017) established higher As concentration in the liver of bleak than in the muscles and skin. Shukerova et al. (2017) investigated *Alburnus alburnus* from the Bulgarian section of the Danube River for cadmium content and found higher Cd content in liver than in muscle and skin. Chunchukova et al. (2017a) analyzed the lead content in liver, skin and muscles of bleak from the Bulgarian section of the Danube River, in the area of the Vetren village, and found the highest lead content in the liver. Burada et al. (2017) measured the concentrations of heavy metals and metalloids (chromium, copper, mercury, nickel, lead and arsenic) in muscles of fifteen fish species, and they found the highest level of lead in *A. alburnus*. Duck et al. (2008) investigated the concentrations of Cd and Zn in tissues and organs (gills, bones, muscles, digestive system, liver and kidneys) of *A. alburnus* from the Topolnitsa dam, in Bulgaria. They found the highest concentration of Cd and Zn in kidneys and liver of *A. alburnus*.

CONCLUSIONS

This study provides new data on the content of heavy metals/metalloids (Cu, Cd and As) in *A. alburnus* for the upstream Danube River from the Bulgarian section. The concentration of the

investigated elements in tissues and organs of bleak decrease in the order: liver>skin>muscles. The study showed exceedances of Cd and As in the liver, skin and muscles of the examined specimens of *A. alburnus*. The obtained results do not indicate exceedances of Cu in the examined liver, skin and muscle samples. Exceedances of Cd and As, and of Cu and Cd were found in water samples and sediment samples, respectively. Due to the high values of bioconcentration factor and the positive linear correlations, *A. alburnus* can be used as a bioindicator for Cu, Cd and As content. The results for Cd, as well as those for As, are the most significant.

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PARASITES AND PARASITE COMMUNITIES OF THE COMMON NASE (*CHONDROSTOMA NASUS* (LINNAEUS, 1758)) FROM THE DANUBE RIVER

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Abstract

In 2019, studies on the parasites and parasite communities of Chondrostoma nasus (Linnaeus, 1758) from the Danube River, Bulgaria (Koshava and Kudelin villages, Vidin region) were conducted. After ecoparasitological studies of a total of 155 specimens of C. nasus from the two biotopes, eight species of parasites were found: two species of the class Trematoda (Allocreadium isoporum (Looss, 1894), Sphaerostomum bramae (Müller, 1776)), one species of the class Cestoda (Bothriocephalus acheilognathi (Yamaguti, 1934, immature specimens)), one species from the class Acanthocephala (Pomphorhynchus laevis (Müller, 1776)) and four species from the class Nematoda (Raphidascaris acus (Bloch, 1779), larvae; Contracaecum sp., larvae; Hysterothylacium sp., larvae; Pseudocapillaria tomentosa (Dujardin, 1843)). The majority of parasite species (seven species) were detected during the spring from biotope Kudelin. The dominant parasite species in all three seasons was the nematode Contracaecum sp. C. nasus was reported as a new host for A. isoporum, S. bramae, B. acheilognathi, R. acus, Contracaecum sp., Hysterothylacium sp., P. tomentosa from the Bulgarian section of the Danube River. New data on the structure of parasite communities of the river freshwater ecosystem were presented.

Key words*: Bulgaria, Chondrostoma nasus, Danube River, helminths, helminth communities.*

INTRODUCTION

The Danube River is among the longest rivers in Europe, it takes second place (2,857 km long). The river passes through ten European countries, including Bulgaria (Juhásová et al., 2019). Flowing across much of the continent, the Danube River connects the countries from Western, Central and Eastern Europe (Hock and Kovdcs, 1987). Along its course (from the Black Forest to the Black Sea), the river provides a wide variety of habitats.

The river is home and an important place for the conservation of many plant and animal species. (http://www.danubeparks.org).

In comparison to all European rivers, the Danube River is characterized by the most significant diversity of fish species, with more than 100 reported species. Fish species from six families - Cyprinidae, Percidae, Gobiidae, Cobitidae, Salmonidae and Acipenseridae, are dominant in the ichthyofauna of the Danube River (Keckeis & Schiemer, 2002; Kováč, 2015). Various authors have studied the ichthyofauna of the Danube River (Pehlivanov, 2005; Polačik et al., 2008; Lenhardt et al., 2010; Sandu (Calin) and Oprea, 2013; Bănăduc et al., 2014; Zorić et al., 2014; Kováč, 2015, etc.). Not only individual fish species but also their parasites are an object of study. The role of parasites in different ecosystems is significant. They influence on species diversity; respond to changes in the environment; provide information on food chains and the state of the ecosystem. The presence of more parasite species is a sign of the state of the whole ecosystem (Sures et al., 2017).

Different authors investigate the parasites and parasite communities of freshwater fish species from the Danube River (Cojocaru, 2003; Cakić et al., 2008; Cojocaru, 2009; Nachev and Sures, 2009; Đikanović et al., 2013; Kirin et al., 2013; Kvach et al., 2013; Kirin et al., 2014; Đikanović et al., 2015; Chunchukova et al., 2016; Kvach et al., 2016; Kvach et al., 2017; Chunchukova and Kirin, 2018; Chunchukova et al., 2018; Đikanović et al., 2018; Juhásová et al., 2019; Radačovská et al., 2019), but few authors provide information on parasites of the common nase (*Chondrostoma nasus* (Linnaeus, 1758))

from the Danube River (Cojocaru, 2007; 2009; 2010; Đikanovic et al., 2011; Kirin et al., 2013). New data on the parasites and parasite communities of common nase (*Chondrostoma nasus* (Linnaeus, 1758)) from the Bulgarian territory of the Danube River in its upper section were presented.

MATERIALS AND METHODS

During the spring, summer and autumn of 2019, fish and fish parasites were collected and examined from the Danube River (Koshava village and Kudelin village, Vidin region, designated as Koshava biotope and Kudelin biotope) (Figure 1).

Figure 1. Danube River (Kudelin village and Koshava village)

The village of Kudelin (44°11′30″N, 22°40′5″E) is the first settlement on the Bulgarian section of the Danube River (844 river km). The village of Koshava (44°4′0″N, 23°2′0″ E) is situated along the Danube River (807 river km).

A total of 155 specimens of *C. nasus* were collected throughout the year, of which 91 specimens in spring, 32 specimens in summer and 32 specimens in autumn (Table 1).

Table 1. Number of *Chondrostoma nasus* specimens studied by biotopes from the Danube River

Season (N = 155)	Kudelin	Koshava
Spring (N = 91)	49	42
Summer (N = 32)	32	–
Autumn (N = 32)	32	–

The fish were caught under a fishing permit for scientific research by the Executive Agency for Fisheries and Aquaculture, the Ministry of Agriculture, Food and Forests in Bulgaria. Species belonging to the studied fish specimens were determined by Karapetkova and Jivkov (2006); Kottelat and Freyhof (2007). The scientific name of the species is written by Froese and Pauly (2019). Metric data (weight (g) in grams, maximum body length (L) in centimetres and maximum body width (H) in centimetres) for all examined specimens *C. nasus* were determined (Table 2).

Table 2. Metric data (L, H and g) of the examined specimens *C. nasus* by biotopes from the Danube River

Chondrostoma nasus	Spring		Summer	Autumn
	Koshava	Kudelin	Kudelin	Kudelin
L Average ± SD	29.91 ± 4.95	31.15 ± 3.55	26.59 ± 3.06	30.25 ± 2.88
H Average ± SD	6.52 ± 1.30	6.54 ± 0.85	5.90 ± 0.61	6.70 ± 0.91
g Average ± SD	244.98 ± 129.71	277.11 ± 78.73	162.09 ± 46.23	274.25 ± 77.77

The collected specimens of common nase from both biotopes were examined for multicellular endoparasites. Helminthological investigations were performed according to Petrochenko (1956); Zashev and Margaritov (1966); Kakacheva-Avramova (1983); Bauer (Ed.) (1987); Moravec (2013). The isolated endoparasites were fixed and stored in 70 % ethyl alcohol. Permanent microscope preparations were prepared by the representatives of the class Trematoda and class Cestoda according to the method of Georgiev et al. (1986) and Scholz and Hanzelova (1998), and temporary microscope preparations were prepared by the representatives of class Nematoda and class Acanthocephala (Zashev

and Margaritov, 1966; Moravec, 2013). Prevalence (P %), mean intensity (MI) and mean abundance (MA) were determined for each parasite species. The structure of the component parasite communities was determined according to the criteria proposed by Kennedy (1993) and Bush et al. (1997). Based on prevalence (P %), species are divided into accidental (P % < 10), component (10 < P % < 20), and core (P % > 20). The infracommunities are analyzed based on indicators: total number of species, the mean number of endoparasites, the Brillouin's diversity index (HB) (Magurran, 1998). The calculations were performed with MS Excel (Microsoft 2010) and Statistica 10 (StatSoft Inc., 2011).

RESULTS AND DISCUSSIONS

A total of 155 specimens of *Chondrostoma nasus* (Linnaeus, 1758) from the Danube River were captured and investigated. The common nase is a species of the family Cyprinidae. On the territory of Bulgaria, the species was found in the Danube River and its tributaries.

The common nase is a freshwater fish that can be found in rivers with a moderate course. Fish eat plant materials. It reaches maximum body length up to 50 cm and weight up to 1 kg, rarely from 2 to 2.5 kg (Karapetkova and Jivkov, 2006; Kottelat and Freyhof, 2007).

Helminth community structure

Endoparasites were found in 125 specimens (80.65%) of all 155 examined specimens common nase from the Danube River.

In the spring, 74 specimens (81.32%) of 91 studied specimens *C. nasus* were infected, in the summer, 24 specimens (75%) of 32 specimens *C. nasus* were infected, and 27 specimens (84.38%) of 32 specimens *C. nasus* were infected in the autumn.

In all 155 studied specimens *C. nasus*, eight species of parasites were established: two species of the class Trematoda (*Allocreadium isoporum* (Looss, 1894), *Sphaerostomum bramae* (Müller, 1776)); one species of the class Cestoda (*Bothriocephalus acheilognathi* (Yamaguti, 1934), immature); one species from the class Acanthocephala (*Pomphorhynchus laevis* (Müller, 1776)); four species from the class Nematoda (*Raphidascaris acus* (Bloch, 1779), larvae; *Contracaecum* sp., larvae; *Hysterothylacium* sp., larvae; *Pseudocapillaria tomentosa* (Dujardin, 1843)) (Table 3).

Table 3. Species diversity of *Chondrostoma nasus* parasites by seasons and biotopes from the Danube River

Parasite species	Spring		Summer	Autumn
	Koshava	Kudelin	Kudelin	Kudelin
Allocreadium isoporum (Looss, 1894)	•	•		
Sphaerostomum bramae (Müller, 1776)				•
Bothriocephalus acheilognathi (Yamaguti, 1934), immature	•	•		
Pomphorhynchus laevis (Müller, 1776)	•	•		
Raphidascaris acus (Bloch, 1779), larvae	•	•	•	
Contracaecum sp., larvae	•	•	•	•
Hysterothylacium sp., larvae		•		
Pseudocapillaria tomentosa (Dujardin, 1843)		•		

Component community

In the component community of *C. nasus* from the Danube River (Koshava and Kudelin biotopes), nematodes (4 species with 2,056 specimens) are represented with the most significant number of specimens, followed by trematodes (2 species with 15 specimens), cestodes (1 species with 10 specimens) and acanthocephalans (1 species with 7 specimens).

Five species of parasites were detected in common nase from Koshava biotope: *Allocreadium isoporum*, *Bothriocephalus acheilognathi*, *Pomphorhynchus laevis*, *Raphidascaris acus*, larvae and *Contracaecum* sp., larvae. The nematodes *Contracaecum* sp.

(P % = 40.48) and *R. acus* (P % = 30.95) are core parasite species in the parasite communities of common nase. The trematode *A. isoporum* (P % = 11.90) is a component parasite species. Whereas the cestode *B. acheilognathi* (P % = 7.14) and the acanthocephalan *P. laevis* (P % = 2.38) are accidental parasite species in the parasite communities of common nase from Koshava biotope. The highest mean intensity (MI) has *R. acus* (MI = 8.69), and the highest mean abundance (MA) has *Contracaecum* sp. (MA = 3.48) (Table 4).

Table 4. Species diversity and main ecological terms of parasites and parasite communities of *Chondrostoma nasus* from the Danube River, Koshava biotope

Parasite species	Koshava N = 42					
	n	p	MI	MA	P %	Range
Allocreadium isoporum	5	5	1.00	0.12	11.90	1
Bothriocephalus acheilognathi, immature	3	7	2.33	0.17	7.14	1–4
Pomphorhynchus laevis	1	6	6.00	0.14	2.38	6
Raphidascaris acus, larvae	13	113	8.69	2.69	30.95	1–34
Contracecum sp., larvae	17	146	8.59	3.48	40.48	1–79

N – number of studied fish hosts, n – number of infected fish hosts, p – number of fish parasites, MI – mean intensity, MA – mean abundance, P % – prevalence.

Eight species of parasites were detected in common nase from Kudelin biotope: *Allocreadium isoporum*, *Sphaerostomum bramae*, *Bothriocephalus acheilognathi*, immature, *Pomphorhynchus laevis*, *Raphidascaris acus*, larvae, *Contracaecum* sp., larvae, *Hysterothylacium* sp., larvae and *Pseudocapillaria tomentosa*. The nematodes *Contracaecum* sp. (P % = 66.37) and *R. acus* (P % = 20.35) are core parasite species in the parasite communities of common nase from this biotope. The other six parasite species are accidental in the parasite communities of common nase from Kudelin biotope. The highest mean intensity (MI) and the highest mean abundance (MA) has *Contracaecum* sp. (MI = 22.68; MA = 15.05) (Table 5).

Table 5. Species diversity and main ecological terms of parasites and parasite communities of *Chondrostoma nasus* from the Danube River, Kudelin biotope

Parasite species	Kudelin N = 113					
	n	p	MI	MA	P %	Range
Allocreadium isoporum	4	6	1.50	0.05	3.54	1–2
Sphaerostomum bramae	3	4	1.33	0.04	2.65	1–2
Bothriocephalus acheilognathi, immature	2	3	1.50	0.03	1.77	1–2
Pomphorhynchus laevis	1	1	1.00	0.01	0.88	1
Raphidascaris acus, larvae	23	90	3.91	0.80	20.35	1–14
Contracecum sp., larvae	75	1,701	22.68	15.05	66.37	1–315
Hysterothylacium sp., larvae	2	4	2.00	0.04	1.77	1–3
Pseudocapillaria tomentosa	1	2	2.00	0.02	0.88	2

N – number of studied fish hosts, n – number of infected fish hosts, p – number of fish parasites, MI – mean intensity, MA – mean abundance, P % – prevalence.

Only from Kudelin biotope were taken samples during the three seasons (spring, summer and autumn). During all seasons in this biotope were found parasite species on *C. nasus* from the Danube River. Invasion with *Contracaecum* sp. were found throughout the study period. *A. isoporum*, *B. acheilognathi*, *P. laevis*, *P. tomentosa,* and *Hysterothylacium* sp., were found only during the spring season. *S. bramae* was established only in the autumn. One specimen of *P. laevis* was found in one specimen of *C. nasus* in the spring. In the component community of common nase from Kudelin biotope, *Contracaecum* sp. has the highest number of specimens (1,701) and the highest mean intensity (MI) in all seasons (spring MI = 36.38; summer MI = 17.88 and autumn MI = 14.78). The highest number of parasite species (7 species) and the highest number of parasite specimens (978) were collected and established in the spring. The dominant parasite species in all three seasons is *Contracaecum* sp. (Table 6).

Table 6. Seasonal differences in species composition on endoparasites and invasion indices of *Chondrostoma nasus* from the Danube River, Kudelin biotope

Season	**Spring (N = 49)**				**Summer (N = 32)**				**Autumn (N = 32)**			
Parasite species	**n/p**	**MI**	**MA**	**P % (Range)**	**n/p**	**MI**	**MA**	**P % (Range)**	**n/p**	**MI**	**MA**	**P % (Range)**
A. isoporum	4/6	1.50	0.12	8.16 (1–2)	–	–	–	–	–	–	–	–
S. bramae	–	–	–	–	–	–	–	–	3/4	1.33	0.13	9.38 (1–2)
***B. acheilognathi*, immature**	2/3	1.50	0.06	4.08 (1–2)	–	–	–	–	–	–	–	–
P. laevis	1/1	1.00	0.02	2.04 (1)	–	–	–	–	–	–	–	–
***R. acus*, larvae**	22/89	4.05	1.82	44.90 (1–14)	1/1	1.00	0.03	3.13 (1)	–	–	–	–
***Contracaecum* sp., larvae**	24/873	36.38	17.82	48.98 (3–315)	24/429	17.88	13.41	75.00 (1–65)	27/399	14.78	12.47	84.38 (1–66)
***Hysterothylacium* sp., larvae**	2/4	2.00	0.08	4.08 (1–3)	–	–	–	–	–	–	–	–
P. tomentosa	1/2	2.00	0.04	2.04 (2)	–	–	–	–	–	–	–	–

N – number of studied fish hosts, n – number of infected fish hosts, p – number of fish parasites, MI – mean intensity, MA – mean abundance, P % – prevalence.

Infracommunity

Of all 155 examined specimens *C. nasus* from the Danube River (Koshava and Kudelin biotopes), 30 specimens (19.35 %) were not infected, and 125 specimens (80.65 %) were infected, as of them the 104 specimens (67.10%) were infected with one species of parasite, 18 specimens (11.61%) with two species of parasites, 2 specimens (1.29%) – with three species of parasites and 1 specimen (0.65%) – with four species of parasites (Table 7).

In the infracommunities of *C. nasus* from the Danube River, Koshava biotope, the number of endoparasite specimens ranged from 1 to 79 in one specimen host.

In contrast, in the infracommunities of *C. nasus* from the Danube River, Kudelin biotope, the number of endoparasite specimens ranged from 1 to 315 in one specimen host.

A total of 2,088 specimens of endoparasites were studied.

The Brillouin's diversity index for the studied sample of the two biotopes is 0.37, ranging from 1.04 to 0.01 for the spring and autumn seasons, with an average value of 0.05 for the summer season (Table 7).

Table 7. Infracommunity of *Chondrostoma nasus* from the Danube River

Number of specimens *Chondrostoma nasus*	Number of parasite species				
	0	**1**	**2**	**3**	**4**
	30	104	18	2	1
Total number of species (Mean number of species ± SD)	8 (0.97 ± 0.65)				
Total number of specimens (Mean number of specimens ± SD)	2,088 (160.62 ± 465.51)				
Brillouin's diversity index (HB)	0.37 ± 0.58				

CONCLUSIONS

As a result of the study of 155 specimens *C. nasus* caught from the Danube River, 8 parasite species were identified: *A. isoporum*, *S. bramae*, *B. acheilognathi*, *P. laevis*, *R. acus*, larvae, *Contracaecum* sp., larvae, *Hysterothylacium* sp., larvae, *P. tomentosa*. *C. nasus* was reported as a new host for *A. isoporum, S. bramae, B. acheilognathi, R. acus, Contracaecum sp., Hysterothylacium sp.* and *P. tomentosa* from the Bulgarian section of the Danube River.

The largest number of parasite species (8 species) were found in the studied specimens *C. nasus* from Kudelin biotope.

In the component community of common nase from Koshava biotope, *R. acus* has the highest mean intensity (MI = 8.69) and *Contracaecum* sp. has the highest mean abundance (MA = 3.48). *Contracaecum* sp. (P % = 40.48) and *R. acus* (P % = 30.95) are core species in the endoparasite communities of *C. nasus* from Koshava biotope. *C. nasus* from Kudelin biotope was examined all three seasons.

The species diversity of endoparasites was highest during the spring study period. Of them, with the highest mean intensity and mean abundance is distinguished *Contracaecum* sp. (MI = 36.38; MA = 17.82). *Contracaecum* sp. is core species in the parasite communities of common nase during spring, summer and autumn (P % = 48.98, P % = 75.00 and P % = 84.38, respectively). *R. acus* is also core species in the parasite communities of common nase but is only found for the spring season (P % = 44.90).

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HELMINTHS AND HELMINTH COMMUNITIES OF ROUND-SCALED BARBELL (*BARBUS CYCLOLEPIS* HECKEL, 1837) AND ITS BIOINDICATOR ROLE

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Abstract

During 2018, 22 specimens of the round-scale barbell from the Tamrashka River (Bulgaria) were examined for helminths. B. cyclolepis is an endemic fish species of the Maritsa River Water Collection. Five parasite species (Allocreadium isoporum Ergens & Lom, 1970; Caryophyllaeides fennica (Schneider, 1902) Nybelin, 1922; Pomphorhynchus laevis (Müller, 1776); Rhabdochona hellichi (Šramek, 1901) Chitwood, 1933; Rhabdochona gnedini Skrjabin, 1948) belonging to four classes and four families were fixed. The Tamrashka River is a new habitat for All. isoporum, C. fennica, P. laevis, Rh. hellichi and Rh. gnedini of B. cyclolepis in Bulgaria. The dominant structure of the parasite communities was discussed based on the level of the component community. The bioindicator role of the studied parasite populations and communities are presented.

Key words*: Aegean Water Basin, B. cyclolepis, bioindication, helminths, helminth communities*.

INTRODUCTION

Barbus cyclolepis Heckle, 1837, is an endemic fish species of the Maritsa River Basin (Kolev, 2016). The Tamrashka River is one of the most significant right tributaries of the Maritsa River, Aegean Water Basin. The river springs west of Modar Peak (about 1800 m above sea level) in the Chernatitsa Ridge, Western Rhodopa Mountain, and Southern Bulgaria. After the village of Parvenets, the Tamrashka River enters the Upper Thracian Plain under the name Parvenetska River. It flows into the Maritza River at 164 meters above sea level western of the town of Plovdiv. The freshwater fish, including *Barbus cyclolepis*, and its helminths are used as biological elements for bioindication (MacKenzie et al., 1995; Lambert and El Gharbi, 1995; Kelepertzis et al., 2012; Kirin et al., 2013). At the same time, the studies on the helminths and helminth communities of *B. cyclolepis* are extremely limited. In Bulgaria to this time, have not studied on the bioindicative importance of Round–scaled barbell, its helminths and helminth communities. The study aims to present the results from the examination of helminth and helminth communities of endemic fish species *Barbus cyclolepis* Heckle, 1837 from the Tamrashka River, Bulgaria.

MATERIALS AND METHODS

During 2018, 22 specimens of *Barbus cyclolepis* Heckel, 1837 from the Tamrashka River, Bulgaria were examined for helminths. The fish were caught by angling according to permission from Ministry of Agriculture, Food and Forestry of the Republic Bulgaria. The scientific name of the fish was present, according to Bianco (1998); Froese and Pauly (Eds.) (2019). The fish were caught in a section of river between the villages of Hrabrino and Parvenets ($40^{0}03'01.60$N and $24^{0}3838.57$E), in Rodopa Mountain, about 17.26 km far away from the town of Plovdiv, Southern Bulgaria. The helminthological studies were carried out according to the Zashev and Margaritov (1966); Bauer (Ed.) (1987); Moravec (2013). The detected and isolated helminth specimens were fixed in 70% of ethyl alcohol. Species diversity of representatives from classes Trematoda and Cestoda were determined on permanent slides carried out by the methods of Georgiv et al. (1986) and Scholz and Hanzelová (1998) and

from classes Acanthocephala and Nematoda – on temporary slides carried out by the method of Moravec (2013). Helminth community structure was analysed by two levels: on the level of component community (prevalence (P%); mean intensity (MI) for the determined species) and on the level of infracommunity (total number of fish species; total and mean number of fish specimens; Brillouin's diversity index (HB) and Pielou's evenness index (E)).

In the component community, the found species were divided as core species (P% > 20), component species (P% > 10) and accidental species (P% < 10), according to the criteria of Magurran (1988); Bush et al. (1997) and Kennedy (1997). The obtained results were statistically processed using the software products Statistica 10 (StatSoft Inc., 2011) and MS Exel (Microsoft 2010).

RESULTS AND DISCUSSIONS

Fish communities

The Round–scaled barbell or Maritsa barber (*Barbus cyclolepis* Heckel, 1837; Cyprinidae) inhabits the bottom of reservoirs, the middle and upper part of the rivers with sandy–gravelly or rocky bottom. In the spring it goes down to the deeper parts of the rivers, and in the summer it goes up to the fast streams.

The Maritsa barbell measures up to 30 cm in length and weighs up to 1 kg. The colour of the fish depends mainly on the environment. Most often the colour of fish is silvery, streaked with fine dark spots.

The fins are yellowish, only in certain cases, turning pure orange. The Round-scaled barbell feeds mainly on demersal organisms.

The species prefers for food larvae and larvae of insects, all crustaceans, invertebrates, and also worms, caterpillars and insects. Sexually mature at 2-3 years. The fish species propagated mainly in the period April – May.

The species lives up to 10 years. It is most widespread fish species in the rivers Maritsa, Mesta, Struma and their tributaries, also in rivers in north-eastern Greece (Bianco, 1998; Karapetkova and Zhivkov, 2006; Kottelat and Freyhof, 2007; Kolev, 2013; 2016). *B. cyclolepis* is an IUCN as non–threatened species (= LC = Least Concern) (Froese & Pauly (Eds.), 2019). The species is included in Annexes 2 and 4 of the Biodiversity Law of the Republic of Bulgaria. *B. cyclolepis* is an endemic species for the Maritsa catchment area (Kolev, 2016).

Helminth community structure

The helminths and helminth communities of 22 specimens *B. cyclolepis* from the Tamrashka River (Aegean Water Basin, Bulgaria) were examined. Five species of helminths (*Allocreadium isoporum (*Ergens and Lom, 1970); *Caryophyllaeides fennica* (Schneider, 1902; Nybelin, 1922); *Pomphorhynchus laevis* (Müller, 1776); *Rhabdochona hellichi* (Šrameć, 1901; Chitwood, 1933; *Rhabdochona gnedini (*Skrjabin, 1948), belonging to 4 classes, four orders and four families have been found. Only from the class Nematoda, two species of helminths were fixed. The other three classes are represented by one species of helminths (Table 1).

Table 1. Biodiversity and ecological indices of helminths and helminth communities of *Barbuscyclolepis* from the Tamrashka River

Barbus cyclolepis (N[1] = 22) Helminth species	n[2]	p[3]	P%[4]	MI[5]
Trematoda Class Order Fasciolida Family Allocreadidae				
Allocreadium isoporum (Looss, 1894)	15	54 1-12	68.18	3.6
Cestoda Class Order Caryophyllaeidea Family Caryophyllaeidae				
Caryophyllaeides fennica (Schneider, 1902) Nybelin, 1922	2	2	9.09	1
Acanthocephala Class Order Echinorhynchidae Family Pomphorhynchidae				
Pomphorhynchus laevis (Müller, 1776)	9	27 1-4	40.9	3.0
Nematoda Class Order Spirurida Family Rhabdochonidae				
Rhabdochona hellichi (Šramek, 1901) Chitwood, 1933	2	3 1-2	9.09	1.5
Rhabdochona gnedini Skrjabin, 1948	1	4	4.54	4

[1]N = total number of examined fish specimens.
[2]n = total number of infected fish specimens.
[3]p = total number of helminth specimens.
[4]P% = prevalence.
[5]MI = mean intensity.

The adult specimens of *Allocreadium isoporum* (Looss, 1894) are developing in different species of Cyprinidae. The parasite's life cycle

involves the participation of two intermediate hosts. The first intermediate hosts are snails of *Sphaerium* genus, and the second are larvae's of insects of Ephemera genera, Linnaeus, 1758 (Ephemeridae), *Anabolia* Cuvier, 1827 (Limpetridae) and *Chaetopterix* (Limnephilidae) (Bauer, 1987; Kakacheva–Avramova, 1983). The species was reported from *B. cyclolepis* in Bulgaria (from Syuyutlijka rivers, Asenitsa – Kakacheva–Avramova, 1965; from Vacha River – Margaritov, 1965; from Tundzha River – Kakacheva–Avramova, 1972; from Luda Yana River – Kirin, 2002a). According to this research, the Tamrashka River is a new habitat for the *All. isoporum* trematode species as parasite species of *B. cyclolepis*.

Caryophyllaeides fennica (Schneider, 1902) Nybelin, 1922 is an intestinal parasite of *Squalius cephalus* (Linnaeus, 1758), *Leuciscus idus* (Linnaeus, 1758), *Barbus barbus* (Linnaeus, 1758), *B. meridionalis petenyi* Heckel, 1852, *Rutilus rutilus* (Linnaeus, 1758), *Abramis brama* (Linnaeus, 1758), *Scardinius erytrophthalmus* (Linnaeus, 1758), *Aspius aspius* (Linnaeus, 1758), *Gobio gobio* (Linnaeus, 1758) and other fish species of Cyprinidae. *Stylaria lacustris* (Linnaeus, 1767) (Oligochaeta) is an intermediate host of the parasite (Bauer, 1987; Kakacheva–Avramova, 1983). *St. lacustris* is a bioindicator for β–mesosaposity. The species refers to the relatively tolerant forms (group C) in terms of the conditions in the habitats (Rusev, 1993; Peev and Gerasimov, 1999; Belkinova et al., 2013). *C. fennica* was reported of *B. cyclolepis* in Bulgaria (from Topolnica River – Margaritov, 1965; from Asenica, Harmanlijska, Topolnitsa, Syuyutlijka and Sushitsa rivers, Bedechka – Kakacheva–Avramova, 1965; from Tundzha River – Kakacheva–Avramova, 1972; from Arda River – Kirin, 2003). The Tamrashka River is a new habitat for *C. fennica* from *B. cyclolepis*.

Pomphorhynchus laevis (Müller, 1776) develops as a marita in a lot of freshwater fish species of Cyprinidae, Salmonidae, Percidae, Siluridae, etc. The developmental cycle is related to the participation of an intermediate host – the *Gammarus pulex* crustaceans (Linnaeus, 1758) (Bauer, 1987; Kakacheva–Avramova, 1983). *G. pulex* is a bioindicator for x–β–mesosaprobity as well as relatively tolerant forms (Group C) in terms of environmental conditions in habitats (Rusev, 1993; Peev & Gerasimov, 1999; Belkinova et al., 2013). Small fish species of Cyprinidae have been established as a reservoir host. The species has been reported by *B. cyclolepis* in Bulgaria (from Tundzha River – Kakacheva–Avramova, 1972). The Tamrashka River is a new habitat for *P. laevis* from *B. cyclolepis*.

Rhabdochona hellichi (Šramek, 1901) Chitwood, 1933 is an intestinal parasite species of different species of freshwater fish of the families of Cyprinidae (*B. barbus*, *B. meridionalis*, *B. petenyi*, etc.), Salmonidae (*Salmo trutta fario* Linnaeus, 1758, *Oncorhynchus mykiss* (Walbaum, 1792), *Thymallus thymallus* (Linnaeus, 1758)), Acipenseridae, Siluridae, etc., which are the final hosts of the species (Bauer, 1987; Kakacheva–Avramova, 1983; Moravec, 2013). In the scientific literature, no data on the species development cycle are reported (Moravec, 2013). *Rh. hellichi* has been reported as a parasite of *B. cyclolepis* in Bulgaria (from Tundzha River – Kakacheva–Avramova, 1972; from Arda River – Kirin, 2003). According to the study, the Tamrashka River is a new habitat for *Rh. hellichi* from *B. cyclolepis*.

Rhabdochona gnedini Skrjabin, 1948 is an intestinal parasite of many fish species of Cyprinidae (*B. barbus*, *B. cyclolepis*, *B. meridionalis*, *Luciobarbus bocagei* (Steindachner, 1864), *L. caspius* (Berg, 1914), *R. rutilus*, *Sq.cephalus*, *Sq. svallize (*Heckel and Kner, 1858), Salmonidae (*S. t. fario*, *S. trutta trutta* Linnaeus, 1758, *S. marmoratus* Cuvier, 1829, *S. obtusirostris* (Heckel, 1851), *O. mykiss*, *Hucho hucho* (Linnaeus, 1758), *Th. thymallus*), Percidae (*Zingel streber* (Siebold, 1863), *Z. zingel* (Linnaeus, 1766)), Siluridae (*Silurus glanis* Linnaeus, 1758) and others (Moravec, 2013). The species development cycle has not been studied (Moravec, 2013). The species was reported as a parasite of *B. cyclolepis* in Bulgaria (from rivers Vacha and Maritsa as *Rh. sulaki* – Margaritov, 1965).The Tamrashka River is a new habitat for *Rh. gnedini* from *B. cyclolepis*.

Component communities

With the highest prevalence were distinguished two helminth species of *B. cyclolepis*: *All. isoporum* (P% = 68.18) and *P. laevis* (P% =

40.90). They are core species for the helminth communities of *B. cyclolepis*. The other three species of helminth (*C. fennica*, *Rh. hellichi* and *Rh. gnedini*) are accidental species on these communities. The highest mean intensity was fixed for *Ph. gnedini* (MI = 4.0), followed by those of *All. isoporum* (MI = 3.6), *P. laevis* (MI = 3.0), etc. As a result of this study, only one specimen of *C. fennica* was found. The population of *Rh hellichi* also showed low mean intensity (MI = 1.5) (Table 1).

Infracommunities

All examined fish specimens of *B. cyclolepis* from Tamrashka River were infected. In 15 specimens of fish, one species of helminth was found, and in 7 specimens of Round-scaled barbell, two species of helminths were found. A total of 90 helminth specimens were studied. The helminth infection was presented from one to 12 specimens, the mean of 3.03 specimens per one specimen of infected fish (Table 2).

Table 2. Infracommunities data

Number of helminth species		
Total number of species	5	
Number of fish	15	7
Number of helminth species	1	2
Number of helminth specimens		
Total number of specimens	90	
Mean ± SD	3.03 ± 2.24	
Range (min - max)	1 – 12	
HB ± SD (Brillouin'sdiversity index)	0.929 ± 1.23	
E ± SD (Pielou's evenness index)	0.624 ± 0.25	

No parasites causing dangerous diseases to fish, humans or other hosts have been identified, as reported by some authors from different hosts (Kakacheva-Avramova and Nedeva, 1979; Margaritov, 1959; Pekova et al., 2017a; Pekova et al., 2017b; Mitev et al., 2020). Other authors also reported the helminth species, found in this study in the country except for *Ph. gnedini*. Some of them from Southern Bulgaria are presented in Table 3.

Table 3. Some other species of fish from freshwater ecosystems in Southern Bulgaria, hosts of helminths reported of *Barbus cyclolepis* from the Tamrashka River

Helminth species	Other fish species	Authors
Allocreadium isoporum (Looss, 1894)	*Gobio gobio* (Linnaeus, 1758)	Kakacheva, 65
	Squalius orpheus Kottelat & Economidi, 2006 (*Leuciscus cephalus*)	Kakacheva-Avramova, 65; Margaritov, 65; Kirin, 2000; 2001a,b; 2002c
Caryophyllaeides fennica (Schneider, 1902) Nybelin, 1922	*Alburnus alburnus* Linnaeus, 1758	Kirin, 2003
	Barbus barbus	Margaritov, 1959
	Squalius orpheus Kottelat & Economid, 2006 (*L. cephalus*)	Kakacheva-Avramova, 1965; Kirin, 2002c; Kirin et al., 2013
	Vimba melaniops	Kakacheva-Avramova, 1965
Pomphorhynchus laevis (Müller, 1776)	*Sander lucioperca*	Nedeva & Grupcheva, 1996
	Squalius orpheus Kottelat & Economid, 2006 (*L. cephalus*)	Kirin, 2000; 2001b
	Salmo trutta fario Linnaeus, 1758	Kakacheva-Avramova & Nedeva, 1979
Rhabdochona hellichi (Šramek, 1901) Chitwood, 1933	*Barbus meridionalis petenyi* Heckel, 1852	Kakacheva_Avramova, &Nedeva, 1978
	Salmo trutta fario Linnaeus, 1758	Kakacheva-Avramova & Nedeva, 1978; 1979; 1982; Kirin, 2002a
	Oncorhynchus mykiss (Walbaum, 1792)	Kakacheva-Avramova & Nedeva, 1979; 1982
Rhabdochona gnedini Skrjabin, 1948 (*Rh. sulaki*)	-	-

There are a few types of research of the parasites and parasite communities of *B. cyclolepis* from freshwater ecosystems in other countries. They mainly refer to representatives of Monogenea class (Šimková et al., 2007 – including the materials from Bulgaria; Lambert and El Gharbi, 1995).

The studied Tamrashka River is not exposed to anthropogenic pressure or other negative effects. The adjacent territories are characterized by high biodiversity, a number of protected, endemic and relict species. Determined helminth species of *B. cyclolepis*, total number of species and total number of specimens, parasites life cycles, calculated diversity indices (HB = 0.929, Brillouin's diversity index) and evenness indices (E = 0.624, Pielou's evenness index) is a reliable evidence of β–mesosaprobity conditions in the freshwater ecosystems of the

Tamrashka River and of the very good ecological status of the investigated freshwater biotopes (Tables 1 – 2; Rusev, 1993; Peev and Gerasimov, 1999; Belkinova et al., 2013).

CONCLUSIONS

The Tamrashka River is a new habitat for the helminth species of *Barbus cyclolepis*: *Allocreadium isoporum*, *Caryophyllaeides fennica*, *Pomphorhynchus laevis*, *Rhabdochona hellichi*, *Rhabdochana gnedini*. *All. isoporum* and *P. laevis* are component species of the helminth communities of *B. cyclolepis*. With the highest mean intensity are distinguished the species *Rh. gnedini* and *All. isoporum*. The analyzed results of the studied helminth and helminth communities ofthe Round–scaled barbell showed the very good ecological condition of the investigated freshwater ecosystem.

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MEAT QUALITY OF TRADITIONALLY SMOKED TROUT FROM TRECĂTOAREA URSULUI SALMONID FARM, BRAȘOV COUNTY

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Abstract

The consumption of fish meat in Romania is on an upward trend, due to the establishment of salmonid units on the territory of the country. The trout`s commercialization is mainly in fresh or chilled form, but for the diversification of the products offered to the market, it is also sold in preserved form, either by salting or by smoking. The analyses performed on the chemical composition of trout meat were as follows: water content, dry matter, fat content, protein content, non-nitrogenous extractive substances and minerals The chemical analysis was made in all phases of the technological flow of conservation by smoking: fresh meat, salted meat, desalted meat and smoked meat throughout the technological flow for the Rainbow and Brook trout species, reared in the fish farm. Therefore, the chemical analyses of the meat pointed out that the highest percentage of water content is found in the Rainbow trout comparative to Brook trout in all stages of the technological flow. On the contrary, the dry matter content was higher in the Brook than in Rainbow trout. As for the fat content we noticed that the Brook trout registered higher values than the Rainbow trout. For the protein content, the obtained values were quite close to both species of salmonids. Both Rainbow trout and Brook trout traditionally smoked meat registered values that are within the limits cited in the scientific literature.

Key words*: quality, salmonid, smoked trout, traditionally.*

INTRODUCTION

Among the products of animal origin, fish meat is a special food source, for which the preferences and tastes of consumers have been and are constantly growing. Due to its high biological, fat and mineral protein content, which can be added to the special dietary properties and the suitability for a wide variety of culinary preparations, fish meat is highly appreciated (Iurca and Răducu, 2005).

To obtain a proper fish meat production at efficient technical-economic parameters, this requires a correct appreciation technique and a better knowledge of the biochemical processes that characterize the fish meat (Petrea et al., 2012).

According to the official statistics (Ministry of Agriculture and Rural Development - MARD), the consumption of fish meat in Romania is on an upward trend, due to the establishment of salmonid units in the territory of the country with European funds (Petrea et al., 2012).

The trout's capitalization for selling purposes is mainly in fresh or chilled form, but for the diversification of the products offered to the market, it is also sold in preserved form, either by salting or by smoking procedures. The quality of fish meat depends on the farm production capacity, the quantity and quality of the administered feed, the environmental conditions, the technologies of exploitation and not lastly, the biological material (Cocan and Mireşan, 2011). Fish meat quality can be maxim if optimal environmental conditions are taken into account (Cinemre et al., 2006; Fornshell, 2002; Uiuiu et al., 2019).

The productive capacity and the economic efficiency also depend on the anatomic-morphological characteristics of the exploited species, as well as on some physiological and microstructural features (Topuz et al., 2017; (Cocan and Mireșan, 2018).

In Romania, aquaculture production is carried out almost exclusively in classic landscaping, such as ponds and pools, where extensive and semi-intensive breeding technologies are applied. The bio productive potential of these types of arrangements is limited, which is why it is necessary to implement new fish breeding

technologies, super intensive systems, to ensure both quantity and quality for the consumer requirements of fish meat. Having certain limits of variation, determined by factors such as species, age of fish, harvest season, geographical area, environmental conditions and operating systems, as well as the feed used (Ihuț et al., 2018), fish meat has special organoleptic qualities and high nutritional value.

MATERIALS AND METHODS

The biological material used in this study was sampled from Trecătoare Ursului trout farm, Râșnov, Brașov County and it was represented by Rainbow trout (*Oncorhynchus mykiss*) and Brook trout (*Salvelinus fontinalis*) species.

The experiment took place in July 2019. In each stage of the technological flow of the traditional smoking process, 10 specimens from each species were analysed in each stage of the smoking process per lot (100 fishes/lot), with a body weight of approximately 250 grams per individual, resulting a total of 80 analysed individuals.

The traditional smoking of the specimens was done following the following technological flow: harvesting (with the help of fishing nets), stunning (mechanical stunning percussion in the dorsal-aboral region of the head), evisceration (the extraction of organs together with the peritoneum of the general cavity and the formed blood clots), washing (removal of mucositides and impurities adhering to the fish surface, elimination of blood and viscera), salting (the salting time is at least 16 hours, under refrigeration conditions, 2–4ºC) and desalting (in desalination (washing), the amount of salt remaining on products is max. 3%), drying (the salted fish is washed and left to dry, an interval between 2 and 4 hours), smoking (the fuel used for smoking is made of beech and cherry wood, stifled when necessary, with nettle and fir satin. It is used a cold smoke for one and a half hour, after a warm smoke at 65-70ºC and then hot smoking at 70-85ºC for a maximum of 2 hours) and packaging and storage (it is made in cold spaces, under refrigeration conditions, to ensure the 45 days validity of the product).

The specimens used in the experiment were clinically healthy, in order to not interfere with the determination of chemical composition of the trout meat throughout the technological flow related to traditional smoking conservation procedures. For this, specimens of each species were sacrificed, and the meat was separated manually from fins, skin, bones and viscera. The separated meat was macerated well using pestle and used for further analyses.

The analyses determined were: water (Water%), dry matter (SU%), fat (Gb%), protein (Pb%), non-nitrogenous extractive substances (SEN%) and mineral substances (Ash%).

The determination of the water content (Water%) was done by drying the samples in the oven at 103ºC (mandatory in case of litigation). The dry matter content (SU%) was determined by the percentage difference of the mass of the sample to be analysed and the water content, determined by drying the sample in the oven.

The determination of the crude protein (Pb%) was done by the Kjeldahl method. The total nitrogen in the sample to be analysed was converted into ammonium ions under the catalytic action of copper sulphate in sulfuric acid and potassium sulphate. After alkalization, ammonia was introduced with water vapor, which was captured in a hydrochloric acid solution, subsequently titrated with 10n sodium hydroxide.

The determination of fat was done by extraction with organic solvent (petroleum ether) using the Soxhlet apparatus. After extraction it fallowed the solvent removal, drying and weighing of the extracted fat. The determination of non-nitrogenous extractive substances was done by mathematical calculation and the determination of mineral substances (Ash%) was done by calcining the samples to be analyzed at a temperature of 600ºC, for 5 hours. All the chemical analyses of the fish meat were carried out by well-established methods used to determine these parameters (Iurca & Răducu, 2006), and the results have been statistically processed and are presented in the following section.

RESULTS AND DISCUSSIONS

In order to determine the chemical composition of traditionally smoked trout meat, samples were taken from 10 specimens from each lot of fish prepared for smoking.

The analyses determined were made throughout the entire technological flow related to

traditional smoking conservation procedures (fresh, salted, desalted and smoked meat).
The chemical analyses of the fresh meat from the two species of salmonids exploited within the Trecătoare Ursului trout farm, showed that the highest percentage of water is found in the Rainbow trout 74.18±0.083%, followed by the Brook trout with a water content of 72.88±0.112% (Tabel 1).

Table 1. The chemical composition of fresh trout meat (n=10)

Species	Specification	X ± sx	V%	s
O. mykiss	Water (%)	74.18±0.083	1.12	0.834
	Dry matter (%)	25.82±0.083	3.23	0.834
	Fat (%)	4.25±0.083	19.58	0.832
	Protein (%)	16.72±0.058	3.46	0.579
	SEN (%)	0.27±0.005	16.70	0.046
	Ash (%)	4.58±0.041	8.87	0.406
S. fontinalis	Water (%)	72.88±0.112	1.54	1.121
	Dry matter (%)	27.12±0.112	4.13	1.121
	Fat (%)	5.20±0.023	4.40	0.229
	Protein (%)	16.16±0.071	4.40	0.711
	SEN (%)	0.21±0.008	38.72	0.081
	Ash (%)	5.54±0.031	5.51	0.305

* X ± sx – Mean ± Standard error of mean; V% - coefficient of variability; s – standard deviation; n – number of samples from every lot.

The dry matter content, which is inversely proportional to the water content, was higher in Brook trout (27.12±0.112%) than in the Rainbow trout (25.82±0.083%). The fat content showed the highest value in the Brook trout (5.20±0.023%) and in the rainbow trout (4.25 ± 0.083%). Regarding the content in crude protein, the obtained values were quite close to both species of salmonids, however with a maximum value recorded in the case Rainbow trout (16.72±0.058%) and the Brook trout (16.16±0.071%). The non-nitrogenous extractive substances were in the Rainbow trout 0.27±0.005%, and in the case of the Brook trout, 0.21±0.008%. The minerals obtained after the calcination, presented the following values: Brook trout - 5.54±0.031%; and Rainbow trout - 4.58±0.041%.
Noteworthy is the coefficient of variability reduced for all species, for the determinations. These low values of the coefficients of variability do nothing but certify the accuracy of the determinations made, even if the number of samples has been reduced.
The chemical analyses of the salted meat from the two species of salmonids exploited within the Trecătoare Ursului trout farm are shown in Tabel 2.
Analysing the data regarding the chemical composition of the meat subjected to salting process, it is possible to observe a decrease in the water content, compared to the fresh meat, regardless of the species. Thus, the lowest water content was recorded in the Brook trout - 69.54±0.092%, the Rainbow trout - 71.12±0.076%.

Table 2. The chemical composition of salted trout meat (n=10)

Species	Specification	X ± sx	V%	s
O. mykiss	Water (%)	71.12±0.076	1.07	0.761
	Dry matter (%)	28.88±0.076	2.64	0.761
	Fat (%)	3.16±0.016	5.16	0.163
	Protein (%)	15.06±0.075	4.95	0.746
	SEN (%)	0.27±0.007	24.34	0.065
	Ash (%)	10.39±0.043	4.11	0.427
S. fontinalis	Water (%)	69.54±0.092	1.32	0.921
	Dry matter (%)	30.46±0.092	3.02	0.921
	Fat (%)	4.33±0.062	14.22	0.616
	Protein (%)	16.98±0.149	8.78	1.492
	SEN (%)	0.32±0.008	25.65	0.082
	Ash (%)	8.82±0.062	7.01	0.619

* X ± sx – Mean ± Standard error of mean; V% - coefficient of variability; s – standard deviation; n – number of samples from every lot

Due to the fact that the dry matter content is inversely proportional to the water content, it was higher than compared to the fresh meat data values. After salting, the highest value was obtained in the case of the Brook trout (30.46±0.092%), the rainbow trout (28.88±0.076%).
As a result of the salting process, the percentage of fat was reduced, by about one percent, regardless of the species. Thus, in the case of the Brook trout, the fat content of the salted meat was 4.33±0.062%, and for the Rainbow trout it was of 3.16±0.016%. Throughout salting process, the following values were obtained regarding the level of the protein according to the species, Brook trout - 16.98±0.149%, respectively Rainbow trout - 15.06 ± 0.075%.

The non-nitrogenous extractive substances (SEN%) had a weight of 0.32 ± 0.008% in the case of the Brook trout and 0.27 ± 0.007% in the case of the Rainbow trout and the mineral substances (ash) had a very significant weight following the salting of the meat, resulting for the Rainbow trout a weight of 10.39±0.043%, for the Brook trout 8.82±0.062%. The coefficient of variability was low for all species, aspect which show us the accuracy of the determinations made.

For future stage of smoking, the trout were washed and desalted, respecting the technological flow. The results of the analyses for the desalted trout can be seen in Table 3.

Table 3. The chemical composition of desalted trout meat (n=10)

Species	Specification	X ± sx	V%	s
O. mykiss	Water (%)	73.81±0.099	1.34	0.989
	Dry matter (%)	26.19±0.099	3.78	0.989
	Fat (%)	3.05±0.032	10.44	0.318
	Protein (%)	15.57±0.104	6.69	1.042
	SEN (%)	0.21±0.005	25.03	0.051
	Ash (%)	7.36±0.035	4.73	0.348
S. fontinalis	Water (%)	71.23±0.094	1.32	0.942
	Dry matter (%)	28.78±0.094	3.27	0.942
	Fat (%)	4.59±0.04	8.71	0.400
	Protein (%)	16.99±0.128	7.56	1.285
	SEN (%)	0.51±0.011	20.63	0.106
	Ash (%)	6.68±0.058	8.63	0.576

* X ± sx – Mean ± Standard error of mean; V% - coefficient of variability; s – standard deviation; n – number of samples from every lot

Following the desalting process, as an important stage of the technological flow for obtaining the traditional smoked trout, changes in the chemical composition of the meat have appeared again in the two species of salmonids exploited within the Trecătoarea Ursului trout farm.

Therefore, the water content increased slightly due to carcass washing and easy assimilation through osmosis processes. In this case, the rainbow trout presented the highest water content - 73.81±0.099% and Brook trout - 71.23±0.094%.

As we expected, with the slight increase in the water content, the weight of the dry matter (SU%) was reduced, the Rainbow trout having a lower value (26.19±0.099%), compared to the Brook trout (28.78±0.094%).

The fat content (Gb%) does not show major changes compared to the previous stage of the technological flow, the highest value being obtained in the Brook trout, 4.59±0.04% and in the Rainbow trout 3.05±0.032%. As in the case of salted meat, the content of protein (Pb%) is situated around 16%. The content of non-nitrogenous extractive substances (SEN%) presented for the Rainbow trout an average value which is almost half when it's compared to the Brook trout (Rainbow trout - SEN% = 0.21 ± 0.005; Brook trout - SEN% = 0.51 ± 0.011. The highest weight of minerals was recorded in the case of meat subjected to desalinisation for the rainbow trout (Ash% = 7.36 ± 0.035).

Going through all stages in chronological order to the technological flow for obtaining traditionally smoked trout in Table 4 is shown the chemical compositions of smoked meat.

Table 4. The chemical composition of traditionally smoked trout meat (n=10)

Species	Specification	X ± sx	V%	s
O. mykiss	Water (%)	66.14±0.045	0.68	0.451
	Dry matter (%)	33.86±0.045	1.33	0.451
	Fat (%)	4.10±0.062	15.19	0.623
	Protein (%)	20.97±0.042	2.02	0.424
	SEN (%)	5.91±0.029	4.86	0.288
	Ash (%)	2.86±0.017	5.86	0.167
S. fontinalis	Water (%)	64.25±0.14	2.18	1.400
	Dry matter (%)	35.75±0.14	3.92	1.400
	Fat (%)	5.66±0.053	9.41	0.533
	Protein (%)	21.86±0.064	2.91	0.636
	SEN (%)	5.19±0.085	16.33	0.848
	Ash (%)	3.04±0.029	9.65	0.293

X ± sx – Mean ± Standard error of mean; V% - coefficient of variability; s – standard deviation; n – number of samples from every lot

The finished product, the traditional smoked trout, after drying, baking and smoking at high temperature, lost its water content, as follows: the rainbow trout has the highest content (66.14 ±0.045%) followed by the Brook fountain (64.25 ±0.140%).

As expected, the percentage of dry matter (SU%) regardless of the analysed species, increased as a result of the smoking and baking process. Therefore, the highest value recorded,

we found in the Brook trout, 35.75±0.140%, and in the Rainbow trout it was 33.86±0.045%.
The content of fat (Gb%) recorded after smoking had the following percentages: Rainbow trout 4.10 ± 0.062% and Brook trout 5.66 ± 0.053%. There is a significant increase in the content of protein in traditionally smoked trout. Therefore, the crude protein (Pb%) showed an increase to 20.97±0.042% for the Rainbow trout and in the case of the Brook trout to 21.86±0.064%.
The content of non-nitrogenous extractive substances (SEN%) presented the following values for smoked trout meat (finished product): Rainbow trout - 5.91±0.029% and Brook trout - 5.19±0.085% and regarding the mineral content of traditionally trout smoked meat, it is 2.86 ± 0.017% in the Rainbow trout and 3.04 ± 0.029% in the Brook trout.
When we analysed the obtain data per the entire technological flow, we noticed that chemical parameters had significant changes during the stages. We can notice that in comparation with the fresh meat, after the salting process occurs, the water content decreased, fact that is primarily due to the osmosis processes. The dry matter content increased, because is inversely proportional to the water content.
As a result of the salting process, the percentage of fat was reduced, by approximately one percentage, regardless of the species and the mineral substances (ash) had a significant weight following this process.
After the salting stage, according to the technological flow discussed in the Material and Method's Section, the trout carcasses were desalted (washed with water and left to dry), therefore, the water content increased slightly due to carcass washing and easy assimilation through osmosis processes. Also, the dry matter content decreased comparative with the salting process. As a result of the desalting, the chemical constituents did not present values different from the other situations, except for the water content which increased by about 1%.
The finished product, the traditional smoked trout, after drying, baking and smoking at high temperature, has lost a high quantity of its water content, with the increase of the dry matter. Also, there is a significant increase in the protein content in smoked trout in comparation with the fresh, salted and desalted meat. This is primarily due to the change of the aminoacidic structure which is in relationship with the heat release during the smoking process.
Both rainbow trout and Brook trout traditionally smoked meat registered values are within the limits cited in the scientific literature (Alçiçek, 2011; Cardinal et al., 2001; Duedahl-Olesen et al., 2010; Arvanitoyannis and Kotsanopoulos, 2011; Tóth & Potthast, 1984; Jittinandana et al., 2002; Popescu et al., 2019) and present no risk for human consumption.

CONCLUSIONS

Chemical analyses of the traditionally smoked trout meat from the Trecătoarea Ursului trout farm were made for two species of salmonids exploited within the farm, in the entire technological flow.
Therefore, the chemical analyses of the meat pointed out that the highest percentage of water content is found in the Rainbow trout comparative to Brook trout in all stages of the technological flow. On the contrary, the dry matter content, which is inversely proportional to the water content, was higher in the Brook trout than in Rainbow trout.
As for the fat content we noticed that the Brook trout registered in all cases higher values than the Rainbow trout. Regarding the protein content, the obtained values were quite close to both species of salmonids, however with a maximum value recorded in the case of Brook trout. The content of non-nitrogenous extractive substances (SEN%) and the minerals content was also higher in the case of Brook trout.
We recommend and propose at the same time the continuation of studies in this field, especially regarding the composition of the smoke and its influence on the quality of the finished products (wood essences, aromatic hydrocarbons, etc.) with direct implications on the food safety for human consumption.

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GROWTH OF SIBERIAN STURGEON (*ACIPENSER BAERII*), RUSSIAN STURGEON (*ACIPENSER GUELDENSTAEDTII*) AND HYBRID (F1 *A. BAERII* X *A. GUELDENSTAEDTII*) REARED IN CAGES

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Abstract

The study clarifies the growth of Siberian Sturgeon, Russian Sturgeon and their hybrid grown on an industrial cage farm. Three groups within each genotype of male individuals with different initial body weight were formed, as follows: Russian Sturgeon - I - 2.504±0.060 kg; II - 3.042±0.063 kg; III - 3.693±0.074 kg; Siberian Sturgeon - I - 1.395±0.034 kg; II - 2.277±0.065 kg; III - 2.925±0.066 kg; Hybrid (Siberian Sturgeon x Russian Sturgeon) – I - 2.081±0.050 kg; II - 3.049±0.051 kg; III - 3.704±0.058 kg. It was found that in I and II group of Siberian Sturgeon the relative growth for the whole period was practically the same, respectively 59.1 and 57.9%. The fish from group III had a significantly smaller growth (24.6%). In Russian sturgeon, in the case of fish that are growing slower at the beginning of the growing season, a mechanism for compensatory growth is established. The smallest fish grew best at the beginning of the period and the second and the fish from third group grew at the end of the growing season. In this species, the total relative growth in group I was 39.5%, group II - 15.2%, group III - 12.0%. The relative growth over the whole period in the hybrid of I, II, III groups was 75.3%, respectively; 23.7%, 26.7%.

Key words*: aquaculture, body weight, cage – farm, genotype, sturgeon.*

INTRODUCTION

The development of sturgeon breeding is conditioned, on the one hand, by the delicacy harvested production and, on the other, by the global critical status of natural populations of sturgeon species, which is why their catch in many countries is prohibited (Bloesch et al., 2005; Vasileva, 2015, etc.).

Unlike in Europe, where in general sturgeon breeding is not sufficiently developed (WWF, 2012), regardless of the importance of the industry, in Bulgaria sturgeon breeding is developing intensively.

The country ranks twelfth in the world for total sturgeon biomass and eighth for caviar production (Bronzi et al., 2019). Initially, the Bulgarian sturgeon breeding was based on the Russian sturgeon, but gradually other species were introduced, such as Beluga, Sterlet, and Stellate sturgeon.

In recent years, the share of Siberian sturgeon has increased (Nikolova, 2019). Along with keeping the species in pure condition, hybridization between them is often used to increase production efficiency.

Ponomareva et al. (2019) note that the study of the characteristics related to the fishery productivity of sturgeon hybrids under specific growing conditions enables them to be successfully implemented in aquaculture production.

Kocabaş et al. (2015) emphasize the lack of information on the growth of sturgeons when reared in aquaculture farms, especially in the growth-out phase. The authors have studied the growth of Russian sturgeon in circular fiberglass tanks for 5 years. The growth of Siberian sturgeon in basins has been studied in the Czech Republic (Kurfürst et al., 2000), in indoor fiberglass tanks in Iran (Zare et al., 2009).

Despite the good development of sturgeon farming in Bulgaria, research in this field is extremely insufficient. Separate studies have been conducted only on natural populations (Peycheva et al., 2004; Dobrovolov et al., 2004, 2005; Tsekov et al., 2008; Tsecov and Tsecov, 2013).

The main technology of sturgeon breeding in Bulgaria involves the separation of fish by sex, with females being used to produce caviar and males not included in breeding flocks being sold

for meat. In this regard, it is necessary to know the patterns of growth, the timing for the rational fattening of male fish in specific conditions when grown on industrial farms.

The purpose of this study is to investigate the growth of male individuals of the Siberian sturgeon (*Acipenser baerii*), the Russian sturgeon (*Acipenser gueldenstaedtii*) and their hybrids (F1 *Acipenser baerii* x *Acipenser gueldenstaedtii*), with different initial body weight, reared on a cage farm.

MATERIALS AND METHODS

The study was conducted at an industrial cage farm.

The cages are located in a warm water reservoir with an average altitude of about 280 m in Southeastern Bulgaria. The reservoir has an area of 16.07 km² and a volume of 532.9 x 106. m³.

At the beginning of the season, experimental groups with average body weight (BW) were formed as follows: 1. For a four-summer old Siberian sturgeon (Ab) - 1.395 ± 0.034 kg (group I); 2.277 ± 0.065 kg (II gr.); 2.925 ± 0.066 kg (III gr.). 2. For six-summer-old Russian sturgeon (Ag) - 2,504 ± 0.060 kg (group I); 3,042 ± 0,063 kg (II gr.); 3,693 ± 0.074 kg (III gr.); 3. six-summer-old Hybrid (Ab x Ag) - 2.081 ± 0.050 kg (group I); 3,049 ± 0,051 kg (II gr.); - 3.704 ± 0.058 kg (III gr.). Each group was grown in a separate cage. The cages are 8x8 m in size, with a depth of water surface of 6 m. Each cage is equipped with double polyamide nets. Planting density was 20 kg m^{-2}.

Samplings were made during the growing season (223 days), weighing 50 randomly selected individuals from each experimental group.

The fish were fed with a factory-specialized completed granulated sturgeon feed. The composition of the feed mixture is shown in Table 1.

The relative increase (K) of fish for each period between samplings was determined by the formula:

$$K = \frac{W_1 - W_0}{W_0} \times 100\%$$

;

where W_0 is the initial body weight, kg; W1 - final body weight for each period, kg.

IBM SPSS Stasistics 21 was used for statistical processing.

RESULTS AND DISCUSSIONS

The results of the experiment show that when reared in a cage industrial farm Siberian sturgeon, Russian sturgeon and their hybrid have good growth capacity. The fish grew throughout the whole growing season, including the October-December period (Table 2).

However, trends in the growth of individual groups are different. In addition, we found in the comparative analysis that the initial body weight does not always linearly determine the final body weight.

Table 1. Proximate composition of fish diet

Indices	Percent of dry weigh
Crude protein, %	46
Crude fat, %	15
Crude fiber, %	1.4
Ash, %	6.5
P, %	1.03
Ca, %	1.4
Na, %	0.3%
Vitamin A, IU.kg^{-1}	10 000
Vitamin C, mg.kg^{-1}	520
Vitamin E, mg.kg^{-1}	200
Vitamin D_3, IU.kg^{-1}	2 303
Gross energy, MJ.kg^{-1}	21.0
Digestible energy, MJ.kg^{-1}	19.2

With significant intergroup differences in body weight at the beginning of the period, a compensatory growth is observed in some of the groups, which practically eliminates the differences.

In Siberian sturgeon, the fish from the first group (with the lowest BW) were significantly different from the other body weight groups from the beginning to the end of the observed period. The difference between the first and second and first and third groups in May was 0.882 kg ($p<0.001$) and 1.530 kg ($p<0.001$), respectively. In December, the difference between the first two groups increased by 56.2%, reaching 1.378 kg ($p<0.001$), and between the first and third groups - practically remained - 1.425 kg ($p<0.001$). Second- and third-group fish are nearly levelling at BW at the end of the season. The difference in the sign in May is 0.648 kg ($p<0.001$) and in December 0.047 kg ($p>0.05$).

Table 2. Growth indices of individual species and hybrids, kg

Group	Period	Ab (n=600)			Ag (n=600)			Ab x Ag (n=600)		
		$\bar{X}$	SE	SD	$\bar{X}$	SE	SD	$\bar{X}$	SE	SD
I	May	1.395	0.034	0.242	2.504	0.060	0.429	2.081	0.050	0.357
	June	1.445	0.054	0.385	3.180**a**	0.075	0.531	2.780	0.060	0.427
	October	2.022	0.084	0.590	3.365**b**	0.090	0.634	3.238	0.086	0.607
	December	2.220	0.084	0.596	3.492**c**	0.062	0.435	3.647**a**	0.112	0.790
II	May	2.277	0.065	0.458	3.042	0.063	0.447	3.049	0.051	0.361
	June	2.716	0.080	0.564	3.202**a**	0.083	0.583	3.423	0.066	0.470
	October	3.531**a**	0.106	0.749	3.279**b**	0.103	0.727	3.607	0.092	0.652
	December	3.598**b**	0.123	0.867	3.503**c**	0.079	0.561	3.771**a**	0.084	0.592
III	May	2.925	0.066	0.468	3.693	0.074	0.498	3.704	0.058	0.411
	June	3.177	0.070	0.496	3.721	0.074	0.526	3.852	0.068	0.480
	October	3.619**a**	0.092	0.650	3.843	0.096	0.676	4.480	0.084	0.595
	December	3.645**b**	0.064	0.451	4.137	0.099	0.699	4.691	0.107	0.758

The differences between the values without index, within the month, in the columns are significant - p <0.01
The values with the same index in the columns are not significant: a; b; c - p> 0.05

The Russian sturgeon, unlike the Siberian sturgeon, the fish with the highest BW (group III) significantly exceeded the other two groups from the beginning to the end of the observed period. The difference between the third and first and third and second groups in May was 1.189 kg ($p<0.001$) and 0.651 kg ($p<0.001$) respectively, and in December, respectively 0.645 kg ($p<0.001$) and 0.634 kg ($p<0.001$). The fish in the second group, compared to the first one, in May have 0.538 kg ($p<0.001$) higher BW, and in June the fish from the two groups practically equalized in the BW, the difference in advantage of the second group is only 0.022 kg ($p>0.05$). In the period June-October, the fish in the second group continued to lag in growth and at the end of the period the difference is 0.086 kg, although insignificant ($p>0.528$), was in advantage of the first group. At the end of the growing season, the relevant average body weight of the fish in the two groups is practically the same, with a difference of 0.011 kg ($p>0.05$) in advantage of the second group.

The hybrid growth trends are similar to those of the Russian sturgeon. The group with the highest body weight at the beginning of the observation (group III) significantly exceed the other two groups throughout the growing season. At the beginning of the season, the difference between the third and the first, and the third and second group is 1.623 kg ($p<0.001$) and 0.655 kg ($p<0.001$) respectively, and in December - 1.044 kg ($p<0.001$) and 0.920 kg ($p<0.001$). Unlike the Russian sturgeon, however, a similarity of BW between the fish in the first and second group was reported at the end of the period in December, and during the whole period the difference gradually decreased: May - 0.968 kg ($p<0.001$); June - 0.643 kg ($p<0.001$); October - 0.369 kg ($p<0.001$); December - 0.124 kg ($p = 390$).

The features of the growth of the fish can be clearly seen in the figures, which show the dynamics of relative growth. Figure 1 shows the growth of the Siberian sturgeon. In general, this species is introduced into Bulgarian aquaculture later, compared to Russian sturgeon, but gradually its share is increasing, displacing other sturgeon species. Falahatkar (2018) notes that Siberian sturgeon is one of the best sturgeon for captive breeding and shows high growth rates under different conditions. There is a successful attempt to cultivate the species on trout farms when feeding on trout mixtures (Köksal et al., 2000). Zare et al. (2009), in a study of the growth of three-year-old Siberian sturgeons, found that fish of the species are tolerant of high density stocking, successfully cultivated in fiber-glass tanks and individuals, with an average initial body weight of 460 g have reached an average final body weight of 668.8 g for a period of 61 days. Yazdani Sadati and Vlasov (2006) found that fish growth was dependent on initial body weight in a study of the growth of one-year-old Siberian sturgeon reared in tanks. The biggest ones had higher growth - up to 1.6 times compared to the average ones and up to 1.3 times compared to the smallest ones.

In our experiment, the highest relative increase in Siberian sturgeon - 59.1% had the smallest fish (group I), with fish from the intermediate group (group II) approaching them considerably - 57.9%. The third group of fish grew considerably less, with a relative increase of

24.6%. The difference in relative growth between the first and third group is 2.4 times. At the beginning of the season, the second group of fish increased most intensively, with a relative increase of 19.3% from May to June. In the same period it was 3.6% in the first group and 8.5% in the third. During the period June - October, the fish of the second group continued to grow intensively. Their relative growth was 29.8%, but the highest growth for the period was reported in the first group - 39.9%. Compared to the first group, the fish from the third group increased almost three times less. Between October and December, growth rates decreased considerably, to 9.9% in the first group and up to 1.9% in the second group. The third group of fish did not increase during this period, with a relative increase of 0.7%.

Analyzing the growth dynamics of Russian sturgeon, it can be seen that there is a compensatory growth in the following periods (Figure 2), with the fish growing less early in the growing season. At the beginning of the season, the smallest fish (the first group) grew best, and from May to June they accumulated the major part (27%) of the growth received during the whole period (39.5%). In the period June - October and October - December, the relative growth decreased to 5.8% and 3.8% respectively. At the beginning of the season in the second fish group a relative increase of 5.3% was reported, in June - 2.4%, and in the period October-December - 6.8%. The same trend for intensive growth at the end of the growing season was observed in the group with the highest initial body weight. The fish in this group from May to June, practically did not grow, with a relative growth of 0.8%. From June to October the relative growth increased to 3.3%, and the main accumulation of body weight (7.7%) was obtained at the end of the growing season (October-December). The relative growth in the Russian sturgeon of the highest body weight group was the lowest for the whole study. Generally, it can be stated that in this species the relative growth for the season was the highest for the smallest fish (39.5%), followed by medium (15.2%) and large (12.0%). Fedorov et al. (2017) have studied Russian sturgeon at different ages and different initial body weight when grown in a pond fish farm in a polyculture with herbivorous fish species. The data provided by the authors show that the smallest fish had the best relative growth followed by the big fish. Those with the average initial body weight grew the worst, with the difference with the smallest ones being over 2 times.

The hybrid highest relative growth for the whole period was found in the first group - 75.3% (Figure 3). In the second group of fish it was 23.7% and in the third group - 26.7%. In the first and second group the main growth was in the period May-June, respectively 33.6 and 12.3%. In the period June-October, the growth in these groups decreased to 16.5 and 5.4% respectively. An opposite trend is observed in the third group. In the period May-June, the relative growth was 4%, while in June-October it increased 4 times, reaching 16.3%. In the period October-December, fish from all experimental groups continued to grow at lower levels of intensity - 12.6%; 4.6% and 4.7%, respectively for the first, second and third group. The resulting relative growth in hybrids with the lowest initial body weight (first group) was highest throughout our whole study.

In general, the interest in various hybridization schemes in the sturgeon breeding is conditioned by the intention to increase production efficiency. Siberian sturgeon hybrids with other sturgeon species are considered dominant in the leading sturgeon breeding countries (Shen et al., 2014; Bronzi et al., 2019). Main studies of hybrid (Siberian x Russian sturgeon) growth indicators are in the juvenile stage (Guo et al., 2011, 2012). The hybrids of Siberian and Russian sturgeon show high productive performance in different production systems (Efimov, 2004; Iskakova and Kulmanova, 2014; Miburo, 2018).

They grow well both in mountainous reservoirs with lower water temperatures (Magomaev and Chipinov, 2011) and in RAS (Levina et al., 2015). Growing in RAS allows hybrids to show their high performance for high productivity. Thus, according to Ponomareva et al. (2019), individuals with an average body weight of 0.726 kg were obtained from fish with an initial body weight of 0.020 kg for 8.5 months from a study of the growth of hybrid Russian and Siberian sturgeon in RAS. Our study shows that under the conditions of an industrial cage farm located in a warm water reservoir in Southern

Bulgaria, the hybrid of Siberian and Russian sturgeon grows well and may be one of the most perspective for cultivation. At the same (six-year-old) age, hybrids had a higher relative growth than Russian sturgeon by 25.7%, 7.7%, and 13.1%, respectively, for the lowest, middle, and highest weight group.

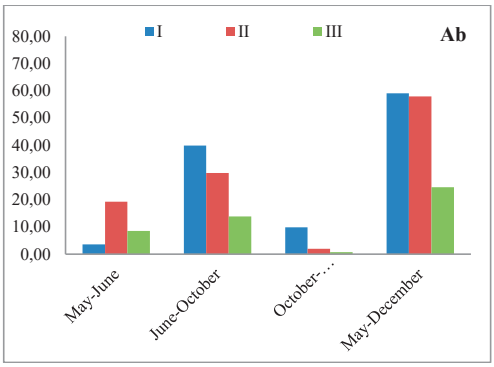

Figure 1. Relative growth of Siberian sturgeon from different groups, %

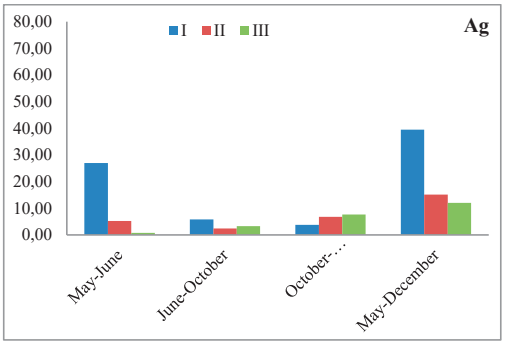

Figure 2. Relative growth of Russian sturgeon from different groups, %

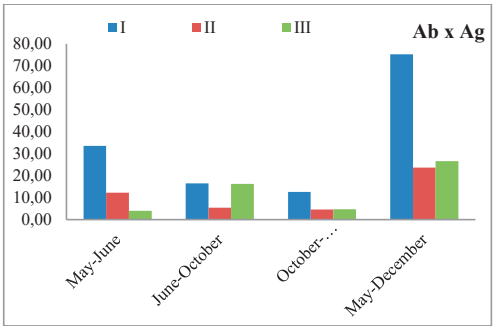

Figure 3. Relative growth of the hybrid from different groups, %

CONCLUSIONS

Siberian sturgeon, Russian sturgeon and their hybrid have good growth capacity when reared on an industrial cage farm. The fish have been growing throughout the growing season, including the October-December period.

It was found that in I and II group of Siberian Sturgeon the relative growth for the whole period was practically the same, respectively 58.6 and 57.9%. The fish from group III had a significantly smaller growth (24.57%).

In Russian sturgeon, in the case of fish that are growing slower at the beginning of the growing season, a mechanism for compensatory growth is established. The smallest fish grew best at the beginning of the period and the second and the fish from third group grew at the end of the growing season. In this species, the total relative growth in group I was 39.46%, group II - 15.15%, group III - 12.02%.

The relative growth over the whole period in the Hybrid of I, II, III groups was 75.25%, respectively; 21.71%, 26.65%.

The hybrid of Siberian and Russian sturgeon grows well under the conditions of an industrial cage farm located in a warm water reservoir in southern Bulgaria and may be one of the most perspective for cultivation.

At the same (six-year-old) age, hybrids had a higher relative growth than Russian sturgeon by 25.7%, 7.7%, and 13.1%, respectively, for the lowest, middle, and highest weight groups.

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HEALTH PROFILE OF *ALOSA IMMACULATA* (BENNET, 1835) DURING ITS SPAWNING MIGRATION IN THE DANUBE

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Abstract

This study characterizes the haematological and parasitological profiles of the Pontic shad during its passage in the freshwater environment (Danube, Mm 74-77) as part of the reproduction migration. The investigations were carried out by classic methods on specimens captured during the 2011 and 2018 migrations. The obtained values for the haematological parameters, in the both samples sets, varied in the following ranges: red blood cells count (RBCc) 1.40÷3.82 (×10^{6}cells/μL), haemoglobin (Hb) 9,29÷16.87 (g/dL), haematocrit (Ht) 32,75÷88.00 (%), mean corpuscular volume (MCV) 146,37÷298.47 (fL), mean corpuscular haemoglobin (MCH) 38.32÷80,33 (pg), mean corpuscular haemoglobin concentration (MCHC) 15.14÷34,95 (g/dL). From parasitological point of view, in the analysed Alosa immaculata, the most frequently encountered parasitosis was produced by Contracaecum aduncum, in direct correlation with the shad's diet. The prevalence of the infestation with this nematode was quite high in both data samplings. Extending the future research regarding the specie's health condition at different times of migration, from Black Sea till Danube upstream, can complete the profile of the physiological reactions of this species with great ecological importance.

Key words*: Alosa immaculata, condition, haematology, health, parasitology.*

INTRODUCTION

In-depth knowledge of the biology of the Pontic shad (or Danube shad) *Alosa immaculata* (Bennet, 1835), a specific migratory species of the Ponto-Caspian basin together with sturgeons, has become of increased interest for Danube's riparian countries, considering its ecological relevance due to transition between marine and freshwater environments and ability to cope with the challenges associated to these habitats' heterogeneity (Ciolac, 2004; Višnjić-Jeftić et al., 2013; Dobrin et al., 2013; Năvodaru and Năstase, 2014; Rozdina et al., 2015). These studies are scientifically interesting, highlighting the great value of the migratory fish species as tools for researching the function of the habitats interconnection during fish life cycle and the ecosystems overall health, diversity and conservation (McDowall, 1997; McIntyre et al. 2015; Tamario et al., 2019), as well as for understanding the patterns in fish behaviour plasticity or phenotypic flexibility, too (Bernatchez and Dodson, 1987; Griffths, 2006; Bloom et al., 2018).

The adults of the Pontic shad, after they have grown and reached sexual maturity in the marine environment of the Black Sea, return in the Danube River for reproduction, early in spring. This anadromous movement of the Pontic shad starts under the influence of some environmental cues like the thermal regime of the water and, especially, by the intensity, amplitude and duration of the spring floods discharged in the river mouths (Năvodaru, 1997; Năvodaru and Waldman, 2003; Ciolac and Patriche, 2004; Rozdina et al., 2010). So, this species presents a physiological plasticity that allows it to cope, simultaneously, with the environment's change, the completion of gonad maturation for spawning, energy consummation for upstream swimming, as well as with the modifications into the feeding regime and parasitological profile during migration.

The latest published data indicate an increased decline of the Pontic shad stocks, suggesting potential issues in the conservation status due to anthropogenic pressures such as water pollution (Višnjić-Jeftić et al., 2010), lower water level (Năvodaru and Waldman, 2003), overfishing mainly in brackish waters from the Danube River mouth area where fish are more exposed waiting for the changeover in their osmotic regulating systems from saline to freshwater.

For maintaining the physiological balance, the organism mobilizes his resources and regulatory mechanisms and, among these, the blood carries a primary homeostatic role. The reaction of the blood's constituents consists in qualitative and quantitative changes reflecting the homeostasis possibilities and the health status (Cristea and Munteanu, 1995; Witeska, 2013; Maceda - Veiga et al., 2015).

In order to know more about the physiological reactions of this species, the present paper brings new data, additionally to those reported in a previous study (Grecu et al. 2018), for describing the blood features in relation to shoal shads' fitness and parasitological status associated to each sampling period.

MATERIALS AND METHODS

The collected specimens of Pontic shad were captured for scientific purposes during the spawning migrations along Cotul Pisicii area (Mm 74), in the same period, respectively first decade of May in 2011 (n=18) and 2018 (n=22). Almost 2 mL of blood samples were collected into tubes containing lithium heparin as anticoagulant, *in situ*, immediately after the catch, by excising the caudal peduncle, carefully avoiding the water contact, and transported at 4-8^{o}C for haematological analyse in the labs. After blood sampling, the shad specimens were transferred, individually, in polyethylene bags for parasitological study. Further, all the analyses were carried out within the Research Centre MoRAS-UDJ Galati (https://erris.gov.ro/ROMANIAN-CENTER-FOR-MODELLIN).

Fish condition

Each shad specimen was measured for the total length (Lt, cm) at a precision of 5 mm, weighed to an accuracy of 1 g scale for total weight (Wt, g) and fish condition was assessed by the length-weight relationship (LWR) $W=a \times L^{b}$ (Jones et al., 1999). The analyse of the growth rate through this indicator by determining the exponent "b" (regression slope) and it's difference from isometric value of "3.0" indicates the direction of condition change.

Haematological analysis

The blood was analysed within 4 hours after the sampling by routine methods used in fish haematology: the red blood cells count (RBCc, x 10^{6} /μL) was determined on Neubauer improved hemocytometer at a magnification of ×400 using Vulpian diluting solution. The haematocrit (Ht, %) was determined with microcapillary tubes centrifuged for 5 minutes at 12000 rpm. The haemoglobin concentration (Hb, g/dL) was spectrophotometrically determined at 540 nm wavelength applying cyanmethaemoglobin method. Using standard formulas, the red blood indices were calculated: the mean corpuscular volume (MCV), the mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC).

Parasitological analysis

The sampled fish were examined for ectoparasites and endoparasites using standard parasitological procedures. The taxonomic classification and identification of the parasites were done according to Munteanu (2005) and Bauer (1984, 1985, 1987). Areas around the fins, nostril, operculum and the buccal cavity were examined for external parasites (monogeneans and crustaceans). Each fish was opened dorso-ventrally and its internal organs were examined for parasites. The entire digestive system was removed and placed in a Petri dish with physiological saline solution. Observations on parasites were performed under stereomicroscope or classic microscopy using the AXIO-Imager A1 (ZEISS) microscope connected to a CCD camera AXIOCAM MR3 for images capture. The usual indicators (prevalence, intensity level, mean intensity) were used to express the infection level after Bush at al. (1997) excepting the intensity of parasitism that was assessed using an arbitrary scale in function of the parasite density into the microscopic field: low (less than 5 parasites identified in a microscopic field); medium (5 to 10 parasites), high (more than 10 parasites).

Statistical analysis

The obtained data were statistically analysed for normal distribution using Kolmogorov-Smirnov test ($p>0.05$, K-S test) and independent t-test ($p>0.05$, t-test) from SPSS Statistics 17.0 program, and descriptive statistics from Microsoft Excel 2016 program. Also, the data heterogeneity was assessed by computing the variation coefficient (CV, %) and the results were presented as mean ± standard deviation (M±SD) for haematological data and mean ± standard error (M±S.E.) for parasitological data.

RESULTS AND DISCUSSIONS

Fish condition

The series of data analysed for each year showed no deviations from the normal distribution ($p>0.05$, K-S test) that permitted us to apply furtherly the parametric tests. Statistics reveals significant differences in Pontic shad size between years (Table 1). Migration sampling from 2011 consisted of shads between 29.00 to 34.50 cm long in size and 210-326 g weight that correspond, according to Năvodaru and Waldman (2003), to a dimensions structure associated to 3- and 4-years old shads. In comparison, the sizes of the 2018 individuals ranged between 23.5 to 30.00 cm length and 111 to 198 g weight that correspond to younger shads, belonging to the 2- and 3-years classes. The obtained equations for LWR in each migration present different "b" coefficients indicating allometric growth ($b\neq3$) but still ranging in the limits determined by other authors (Table 1). Monitoring the LWR in a fish population, for longer periods, based on old and new data gathered over the time, is considered a simplified system in assessing the fish welfare in wild population (Bolger and Connolly, 1989; Jones et al., 1999) because they indicate the tertiary or whole-animal responses to stress (Barton et al., 2002) with relevance in fish population management under consideration. Basically, in the conventional fisheries model, values of $b<3.0$ indicates a decrease in condition or elongation in form, $b>3.0$ indicates an increase in the condition because the increase in weight exceeds the increase in length. The larger the difference from 3.0, the higher change in the condition could be observed. Yilmaz and Polat (2011) found no significant difference in the slopes of Pontic shad females and males within season, meaning that the female and male individuals have the same body shapes in the same seasons. Therefore, the fact that the b value calculated for 2018 sampling is lower than that the 2011 value, for the same season period of sampling (beginning of May), could be attributed to a poorly nutritional status of the 2018 shad individuals from our catch. A possible constraint in our results is related to the small number of specimens used for condition determination. This condition factor is influenced by internal factors like diet, disease, reproductive status or by those external including season, site binding conditions, chemical exposure.

Table 1. Biometrics of the analysed Pontic shads

Statistical parameters	W_t (g)		L_t (cm)		Length-weight relationship (LWR))	
	2011	2018	2011	2018	2011	2018
M±SD.	266.71±39.20[a]	140.86±24.44[b]	31.96±1.55[a]	26.11 ±1.70[b]	$W = 0.0182 * L^{2.7674}$	$W = 0.042*L^{2.4856}$
CV (%)	14.70	17.35	4.85	6.51	$R^2 = 0.8483$ b=2.7674	$R^2 = 0.9388$ b=2.4856
Năvodaru and Waldman (2003)					$W = 0.0563*L^{2.457}$ b=2.457	
Rozdina et al. (2013)					$W = 0.0234*L^{2.7315}$ $R^2 = 0.95$, b=2.7315	
Năvodaru and Năstase (2014)					$W = 0.0142*L^{2.8735}$ $R^2 = 0.8442$, b=2.8735	
Ibanescu et al. (2016)					$W = 0.0526* L^{2.487}$ $R^2 = 0.85$, b=2.487	

Different letters in a row indicate significant differences between data series for the same variable ($p < 0.05$; independent t-test)

Haematological profile

The statistical analyse of the blood data series presents normal distributions ($p>0.05$, K-S test) allowing the application of parametric tests.

The overall data table of the Pontic shad blood parameters shows high values (Table 2), in correlation with the specie's natural motility. The pelagic Pontic shad which actively migrates upstream in the Danube for spawning is an anadromous members of the family Clupeidae. Generally, all the family members are considered comparatively strong swimmers (Clough et al., 2001).

For American shad (*Alosa sapidissima* Wilson, 1811), Leonard and McCormick (1999) concluded that its performance and migratory success is increased by the changes in haematological physiology that occurred during upstream swimming.

In our studies, the RBCc indicates a range of variation from 1.40 to 3.82 ($x10^6$ cells/µL), highlighting the intense process of erythropoiesis required to cover the high oxygen uptake necessary to such an aerobic exercise.

The comparison of the RBCc data shows a significant difference between the years: the smaller values of the 2018 shads could be explained by the dominance of the younger individuals (2 years age) and by the inferior status of the shad's condition.

Similar RBCc values were indicated by several authors: 3.0÷4.2 $x10^6$ cells/µL (Soldatov, 2005), 3.0 $x10^6$ cells/µl (Stancioiu et al., 2006) for pelagic actively migrating species. The increase of the erythropoietic rate in order to maintain the level of functional activity of the organism could also explains the release of juvenile erythrocytes like polychromatophilic normoblasts found into the circulating blood of 2011 specimens, in a percentage of 6.09 ± 1.19 % (Grecu et al., 2018).

The Ht is an accurate blood analyse parameter and considered a valuable primary diagnostic index for fish health. Our data show a positive and direct correlation of high Ht values with the increased RBCc explained above. The range of 2011 Ht values varied between 48-88 % and that of 2018 from 32.75 to 61.4% maintaining the same statistic difference between the samples. These high values, over the known amplitude for majority fish species that ranging from 20% to 45% (Hrubec & Smith, 2010), are comparable with those published by Leonard & McCormick (1999) concerning the American shad. In their study, the authors found significant difference biased by sex (60±0.5 % for males and 54±0.7 % for females) with a clear Ht elevation tendency along the upriver sites. Jawad et al. (2004) found for Indian shad (*Tenualosa ilisha* Hamilton, 1822) a positive and direct relation between haematocrit values and the length of the fish under a polynomial equation. Also, the haematocrit of herring (*Clupea harengus* Linnaeus, 1758) was found positively correlated with body length and temperature (Everaarts, 1978). A similar tendency could be observed on our data, too.

The Hb is considered the most direct measure of oxygen carrying capacity (Huston, 1997; Witeska, 2013), reflecting the aerobic swimming ability of the fish. The high values determined for this parameter (11.76-16.10 g/dL in 2011; 9.29-16.87 g/dL in 2018) indicate that the members of this species are capable of sustained locomotor activity for fulfil the migration process by upstream swimming, without been influenced by their condition status or age. From this point of view, no statistical difference between years was determined. Higher haemoglobin values were also reported for American shads, increasing progressively, during their upriver migration (Leonard and McCormick, 1999).

Table 2. Haematological parameters of the analysed Pontic shads

Haematological parameters		RBCc ($x10^6$ /µL)	Ht (%)	Hb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)
2011	M±SD	2.85 ±0.57 [a]	58.42±8.71 [a]	13.97±1.15	211.53±44.28	50.96±11.21 [a]	24.24±2.92 [a]
	CV (%)	20.10	14.91	8.23	20,93	22.00	12.06
2018	M±SD	2.42 ±0.47 [b]	50.62±6.96 [b]	14.17±1.74	213.64±32.81	60.04±10.02 [b]	28.19±2.84 [b]
	CV (%)	19.42	13.74	12.28	15.36	16.69	10.07

Different letters in a column indicate significant differences between data series for the same variable ($p < 0.05$; independent t-test)

Based on the found haematological parameters, the erythrocyte's indices were further determined, the results reflecting the state of physiological effort and featuring the transport capacity of the erythrocyte in oxygen.

For the MCV, data statistics shows no significant difference between the average annual values. However, the reference range can be greatly extended taking into account the variation limits of the values range (154.45-29.47 fL in 2011; 146.37-277.26 fL in 2018).

The MCH indicates a significant high load with haemoglobin of the erythrocyte for 2018 shads (40.10-80.33 pg) in comparison with those from 2011 (38.32-73.16 pg).

The MCHC varies significantly between years due to differences in size of circulating RBCs. The average value of MCHC in 2018 indicate that the erythrocytes were loaded with a higher amount of haemoglobin (28.19 g/dL), meaning that almost 28% of the erythrocyte volume consists of haemoglobin that carries oxygen to the tissues (23.21-34.95 g/dL) in comparison to 2011 (15.14- 28.14 g/dL).

The data assessment of the erythrocyte's indices shows slightly higher values for 2018, possible indicating the presence of more mature red cells into the circulating blood of 2018 shads. Hrubec and Smith (2010) explained that higher MCV, MCH, and MCHC could be found in older cells due to increase in size and haemoglobin content with time during RBC development.

Parasitological profile

Four species of parasites belonging to 3 systematic groups were identified (Table 3) in both analysed samples of *Alosa immaculata*. The prevalence of total parasite infestation showed the maximum degree of 100 % due to the fact that all the specimens were contaminated with at least one kind of parasite.

The highest prevalence of parasitic infestation belonged to the nematode *Contracaecum aduncum* (syn. *Hysterothylacium aduncum* Rudolphi, 1802) which was found into pyloric caeca, stomach and intestine of 94.44 % of specimens in 2011 and 95.45% in 2018, respectively (Figure 1). The worm burden in 2011 was quite higher in comparison with 2018. This nematode is a typical marine parasite that can only reach freshwaters along with hosts that migrate from the sea to rivers due to the diet spectrum of the shad, mainly composed of crustaceans (Mysidae, Gammaridae etc.) and small fish species which are the primary intermediate hosts for those intestinal nematodes (Munteanu and Bogatu, 2005).

The monogenic worm *Mazocreas alosae* (Hermann, 1782), a specific parasite of the Pontic shad (Figure 2), was determined as the next most common infestation after was isolated in almost 2/3 of specimens even if the presence of this parasite on the shad gills was considered low.

The presence of *Allocreadium isoporum* (Looss, 1894) in the intestine of affected fish (fig. 3) was assessed only in the 2018 samples and was observed an extreme dynamic among individuals, from 2 to 57 parasites/host. Also, only 2 specimens of another digenean trematode were found in the intestine of one shad during 2018, assigned after morphological features as, most probably, *Coitocoecum skrjabini* (Ivanitzky, 1928).

During 2018 sampling examination, traces of the marine pollution by plastics were found in the form of particles ingested and present in the terminal blind sac of the shad stomach (fig. 4). It was a clear proof of the magnitude scale in aquatic pollution by plastics and the fact that it became a very worrying problem of nowadays.

Table 3. Species of parasites identified in the analysed Pontic shads

Systematic group	Species of parasite	Organ/area infested	Prevalence (%)		Parasitic intensity (level)		Mean intensity (M±S.E.)	
			2011	2018	2011	2018	2011	2018
Monogenea	*Mazocreas alosae*	gills	66.67	72.73	Low	Low	4.17±0.67	5.63±0.95
Trematoda	*Allocreadium isoporum*	intestine	-	31.82	-	Medium	-	11.00±7.70
	*Coitocoecum skrjabini**	intestine	-	4.55	-	Low	-	2.00±0.00
Nematoda	*Contracaecum aduncum*	pyloric caeca, stomach, intestine	94.44	95.45	High	High	55.76±7.65	32.38±6.88

*see explanation in the text

Figure 1. General view of *Contracaecum aduncum* attached to the stomach tunica

Figure 2. General view of the monogenean *Mazocreas alosae* detached from the Pontic shad gills

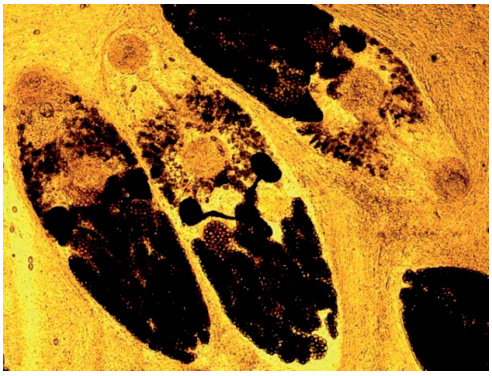

Figure 3. Digenean *Allocreadium isoporum* into the intestinal mucosa of the Pontic shad (x 100)

Figure 4. Plastics particles present into the shad's digestive tract

CONCLUSIONS

Phylogenetically, the migrations of the diadromous fish were caused by various reasons as a life strategy for species survival. In present-day, anthropogenic pressures have seriously impacted the migration pathway continuity or the habitats quality, with unpredictable results that will affect the migration benefit itself.

The Pontic shad *Alosa immaculata* is a typical diadromous fish species with a long-distance locomotor activity from marine saltwater to freshwater as adults and a passive drift for shad's larvae from Danube River towards the Black Sea. Specialists with interest in fisheries management are looking for more information about shad biology that are necessary for species conservation and responsible stocks management.

Any information on physiological, biochemical or behavioural reactions during the migratory activity of the species can bring valid answers on the ability of the fish to cope and acclimate to environmental fluctuation without being forced beyond its limits.

Our goal was to continue the investigation of the Pontic shad patterns in its physiological flexibility for maintaining welfare status during spawning migration. In the condition of our size sampling constraint, data regarding shad condition reflects a small decrease in shad fitness between 2011 and 2018 suggesting the onset of physiological stress during the 2018 migration, with younger age class dominance.

The Pontic shad samples showed an intense erythropoiesis process (ranges with extremely high values of RBCc, Ht and Hb) explaining the high blood oxygen carrying capacity of the species, as is expected in such energetic effort imposed by upstream swimming in Danube River during high flows springs. Our comparative data between 2011 and 2018

exhibited the dependence between haematological variables and fish condition and, possible, fish age.

From parasitological point of view, the analysed *Alosa immaculata* showed a massive infestation with intestinal nematodes, in both sampling, this aspect being related to the feeding regime at the beginning of migration.

Our study results regarding the haematological and parasitological features of the Pontic shad enlarge the biological database of this species, being useful to evaluate the quantitative and qualitative changes that may occur in the alteration of organism' equilibrium state. Future research on Pontic shad should find how its organism regulates the blood's osmotic pressure during the transition from sea to freshwater and other physiological responses during environmental change. These features correlated and integrated with studies regarding genetic diversity in the shad population spur future applied research for species protection.

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STUDY ON THE MODELS OF EVOLUTION OF THE AFRICAN SWINE FEVER OF WILD BOARS IN ROMANIA

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Abstract

The study followed for a period of two years the evolution of African swine fever (ASF) in wild boars in Romania in 188 hunting funds from 41 counties. A total of 1471 positive boars were identified with African swine fever, of which 1217 were found dead and 254 were harvested positive by hunting. Several ways of disseminating the disease specific to the Romanian territory have been identified. A specific transboundary diseases crossing also was described. In parallel, the African swine fever dynamics was compared with the classical swine fever in wild boars. From the analysis of the data obtained, two models of the spread of the disease in the wild fauna were identified. Comparing the harvest of the two hunting seasons, a wave movement was observed from east to west of Romania, the number of cases remaining constant.

Key words*: African swine fever, disease spread, wild boar.*

INTRODUCTION

African Swine Fever (ASF) is an epizootic disease with a major impact on the economy, through the significant damage it causes to the livestock sector (EFSA Journal, 2014). The disease is caused by a virus of African origin confined to wild boar or feral pigs populations (Bosch et al., 2017). After the 1970's and 1980's episodes in the Mediterranean basin, a first outbreak was reported in Georgia in 2007 (Gogin et al., 2013), and then the disease spread to the East and Central Europa (Alexander, 2016).

In Romania, due to the conjugation of specific factors: the varied geographical area, the growth of pigs in individual households, as well as atypical weather phenomena, the epizootic covered in a short time the entire territory of the country, creating major problems for pig farms (Bosch et al., 2014).

Pork exports were blocked and production decreased significantly. Wild boars played an important role in the chain of spreading the disease in domestic pigs (De la Torre et al., 2015).

The present paper tried to identify the ways of spreading of ASF (African Swine Fever) in wild boar, the behavioural characteristics of wild boars and an estimate of the future evolution of the disease in wildlife (Bonney et al., 2014; Massei et al., 2015).

MATERIALS AND METHODS

In the summer of 2018, there were multiple ASF outbreaks in wild boars and domestic pigs in the Danube Delta (DW, 2018). Following the explosive dynamics of the disease, hunting associations under the coordination of veterinarians have prepared a program for surveillance and control of the disease (Acevedo et al., 2014). Wild boar collection teams were organized from 188 hunting funds (Boitani et al., 1995). The hunt was done by the method "on the run" during the day, or on the prowl at night, using night vision equipment.

Hunters have been trained on how to safely transport corpses to the carcass collection centres without eviscerating them on the spot - to prevent the virus from spreading in the surrounding environment (Commission Implementing decision (EU) 2019/100, 2019).

At the same time, the detection of wild boar corpses in the respective areas and the collection of long bones were encouraged in order to highlight any viruses in the bone marrow.

ELISA and PCR tests were used in county laboratories to identify ASF virus (Commission Decision 2003/422/EC, 2003; Soto, 2017).

RESULTS AND DISCUSSIONS

Evaluating the data obtained from the 188 hunting funds, an evolution of the disease was found in a wave shape, starting from the south-eastern border of the country and continuing to the west. From the data collected on the field, a significant increase was observed in the number of dead pigs in the Danube Delta starting with July 2018, as a result of massive hunts executed on the Ukrainian shore of the Danube.

The frightened wild boars changed their travel routes and crossed the Chilia Chanel in large numbers, or threw themselves into the sea and drifted along the Black Sea coast. With the dry season, the animals chose to migrate westward along the Danube River, using the left bank of the water, which is below, and form a meadow. In the following weeks the wild boars climbed the tributaries of the river: Siret, Ialomita, Arges, Olt. These rivers with surrounding meadows have provided a very good habitat for animals providing them with moisture and access to food, especially agricultural crops. During this migration, the sick animals came into contact with other local wild boar populations, infecting them as well (Howey et al., 2013). During the winter of 2018-2019, the disease crossed the Carpathian Mountains and appeared in eastern Transylvania. Figure 1 presents the counties of Romania with the number of wild boars found positively for ASF in 2018.

Figure 1. ASF in wild boars in Romania, 2018

The end of January 2019 was characterized by a weather anomaly: the frozen rain. The phenomenon had unusual consequences for the ecosystem. The flower buds of the oaks froze and thus the trees did not bear fruit in 2019, depriving the fauna in the hilly and mountainous areas of an important food: acorns. As a result, wild boars in search of food descended on the households of the villagers and further spread the disease. Figure 2 presents the counties of Romania with the number of wild boars found positively for ASF in 2019.

Figure 2. ASF in wild boars in Romania, 2019

Unlike (CSF) classical swine fever in which the domestic pig transmits the disease to the wild boar, in the case of ASF the disease is transmitted from wild boar to domestic pig.

This pattern of spreading the ASF was different in the northeastern part of the country where the plague virus also entered from Ukraine, the border being mountainous, forested and difficult to access and advancing from north to south (Bueno et al., 2009).

Following the number of positive cases from the hunted wild boars, especially from the corpses identified in the territory, a decrease in the number of positive cases was observed over time. The situation at the beginning of 2021 shows a disappearance of sick animals or carcasses positive for ASF in the regions where the epizootic was reported three years ago. For example, in the counties of Tulcea and Constanta (Danube Delta) only a few cases were reported, while in the west of the country where in 2018 and 2019 there were 0 cases, currently the disease is evolving.

In the remote mountainous areas the number of animals harvested by hunting was small; instead there was a decrease in the density of wild boars due to high mortality (Figure 3).

Figure 3. Wild boars shooting of different ages

It was gradually concluded that the reduction in the number of wild boars by massive hunting, including during the breeding season, does not give the expected result.

Herds of wild boars have a well-established migration area, the specimens following certain routes that describe ellipses with a radius of 16-20 km and no more.

If the virus has entered a population, it is recommended that the animals be left in place and not be frightened by shooting (even if shock absorbers are used).

Wild boars feel the dead animal and instinctively leave that place migrating and thus spreading the disease.
Particularly in Romania there is an aggravating factor in the spread of the plague virus, due to the increased number of large carnivores: the Carpathian bear. These are the only predators or scavengers that manage to break the long bones of wild boar carcasses (Toïgo et al., 2008).

Knowing that ASF virus is abundant in the blood and bone marrow, through this additional element, the spread of the disease also increases. Comparing the data on the evolution of classical swine fever in wild boar, a specific dynamic was observed. If the number of animals falls below 0.5% per km2 the disease disappears naturally (Figure 4).

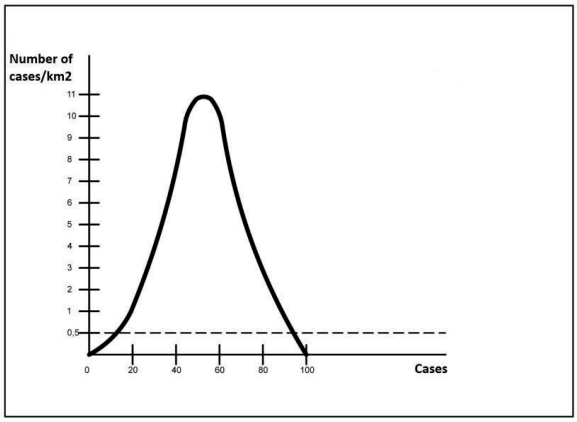

Figure 4. Classical swine fever dynamics in wild boars

In the case of ASF, in the same situation of 0.5% per km2, due to the much longer period of preservation of the virus in the blood and in the bone marrow, the corpses remain a source of contamination for a much longer period of time (up to 9 months) (Figure 5).

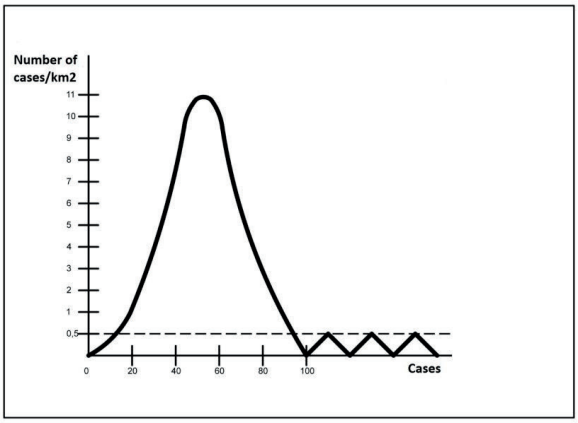

Figure 5. ASF dynamics in wild boars

In this way the virus is transmitted from one year to another, surviving the winter period. If in the case of classical swine fever the elimination of the disease from households decreases the pressure on wild boars, in the case of ASF there is a continuous passage of the virus between domestic pigs and wild boars.

Thus, wild boars infect domestic pigs in various ways: contamination of crops, direct contact with domestic animals, if they are left free, or if shelters are not protected by fences and wild boars are attracted to the smell of sows in heat.
On their turn, domestic pigs can be a source of contamination through carcasses, mismanaged

manure or contaminated by-products. In this way the disease can be maintained in wild boar herds from one year to another.

CONCLUSIONS

It was gradually concluded that the reduction in the number of wild boars by massive hunting, including the breeding season, does not eradicate the African swine fever disease.
If in the case of classical swine fever the elimination of the disease from households decreases the pressure on wild boars. In the case of African swine fever there is a continuous passage of the virus between domestic pigs and wild boars.
Fencing the north-east border, combined with a high surveillance system, may reduce a new wave of African swine fever in Romania.

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HAEMATOLOGICAL PROFILE OF RAINBOW TROUT UNDER DIFFERENT FEEDING INTENSITIES

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Abstract

The aim of this study was to evaluate the haematological profile of rainbow trout after applying of different intensities of feeding (FL1-2.5% BW day^{-1}, FL2-3% BW day^{-1}, FL3-3.5% BW day^{-1}, FL4-4 % BW day^{-1}, FL5-4.5 % BW day^{-1} and, ad libitum-FL6). Fish were reared in a recirculating aquaculture system (RAS), provided with twelve rearing units (in duplicate) for 44 days. Blood samples were collected from twelve fish on each experimental variant, and were made before and after the experimental trial. Red blood cell counts (RBCc), haematocrit values (Hct), haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total serum glucose (Glu) and total proteins (TP) were determined. Also, in order to determine the leukogram and the absolute number of leukocytes, blood samples were collected and used immediately to make smears. At the end of the experiment, ANOVA test revealed no significant changes ($p>0.05$) in the RBCc, Hct, Hb, MCV, MCH, MCHC and glucose concentration (Glu), while the total proteins (TP) from blood serum increased significantly ($p<0.05$) with the increasing of the feeding intensity, except for ad libitum feeding, were total serum protein decreased. Regarding the total number of leukocytes, in general, it has not been registered statistically significant changes ($p>0.05$). Lymphocytes (relative and absolute) have remained relatively constant, without significant variation ($p>0.05$). The main conclusion that emerges from this current study shows that in the case of rainbow trout reared under recirculating aquaculture system conditions, feeding intensity does not significantly influence the haematological profile.

Key words*: feeding intensity, haematological profile, rainbow trout, recirculating aquaculture system.*

INTRODUCTION

In the last years, global fish production has notably increased, intensive farming having a big contribution to the development of aquaculture.

In Romania, rainbow trout is a major farmed species, due to its adaptability at intensive farming conditions.

Between all the technological aspects from a fish farm, feeding is one of the most important factors which can affect fish growth performance, welfare, and physiological status. In salmonid aquaculture, feeding cost represents the highest cost from the farm operations, accounting for around 50% of the total cost of production (Rauw et al., 2016), that`s why it is crucial to determine the optimal feeding rate.

Choosing the optimal feeding intensity is very important in the case of recirculating production systems because non-consuming feeds represent the main factor of deterioration of water quality and implicit of fish welfare. Feeding intensity can be influenced by many factors, such as time of day, season, water temperature, dissolved oxygen levels, and other water quality variables (Craig and Helfrich, 2002). Generally, the optimal growth of fish and welfare is directly associated with feed and feeding conditions (Peres and Oliva, 2014). Because feed management can affect the blood cells, the examination of fish haematology may be helpful in the assessment of nutritional status and diagnosis of diseases (Gingerich et al., 2010).

In this context, the objective of the present study was to evaluate the haematological profile of rainbow trout after applying of different intensities of feeding (FL1-2.5% BW day^{-1}, FL2- 3% BW day^{-1}, FL3-3.5% BW day^{-1}, FL4-4 % BW day^{-1}, FL5-4.5 % BW day^{-1}, and *ad libitum*-FL6).

MATERIALS AND METHODS

Experimental design and feeding trial. The study was carried out at Faculty of Food Science and Engineering, University of Galați, Romania. Fish were procured from a fish farm from

Prejmer, Brașov County, România. The experimental fishes were first acclimatized to laboratory conditions for two weeks (14 days) and were fed with commercial fish feed (54% C.P) (Table 1).

Table 1. The composition of the experimental diet

Composition	U.M	Quantity
Crude protein	%	54
Crude lipids	%	18
Cellulose	%	1
Ash	%	10
Phosphorus	%	1.4
Digestible energy	Mj/kg	19.4
Vitamin A	UI	14000
Vitamin D3	UI	2300
Vitamin E	mg	250
Vitamin C	mg	500
Lysine	%	3.5
Methionine	%	1.5
Cystine	%	0.7
Ingredients: Fish meal, fish oil, hemoglobin, full-fat soy, soybean oil, wheat gluten, sunflower meal, wheat, and wheat products.		

After two weeks, 360 rainbow trout fingerlings (average weight of 34.17±0.11 g) were randomly distributed in a recirculating aquaculture system, composed of twelve rearing units, with a capacity of 132 L, each. The experimental design and data regarding the growth performance indicators were published in our previous paper (Crețu et al., 2019).

Fish were fed manually twice per day at 9 a.m. and 6 p.m. at different feeding levels: FL1-2.5% BW day^{-1}, FL2- 3% BW day^{-1}, FL3-3.5% BW day^{-1}, FL4-4 % BW day^{-1}, FL5-4.5% BW day^{-1} and, *ad libitum*-FL6, with the same diet used during acclimatization. In the *ad libitum* feeding, fish were fed until the first two or three pellet remains to the bottom of the rearing units.

Collection and Determination of Experimental Fish Blood Samples. At the beginning of the experiment and at the end of the experiment, from each experimental variant, 7 fish were randomly sampled in order to evaluate the haematological profile. Fish were anesthetized and blood samples were taken from the caudal vein employing a heparinized syringe. After sampling, a part of the blood was placed in heparinized Eppendorf tubes and another part in un-heparinized tubes for determining the glucose and total protein. At the same time, for determining the leukogram and the absolute number of leukocytes blood smears were made, fixed and colored with May-Grünwald Giemsa panoptic method (MGG). The type of leukocytes was determined based on identification characters listed by Svobodová et al., 1991. The number of thrombocytes and the white blood cell count were determined in relation to 10000 erythrocytes and converted to unit blood volume.

The haemoglobin concentration (Hb, g dl^{-1}) was determined based on cyanmethemoglobin spectrophotometry method. In this context, 20µl of the blood sample was taken from the lithium heparinized tube with the aid of a pipette. Mixing was achieved by slow inversion of the tube, for about 20 times with 5 ml of Drabkin's solution. After that, the samples were read at SPECORD 210 Spectrophotometer Analytikjena, at the wavelength of 540 nm.

The red blood cell counts (RBCc, $\times 10^6$ µL^{-1}) was determined by counting the erythrocytes from 5 small squares of Neubauer hemocytometer using Vulpian diluting solution.

The haematocrit (Ht, %) was determined by microcentrifugation of whole blood for 5 minutes at 12000 rpm in a microhematocrit centrifuge.

Using standard formulas (Ghergariu et al., 1985), the erythrocyte constants were calculated: the mean corpuscular volume (MCV), the mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC).

Determination of glucose (Glu) and total protein (TP) from serum was performed spectrophotometric by SPECORD 210 Analytik Jena. For glucose determination we used the colorimetric method with o-toluidine, readings were made at 635 nm wavelength. Total proteins from serum were determined by Biuret method, the readings were done at a 546 nm wavelength.

Statistical analysis. Data obtained from the experiment was expressed in mean±SD and it was subjected to ANOVA test using SPSS 21 version. When the ANOVA reveals significant difference ($p<0.05$), Duncan multiple range test was used to compare differences among individual treatment means.

RESULTS AND DISCUSSIONS

The results of haematological analyses are presented in Table 2. The results of our study are

analysed in in conjunction with the results obtained regarding the performance of fish farming, results that were previously published by Crețu et al. (2019). At the end of the study fish individual weight increased with the increasing of feeding level, ranging from 87 g in FL1 to 125 g in FL6.

Analysing the values obtained for the haematological indices at the end of the experiment it is observed that are within the optimum range of the species, being observed some changes with feeding intensity.

At the end of the experimental period, the hematocrit (PCV, %) values showed an insignificant increase ($p>0.05$) compared to the initial moment for all the experimental variants. Also, comparing the hematocrit values between the six experimental variants, no significant differences were obtained ($p>0.05$), varying between 39.62% and 43%.

Table 2. The haematological parameters of rainbow trout at different feeding intensities

Experimental variants	Average±SD					
	PCV (%)	Hb (g dl^{-1})	RBCc (x10^6 μl^{-1})	MCV (μm^3)	MCH (pg)	MCHC (g dl^{-1})
Initial	38.27±3.95	9.25±0.97	1.41± 0.16	274.46±53.42	66.26±11.69	24.45±4.00
FL1	42.00±3.55^{ac}	8.35±0.31^{ad}	0.90±0.12^{ad}	473.16±49.82^{ad}	94.63±11.97^{ad}	20.03±1.94^{ad}
FL2	41.25±1.03^{ac}	8.46±0.25^{ad}	0.95±0.17^{ad}	445.77±81.09^{ad}	91.80±17.48^{ad}	20.58±0.75^{ad}
FL3	41.25±4.68^{ac}	8.70±0.55^{ac}	0.96±0.18^{ad}	440.32±79.96^{ad}	94.02±21.70^{ad}	21.31±2.58^{ac}
FL4	39.62±2.62^{ac}	8.49±0.33^{ac}	0.91±0.15^{ad}	448.03±80.98^{ad}	95.62±13.74^{ad}	21.49±1.48^{ac}
FL5	40.12±4.19^{ac}	8.47±0.22^{ad}	0.89±0.16^{ad}	465.23±95.77^{ad}	98.31±19.44^{ad}	21.34±2.64^{ac}
FL6	43.00±3.93^{ac}	8.36±0.29^{ad}	1.01±0.16^{ad}	433.12±68.64^{ad}	84.20±11.51^{ad}	19.60±2.04^{ad}
Zorriehzahra et al., 2010	47	9.4	1.33	353	71	20
Ghittino, 1983	24 - 55	7.6 - 16	0.8 – 1.5	276 - 476	55 - 82	14 - 26

RBCc - red blood cell counts, PCV - haematocrit, Hb - haemoglobin concentration
MCV - mean corpuscular volume, MCH - mean corpuscular haemoglobin, MCHC - mean corpuscular haemoglobin concentration

a- No Significant differences between the experimental variants, $p>0.05$.
b- Significant differences between the experimental variants, $p<0.05$.
c- No significant differences between the initial moment of the experiment, $p>0.05$.
d- Significant differences between the initial moment of the experiment, $p<0.05$.

Comparing the haemoglobin concentration with the values from the initial moment it was observed a significant decrease ($p<0.05$) in the experimental variants FL1, FL2, FL4, FL5, FL6, respectively an insignificant decrease ($p>0.05$) in the variant FL3. Regarding the influence of feeding intensity on haemoglobin concentration, no significant differences ($p>0.05$) can be observed in all six experimental variants.

The red blood cells showed no significant differences ($p>0.05$) between the all experimental variants, but a significant decrease was observed ($p<0.05$) in comparison with the initial moment.

The erythrocyte constants were calculated after the determination of the hematological indices. The obtained results revealed some changes in the values, in comparison with the initial moment, and between the six experimental variants.

Comparing the MCV values between the six experimental variants, the ANOVA test showed no significant changes ($p>0.05$). However, as a compensatory reaction, the reduction of the number of red blood cells caused the increase of the MCV values, being observed slightly higher values in the case of FL1 and FL5 (FL1- 473.16±49.82 μm^3; FL5- 465.23±95.77 μm^3), while in the case of intensities of FL2, FL3 and FL4 similar values were recorded (FL2- 445.77±81.09 μm^3; FL3-440.32±79.96 μm^3; FL4- 448.03±80.98 μm^3.

Regarding the obtained values of mean corpuscular haemoglobin concentration (MCH) at the end of the experiment is observed a significantly increasing tendency ($p<0.05$) with the feeding intensity, but no significant differences ($p>0.05$) were recorded between the six experimental variants.

The mean corpuscular haemoglobin concentration (MCHC) it can be observed a significant decrease ($p<0.05$) in the variant FL1, FL2 and FL6 respectively, from 24.45±4 g dl^{-1} (initial) to 20.03±1.94 g dl^{-1}, 20.58±0.75, respectively to 19.60±2.04 g dl^{-1}. In the case of the experimental variants FL3, FL4 and FL5,

there was no significant increase ($p>0.05$) compared to the initial moment.

Comparing the obtained values of MCHC in the six experimental variants, no statistically significant differences ($p>0.05$) were recorded.

In order to determine the influence of the feeding intensity on immune system, another aim of this experiment was to analyse the reaction of the leukocyte`s system.

In Table 3 are given the relative (the leukogram) and the absolute number of the various types of cells that make the leukocytic complex.

On the examined blood smears, we observed a predominance of the lymphocytes, followed by the neutrophils and monocytes. Eosinophils and basophiles were not found. Analysing the variation of the number of different types of the leukocytes some modification was recorded both, in comparation with the initial moment and between the six experimental variants, presented below.

Table 3. The relative and absolute modification of white blood cells (Average±SD)

WBCc	SI units	Average±SD						
		Initial	FL1	FL2	FL3	FL4	FL5	FL6
LK	$\times10^3$ cel μl^{-1}	63.59±14.88	50.79±11.52^{ad}	51.23±10.22^{ad}	54.20±14.26^{ad}	55.18±10.23^{ad}	55.55±14.03^{ad}	55.75±16.23^{ad}
T	$\times10^3$ cel μl^{-1}	2.89±1.20	13.39±7.36^{ad}	10.12±6.36^{ad}	10.04±5.62^{ad}	10.88±7.75^{ad}	10.55±6.45^{ad}	10.29±7.42^{ad}
Ly	$\times10^3$ cel μl^{-1}	58.83±5.97	45.69±10.11^{ad}	46.77±12.02^{ad}	50.41±10.24^{ad}	52.00±11.25^{ad}	52.08±10.65^{ad}	52.53±10.24^{ad}
	%	92.50±1.48	90.00±2.83^{ac}	91.25±1.60^{ac}	93.00±1.12^{ac}	94.25±1.35^{ac}	93.75±1.06^{ac}	94.25±1.31^{ac}
M	$\times10^3$ cel μl^{-1}	1.74±0.34	1.15±0.16^{ad}	0.89±0.12^{ad}	0.82±0.10^{ad}	0.55±0.09^{ad}	0.97±0.08^{ad}	0.56±0.09^{ad}
	%	2.75±0.07	2.25±0.35^{ac}	1.75±1.06^{ac}	1.50±0.71^{ac}	1.00±0.12^{ac}	1.75±0.15^{ac}	1.00±0.10^{ac}
N	$\times10^3$ cel μl^{-1}	3.01±1.07	3.96±1.1^{ac}	3.57±1.02^{ac}	2.97±1.09^{ac}	2.63±1.12^{ac}	2.50±1.16^{ac}	2.66±1.01^{ac}
	%	4.75±0.34	7.75±1.47^{ac}	7.00±1.54^{ac}	5.50±1.71^{ac}	4.75±1.35^{ac}	4.50±1.71^{ac}	4.75±1.35^{ac}

LK- Leukocytes; T- Trombocytes; Ly- Lymphocytes; M- Monocytes; N- Neutrophiles

a- No Significant differences between the experimental variants, $p>0.05$.

b- Significant differences between the experimental variants, $p<0.05$.

c- No significant differences between the initial moment of the experiment, $p>0.05$.

d- Significant differences between the initial moment of the experiment, $p<0.05$.

The results regarding the absolute number of leukocytes ($x10^3$ cells μl^{-1}), show a significant decrease in the circulating blood, compared to the initial moment in all the six experimental variants ($p<0.05$). The absolute number of leukocytes in the six experimental variants, showed an insignificant increase ($p>0.05$) with the increase of the feeding intensity.

The absolute number of thrombocytes revealed a significant increase in comparation with the initial moment of the experiment ($p<0.05$), while no significant differences ($p>0.05$) were recorded between the six experimental variants.

At the beginning of the experiment, the absolute number of lymphocytes recorded an average of $58.83\pm5.97\times10^3$ cells μl^{-1} of blood, showing a significant decrease in all six experimental variants ($p<0.05$). Comparing the absolute number of lymphocytes from the six experimental variants, it can be seen that the intensity of feeding did not lead to significant changes ($p>0.05$).

In comparation with the initial moment, the average of relative numbers of lymphocytes showed a slight increase, statistically insignificant ($p>0.05$) in the experimental variants FL3, FL4, FL5 and FL6, while in the case of variants FL1 and FL2 the percentage of lymphocytes shows an insignificant decrease ($p>0.05$). Also, comparing the values of the average percentage of lymphocytes between the six experimental variants, no significant differences ($p>0.05$) were registered.

The absolute number of monocytes did not show significant differences ($p>0.05$) between the experimental variants, but in comparison with the initial moment a significant decrease ($p<0.05$) was observed in all variants. Slightly higher values of the relative number of monocytes were recorded in variants FL1, FL2 and FL5. In comparison with the initial moment, the relative number of monocytes decreased slightly ($p>0.05$). The relative number of monocytes did not reveal significant differences between the six experimental variants ($p>0.05$).

Compared to the initial moment, the absolute number of monocytes registered a significant decrease ($p<0.05$) in all six experimental variants.

Comparing the absolute number of neutrophils between the six experimental variants the statistical analysis did not reveal significant differences ($p>0.05$). Also, no significant differences were notice in comparison with the initial moment ($p<0.05$).

Determining the value of the biochemical indicators represents an important aspect in defining the normal physiological state of the fish, because it reflects the possible pathological changes that may occur as a result of the organism's defence against stress conditions. Serum protein level is the most important indicator of the nutritional status of the fish, presenting, at the same time, wide quantitative and qualitative variations depending on the species, age, sex, sexual maturation stage, water temperature, quantity and feed quality (Patriche, 2008).

Determining the serum blood glucose is the most efficient and rapid method for assessing fish stress (Popescu et al., 1990, cited by Patriche, 2008). Stress in the environment can also be the cause of increased glucose levels in the serum (Martin et al., 1998, cited by Patriche, 2008). At the same time, nutritional status can have an important influence on the serum glucose levels.

Figures 1 and 2 present the values of total proteins and glucose.

Analyzing the values of the serum parameters, we can observe a significant increase of the serum proteins compared to the initial moment ($p<0.05$). Also, comparing the serum protein values between the six experimental variants, the statistical analysis revealed significant differences ($p<0.05$).

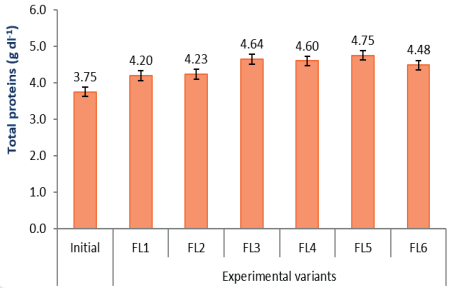

Figure 1. The values of serum total proteins recorded for rainbow trout

Thus, the significant increase of serum proteins was observed with the increase of the feeding level, while in the case of ad libitum feeding is observed their decrease.

Blood glucose content varies between 71.57± 4.43 mg dl^{-1} and 80.32±5.75 mg dl^{-1}. Slightly higher values were recorded in experimental versions FL1 (80.32±5.75 mg dl^{-1}), FL6 (77.26±5.68 mg dl^{-1}), FL2 (77.15±5.59 mg dl^{-1}), but the statistical analysis did not reveal significant differences between the six intensities of feeding ($p>0.05$). Compared to the initial time of the experiment, a significant increase ($p<0.05$) of the blood plasma glucose content was observed in all six experimental variants.

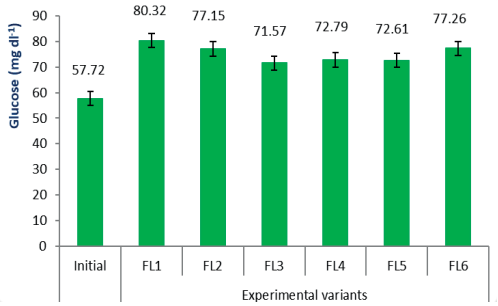

Figure 2. The values of serum glucose recorded for rainbow trout

CONCLUSIONS

Generally, the aim of the haematological studies is to contribute to an understanding of the relationship between blood characteristics and the adaptability of the species to the environment.

After the environmental factors, feeding affect the blood cells and the nutritional status of fish. Knowing that in a farm, feeding costs are very high, maximizing fish growth with lower costs, without affecting fish welfare represents a very important aspect from economical point of view. The results of our haematological study are corroborated with the results obtained regarding the growth performance and show that the feeding levels chosen do not influence the haematological and the immune response of the fish. In this context, we can say that the feeding level of 2.5% BW day^{-1} did not lead to significant changes of the hematological profile of rainbow trout, thus being indicated from the point of view of feeding costs.

The information obtained by the present study can be useful in developing some feeding strategies for rainbow trout in the intensive and semi-intensive culture

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THE IMPACT OF ENVIRONMENTAL LIGHT ON GROWTH PERFORMANCE OF JUVENILE CATFISH (*SILURUS GLANIS*, L., 1758) REARED IN A RECIRCULATING AQUACULTURE SYSTEM

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Abstract

The experiment aimed to determine the impact of environmental light (spectrum, intensity, and photoperiodicity) on the growth performance of juvenile catfish (Silurus glanis, L., 1758), reared in a recirculating industrial aquaculture system (RAS). The experiment was conducted over 60 days and was organized two variants of light intensity, were compared, in replicate, as follows: in first variant (V1) we used green light with an intensity of 80 lx and in the second version (V2) white light with an intensity of 260 lx. The same photoperiodicity regime was applied in both variants 12L:12D. The initial stocking density was 15.66 kg m^{-3}. The indicators of growth performance, obtained at the end of the experimental period, registered the following results: in the experimental variant V1 (white light), the final stocking density was 28.75 kg m^{-3}, SGR 1.44 %/day compared to V2 experimental variant (light green), compared to a final stocking density of 29.99 kg m^{-3}, a value of 1.31% day^{-1} for SGR were recorded. The final results of the study indicate a higher growth performance for the experimental variant with a green light (V2), a fact which demonstrates that juvenile catfish show technological plasticity concerning light regime.

Key words: *growth performance, light regime, RAS, Silurus glanis.*

INTRODUCTION

Light is a complex external and ecological factor whose components include color spectrum (quality), intensity (quantity) and photoperiod (periodicity). From the diversity of factors that determine the dynamics of fish biomass growth in a recirculating industrial aquaculture system, light has particular importance.

Light affects directly or indirectly almost all physiological processes (color or depigmentation), fish behavior, also growth, development and reproduction performances (Mc Cormick et al., 1998; Schreck et al., 2001). Generally, the larvae require a minimum level of light intensity for growth and in the case of certain fish prey for preventing cannibalism.

In the literature, numerous relevant scientific studies mention the fact that certain light colors affect the growth and development processes of different fish species. For example, silver carp (*Hypophthalmichthys molitrix*) and common carp (*Cyprinus carpio*) registered the best growth performances under green light according to Radenko and Alimov, 1991, and Ruchin et al., 2002; guppy (*Poecilia reticulata*) had a better growth under blue light (Ruchin, 2004); pikeperch (*Sander lucioperca*) grown best under red light (Luchiari et al., 2008), while goldfish (*Carassius auratus*) were able to distinguish both the intensity and color of the light (Neumeyer et al., 1991).

Photoperiod is a factor which has a direct effect on fish growth and survival rate (Puvanendran and Brown, 2002).

In fish culture, the importance of photoperiod has been confirmed by several studies. According to Smith et al. (1993), day length is the most important environmental factor acting on the appetite of the Atlantic salmon (*Salmo salar*). Feeding reduction for this species is directly related to the decrease in day length (Jørgensen and Jobling, 1992).

The African Catfish Clarias gariepinus, was catfish which has been thoroughly investigated with the effect of photoperiod was observed on growth, behaviour and stress variables (Appelbaum and Kamler, 2000; Almazan-Rueda et al., 2005).

From the above, it appears that information about *Silurus glanis* growth-related brightness, during the juvenile stage, is not known.

In this context, the main reason of our experiment was to determine the technological plasticity of juvenile European catfish (*Silurus glanis*) grown in a recirculating system, related to light intensity. We have also tried to assess the possible impact of light spectral structure on technological plasticity, respectively growth performance of culture biomass.

MATERIALS AND METHODS

Experimental design. The experiment was conducted for 60-days in a recirculating aquaculture system of the Department of Fisheries and Aquaculture, "Dunărea de Jos" University of Galaţi.

The configuration of the recirculating system for the *Silurus glanis* growth includes essential components: rearing tanks, water treatment equipment (mechanical, biological and chemical filters), disease control equipment, pumps, air conditioning equipment, independent electrical, respectively secondary components, monitoring equipment for water quality, in special, and food distribution system (Figure 1). The system was previously described in our outher paper (Plăcintă et al., 2011).

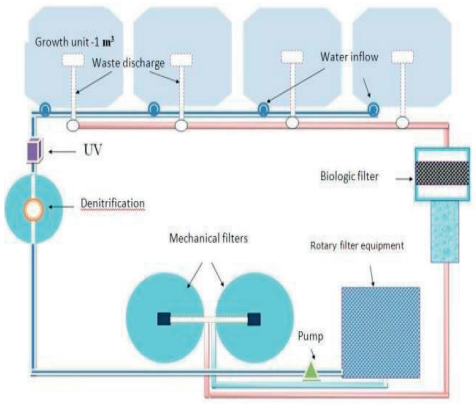

Figure 1. The scheme of the Recirculating aquaculture system

The experiment was organized in two variants, in duplicate. The fish was exposed to photoperiod regimes: Light hours: Dark hours (12 h:12 h) and different light intensity/color.

Biological material and experimental station. The biological material used in the experiment was represented by European catfish provided by CCDP Nucet. Fish were divided into 4 rearing units (124 fish per each variant). The initial stocking density of juvenile catfish was 15.66 kg m^{-3}, with an average weight of 63 g.

Light intensity measured with lux meter TESTO 545 just above the water surface in the center of the rearing tanks, and was 260±1.2 lx (V1 - white light) and 80 ± 0.3 lx (V2 - green light). The Photoperiodicity regime applied in both variants was (12 L:12 D). Constant light intensity was maintained throughout the experiment and illumination was supplied with a fluorescent lamp of 36 watts each suspended 90 cm above the water surface and automatically controlled by a timer.

The polycarbonate sheets by 0.6 mm were used to make the caps for the obstructing light intensity in the rearing units. The only experimental variable was polycarbonate sheets color and both experimental variants were isolated from external factors (solar radiation).

The fish were fed with commercial feed (Classic EXTRA 1P) with a diameter of 2.5 mm, twice daily at 8:00 am and 20:00 pm and the daily ration was 2 % of fish body weight (BW). According to the manufacturers, the feed composition was as follows: protein – 41%; fat – 12%; ash – 6.5%, total phosphorus – 0.9%.

The following water quality parameters were monitored in the system: temperature, dissolved oxygen (every day, with Dissolved Oxygen Meter HANNA Instruments 98186), pH (every day, with pH-meter, model WTW 340) and N-NO_2^-, N-NO_3^-, N-NH_4^+, were measured with Spectroquant Nova 400, using Merck kits.

Calculations. At the end of the experiment, after all, fish were weighed and measured, the following technological efficiency indicators were calculated: growth rate, food conversion ratio, specific growth rate, and protein efficiency ratio using the following equations:

- Weight gain (W) = Final weight (Wt) – Initial weight (W0) (g);
- Food conversion ratio (FCR) =Total feed (F)/Total weight gain (W) (g);
- Specific growth rate (SGR) = 100 × (ln Wt - ln W0)/t (% BW day^{-1});
- Protein efficiency ratio (PER) = Total weight gain (W)/amount of protein fed (P) (g).

For each experimental variant we choose 40 fish and total length (TL) and body weight (W) were used to determine the relationship $W = a \times L^b$, where a is the intercept (the initial growth

coefficient) and “b” is the allometric coefficient (Ricker, 1975). Generally, the index "b" values range between 2-4, mostly 3 and reflects the state of biological material in environmental conditions (Klaus B., et al., 2003).

Statistical analysis. The statistical analysis was performed with the help of the soft SPSS 21 for Windows. The normality of the distribution was verified with the help of the Kolmogorov-Smirnov Z test. The statistical differences between variables were tested with the help of the T-test.

RESULTS AND DISCUSSIONS

Regarding water quality parameter dynamics, no major differences during the day or after feeding were registered. The water quality parameters within the recirculating system had the following average values: temperature – 20.6±1.16˚C, dissolved oxygen- 5.05±1.11 mg L^{-1}, nitrate – 56.71±22.03 mg L^{-1}, nitrite 0.08±0.01 mg L^{-1}, ammonium – 0.11±0.09 mg L^{-1} and pH -7.90±0.15 pH units. The technological growth performance indicators for *Silurus glanis* juveniles are summarized in Table 1 and are also graphically represented in Figures 2 and 3.

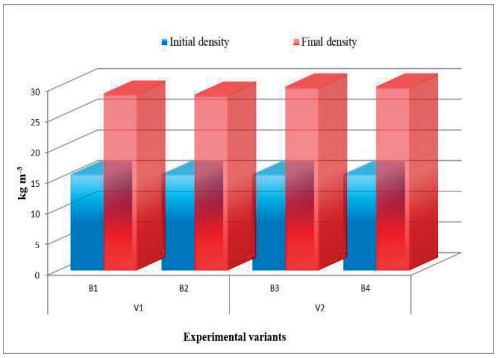

Figure 2. Biomass dynamics for the experimental variants

Both figures and table indicate a better growth of culture biomass in the V2 variant (green light with an intensity of 80 lx).

By analyzing the average values for both V1 and V2 experimental variants, we obtained a greater final stocking density in the case of V2 – 29.99 kg m^{-3}, compared to the V1 experimental variant – 28.75 kg m^{-3} (Figure 2).

Table 1. Technological performance indicators, obtained at the end of the experimental period

Growth performance indicators	Experimental variants					
	V1			V2		
	B1	B3	Average±SD	B2	B4	Average±SD
Initial biomass [g]	7832	7828	7830±2.83	7824	7821	7822.50±2.12
Final biomass [g]	14416	14330	14373±60.81	14984	15004	14994±14.14
Initial fish number	124	124	124	124	124	124
Survival [%]	100	100	100	99.60	100	99.60±0.57
Initial average weight [g $fish^{-1}$]	63.16	63.13	63.15±0.02	63.15	63.08	63.08±0.02
Final average weight [g $fish^{-1}$]	116.26	115.56	115.91±0.49	121	121.41	121.41±0.58
Individual biomass gain [g]	53.10	52.44	52.77±0.47	58.72	57.93	58.33±0.56
SGR (Specific growth rate) [g% day^{-1}]	1.02	1.02	1.01±0.01	1,08	1.09	1.08±0.02
FCR (Feed conversion rate) [g feed g $^{-1}$ biomass gain]	1.43	1.44	1.44±0.01	1.31	1.31	1.31±0.03
Protein efficiency ratio PER (g g^{-1})	1.71	1.69	1.70±0.01	1.87	1.88	1.86±0.05

Regarding the final weight of the fish, the statistical test found no significant differences ($p>0.05$) between the two experimental variants. However, the individual weight of the fish from V2 was higher than those from V1.

Although fish survival was 100% in V1 and 99.60±0.57% in V2, this was not significantly influenced ($p>0.05$) by the applied light regime. Regarding the SGR, FCR, and PER, higher values are encountered in the case of the V2 experimental variant. The SGR values of catfish juveniles for all light conditions were in variant V1 to 1.08 g % day^{-1} in V2.

Regarding, the FCR the best value was obtained at the green light, the mean value being significantly ($p<0.05$) better in comparison with the average value obtained from V2. Also, the mean value of PER was significantly ($p<0.05$) better in the V2, where the green light was applied (Figure 3).

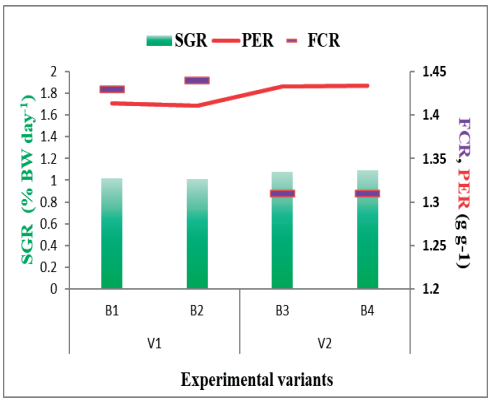

Figure 3. The variation of SGR, PER, and FCR

To evaluate fish condition, we calculate the power regression from the individual weight and length measurements for 40 fish from each experimental variant. (Figure 4 and Figure 5).

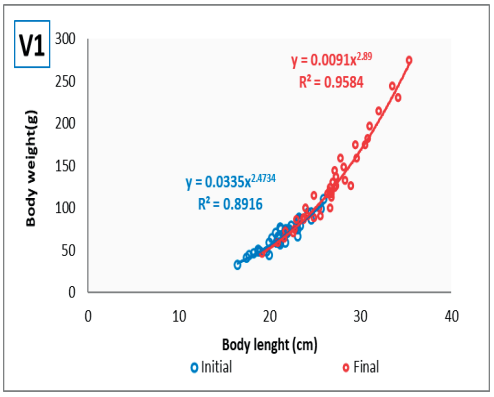

Figure 4. Length-weight regression for the V1 variant

From the correlation between individual length and weight, it can be observed that the condition of catfish individuals was improved in both experimental variants in comparison with the initial moment. However, a negative allometric growth (b<3) can be seen in all experimental groups, the increase in length was faster than the increase in body weight.

The obtained results lead us to state that *Silurus glanis* juvenile's growth performance and metabolic process are influenced in a significant way by light intensity.

According to some authors, obtaining a better growth performance at the lower light intensity conditions is due to the reduction of the stress, as well as repression of the necessary locomotive activities in the dark.

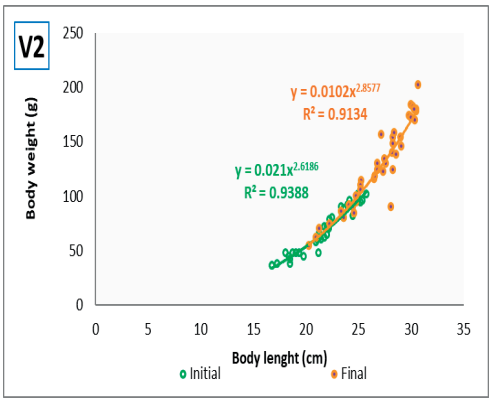

Figure 5. Length-weight regression for the V2 variant

Generally, catfish have different luminous preferences, and it can directly influence its growth, survival, skin pigmentation, behavior, and seed quality (Pedreira et al., 2012; Costa et al., 2016).

Increasing growth performance was also reported in the case of European catfish *Silurus glanis* and African catfish *Clarias gariepinus* at the dark in comparison with the light, mainly due to a more efficient food utilization (Meske & Munster, 1984; Britz and Pienaar, 1992). Also, Meske, 1982, reported improving the growth performance in the case of European eel fry with under 12 h light/dark cycle.

Also, Kawamura et al., 2015, says that the effect of light on fish growth rates could be related to the color preferences of fish and because the catfish is a freshwater fish species that dwell in calm lakes, rivers, floodplains, and swamp areas that flood on a seasonal basis, and that's why prefers weaker light.

Thus, under optimal light conditions, the growth rate increases at the background of minor changes in the daily diet and significant improvement in food conversion.

The results obtained by us are similar to those obtained by other authors. In the case of juvenile catfish *Lophiosilurus alexandri (Steindachner)*, the swimming activity and growth performance were significantly influenced by the light intensity, and better results were obtained at 0 lux in comparison with 218, 278, and 459 lux (Santos et al., 2019). Also, similar results were reported for nocturnal behavioral species, as the African catfish which has better food conversion in dark environments (Rodriguez et al., 2009; Mustapha et al., 2012).

CONCLUSIONS

The main aim of our experiment was for determined the effect of environmental light (spectrum, intensity, and photoperiodicity) on the growth performance of juvenile catfish (*Silurus glanis*, L., 1758), reared in a recirculating industrial aquaculture system (RAS).

According to the results of the our study the main conclusion is that the fish from the variant with the green light with a intensity of 80 lx performed better than those from the white light with an intensity of 260 lx. It is recommended that a lower light intensity should be applied for obtaining a better growth performance of *Silurus glanis* juveniles in recirculating aquaculture systems.

These studies have great potential interest for aquaculture because they should indicate the best photoperiod. Also, future studies are recommended to be made on both the light spectrum and photoperiodicity regime. In the present study, the highest growth rates were obtained under green light.

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ASSESSMENT OF WATER QUALITY FROM ACCUMULATION STÂNCA-COSTEȘTI

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Abstract

Water is one of the natural resources most widespread on Earth, between living organisms and water being an indispensable link. The aim of this research was to assess the water quality of the Stânca-Costeşti accumulation based on the results obtained from the determination of physical- chemical parameters and toxic pollutants. Stânca-Costeşti accumulation is situated on the border between Romania and Moldova in Botosani County. The elements determined for each sample related to the studied section are represented by: pH, dissolved oxygen, biochemical oxygen demand (B.O.D.), nitrite, nitrate, chlorides, sulphates, cadmium, nickel, lead, zinc, copper and chromium. In order to establish the values of determined elements were applied standardized analytical methods. The average concentrations of analyzed heavy metals were below maximum permissible concentration for Ist class of quality. As a general conclusion based on these results, we can say that water from Stânca-Costeşti accumulation fall within the IInd class of quality in terms of the oxygen and in the Ist class of quality in terms of nutrients and heavy metals.

Key words*: Stânca-Costeşti accumulation, water chemical composition, water quality.*

INTRODUCTION

Water is one of the natural resources most widespread on Earth. Between living organisms and water being an indispensable link. Man is made up of 70% of water (Benchea, 2011). Of all types of water, the most important is the freshwater because human life would be impossible without it. Human civilization has been built based on water availability.

Stânca-Costeşti accumulation is situated in Botosani County, on Prut River, on the border between Romania and Moldova (Romanescu and Stoleriu 2017; Stoleriu et al., 2019). Stânca-Costeşti accumulation is located at an average altitude of 200 m, in an area where the major riverbed width of 3-4 km is strangled and reduced to 350-400 m by coral limestone, which basically is a natural dam, presenting optimal natural conditions for creating a retention capacity of around 1.0-1.5 billion m^3.

Stânca-Costeşti accumulation was opened in 1978, being located on the Prut River, about 580 km from its confluence with the Danube. The lake was constructed by a dam with a height of 47 m and a length of 7400 m, retaining a volume of water of about 735 million m^3 at the N.N.R., with an area of 59 km^2 and a maximum depth of 41.5 m, with bottom drain (Vartolomei, 2009).

Stanca-Costesti is the largest accumulation of water on the Prut River, being the second largest in Romania. Stanca-Costesti accumulation was jointly built by the two riparian states (Romania and the Republic of Moldova), to mitigate the effects of the high floods, to prevent damages caused by floods, ensuring the water supply of the population, industry, hydropower production as well as irrigation and fish farming activities.

Natural waters contain various mineral and organic substances, dissolved or in the form of colloids or suspended solids, which occur naturally and do not allow the use of these waters directly for consumption (Dăscălescu et al., 2011; Teodosiu et al., 2009). Studies on the quality of water in the systems of rivers or lakes in Eastern Europe have been carried out by

Albulescu et al., 2010, Dughilă et al., 2010, Benchea et al., 2011, Dăscăliţă, 2011, Romanescu et al., 2012, Lazu et al., 2019a.
Nutrients play a significant role for the environmental state of lakes, mainly because the primary production of lakes is strongly influenced by nutrient availability (Popescu et al., 2019). The aim of this research was to assess the water quality of the Stânca-Costeşti accumulation based on the results obtained from the determination of physical - chemical parameters and toxic pollutants.

MATERIALS AND METHODS

In order to establish water quality from Stânca-Costeşti accumulation (Figure 1), water sampling was carried out in four control sections, namely: from Manoleasa Village, middle of the lake, lake reservoir and dam outlet. Sampling took place in May, June, August and September, sampling points being established in order to provide relevant information for each section of water accumulation.

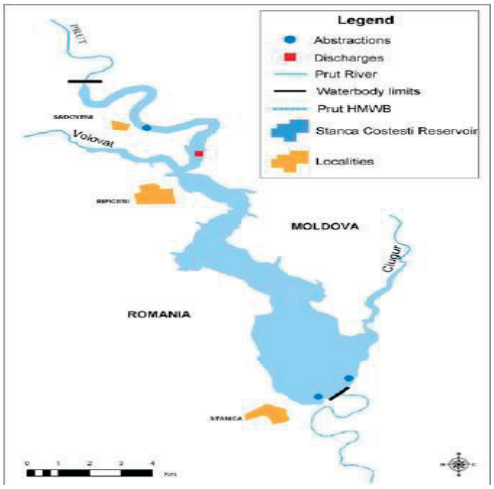

Figure 1. The geographical position of Stânca-Costeşti accumulation
(Source: Petru Şerban and Rădulescu Daniela, 2004)

Collecting the water samples for determination of chemical indicators was performed according to current standards, in sampling containers, from plastic and brown borosilicate glass which prevents light penetration that may have an influence on the organisms present in water and can cause unwanted chemical reactions.
The elements determined for each sample related to the studied section are represented by: pH, dissolved oxygen, biochemical oxygen demand (B.O.D.), nitrite, nitrate, chlorides, sulphates, cadmium, nickel, lead, zinc, copper and chromium.
In order to establish the values of determined elements were applied standardized analytical methods.
Quantitative determination of some pollution indicators (nitrites, nitrates, sulphates) was performed by spectrometry using an ATI UNICAM spectrophotometer.
The levels of heavy metals from the water samples were determined using an atomic absorption spectrophotometer type Shimadzu 6300, and an atomic spectrometer type GBC Avanta.

RESULTS AND DISCUSSIONS

Water pH
Hydrogen ion concentration (pH) - determines the activity of hydrogen ions, hence the ability of the water to be acidic, neutral or alkaline, thus highlighting the level of physico-chemical and biological processes in it (SR ISO 10523-97). The results obtained from the determinations are presented in the table 1.
From the comparison of the data presented in table 1 it can be observed that the pH values were similar during the analyzed periods.
The highest values were obtained, in all four sections taken into control, during 18.06.2019 and 09.08.2019 periods.

Table 1. pH values in the 4 sampling points

Sampling point	15.05.2019	18.06.2019	09.08.2019	10.09.2019	Mean	Maximum
Manoleasa Village	8.23	8.56	8.44	7.90	8.28	8.56
Middle of the lake	8.21	8.48	8.50	8.09	8.32	8.48
Lake reservoir	8.27	8.48	8.64	8.34	8.43	8.64
Dam outlet	8.19	8.19	8.25	8.01	8.16	8.25

The average values of the pH were, in all studied periods and in all 4 sections taken into control of over 8, which indicates the alkaline nature of water from Stânca-Costești accumulation. The maximum value obtained was 8.64 during the period 09.08.2019 at the lake reservoir section.

Dissolved oxygen (DO)

Dissolved oxygen is a very important indicator of surface water quality and depends on a number of factors such as: water temperature, atmospheric pressure, depth, water turbidity and the amount of decomposing organic matter. Analyzing the water quality from Stânca Costești accumulation, in terms of dissolved oxygen we find that it falls within the IInd quality class (table 2).

Table 2. Dissolved oxygen values in the 4 sampling points (mg O_2/l)

Sampling point	15.05.2019	18.06.2019	09.08.2019	10.09.2019	Mean	Maximum
Manoleasa Village	8.20	7.70	5.90	5.33	6.78	8.20
Middle of the lake	7.90	7.60	6.70	6.44	7.16	7.90
Lake reservoir	7.90	6.90	5.80	7.00	6.90	7.90
Dam outlet	7.40	6.10	5.00	5.80	7.21	7.40

From the analysis of the data presented in table 2, we find that the lowest values for dissolved oxygen were recorded in the first two sampling points in September, when the values of this parameter were 5.33 mg O_2/l at Manoleasa Village and 6.44 mg O_2/l in the middle of the lake. For the other two sampling points the minimum values were recorded in August and were 5.00 mg O_2/l at the dam outlet and 5.80 mg O_2/l respectively at the lake dam.

According to season, the dissolved oxygen content in water changes depending on the air temperature, thus the highest oxygen content is recorded in the cold season, because the low temperatures reduce the oxidation and favor the dissolution of atmospheric oxygen in the water. (Păsărin, 2007). The values obtained by us in the case of dissolved oxygen are in accordance with those obtained by Vartolomei (2009), as a result of the analysis of the water quality of Stânca-Costesti accumulation, values that ranged between 6.8 and 10.2 mg O_2/l.

Biochemical oxygen demand (BOD)

The biochemical oxygen demand varies directly in proportion to the amount of organic substances contained in the water and is influenced by the action of the reducing chemical compounds (Dughilă, 2010, Lazu et al., 2019a, Lazu et al., 2019b).

Biochemical oxygen demand represents the amount of oxygen consumed for oxidative degradation by microorganisms of the contained organic substances, at standard temperature (20ºC) and standard time (5 days) in accordance with STAS 6560-82 and SR ISO 6060/96 or SR EN 1899-2/2002 (table 3).

Table 3. The Biochemical oxygen demand values in the 4 sampling points (mg O_2/l)

Sampling point	15.05.2019	18.06.2019	09.08.2019	10.09.2019	Mean	Maximum
Manoleasa Village	5.32	4.54	4.91	3.32	4.52	5.32
Middle of the lake	6.72	3.17	3.05	2.20	3.78	6.72
Lake reservoir	3.16	4.80	3.11	2.15	3.30	4.80
Dam outlet	2.86	3.42	2.02	2.10	2.60	3.42

Biochemical oxygen demand (B.O.D.) in Stânca-Costești accumulation ranged between 2.02 mg O_2/l, in August and 6.72 mg O_2/l in May, with average values oscillated between 2.60 and 4.52 mg O_2/l.

Nitrites (NO_2^-) – are intermediates produced as a result of oxidation of ammonia or reduction of nitrates by bacterial processes (ammonia, under the action of *Nitromonas* and *Nitrobacter* bacteria, in the presence of oxygen gradually transforms into nitrites, then into nitrates) (Bucureșteanu et al., 2008). The nitrogen content in the form of nitrogen ions was determined accordingly to STAS 8900/2-71 and SR ISO 6777-96.

The nitrites were reported in extremely small quantities (Table 4), their average values being between 0.02 0.03 (mg N/l).

Table 4. Nitrites values in the 4 sampling points (mg N/l)

Sampling point	15.05.2019	18.06.2019	09.08.2019	10.09.2019	Mean	Maximum
Manoleasa Village	0.03	0.03	0.06	0.01	0.03	0.06
Middle of the lake	0.03	0.02	0.01	0.01	0.02	0.03
Lake reservoir	0.02	0.02	0.02	0.01	0.02	0.02
Dam outlet	0.02	0.02	0.02	0.01	0.02	0.02

The nitrogen content of the water from the Stânca-Costești accumulation recorded in the 4 sampling points showed values that were in the range 0.01-0.06 mg N/l for the Manoleasa Village sampling point, between 0.01 and 0.03 mg N/l for the sampling point located in the middle of the lake, between 0.01 and 0.02 mg N/l for the lake reservoir sampling point, respectively between 0.01 and 0.02 mg N/l for the dam outlet sampling point.

Nitrates (NO_3^-) – represents an advanced stage of ammonium oxidation or is formed directly from molecular nitrogen. Thus, in the water of Stanca-Costești accumulation the main sources of nitrates are represented by the decomposition of organic waste and less by the use of nitrogen fertilizers in agriculture.

The nitrogen content in the form of nitrogen ions was determined accordingly to STAS 8900/1-71 and SR ISO 7890/1-98 (Table 5).

Table 5. Nitrates values in the 4 sampling points (mg N/l)

Sampling point	15.05.2019	18.06.2019	09.08.2019	10.09.2019	Mean	Maximum
Manoleasa Village	4.10	1.92	2.90	1.95	2.71	4.10
Middle of the lake	3.63	2.81	1.37	1.85	2.41	3.63
Lake reservoir	3.57	2.81	1.40	1.00	2.19	3.57
Dam outlet	3.95	3.00	1.80	1.05	2.45	3.00

The nitrate content recorded during the 4 months taken in the study, shows a decrease of this parameter during the warm period, more precisely in September 2019, when the values recorded at the 4 sampling points did not exceed 2.00 mg N/l, mainly due to the biogenic processes, which intensifies due to the high water temperature.

Chlorides (Cl^-) - the chlorine content in the water from Stanca-Costești accumulation was determined in accordance with STAS 8663-70 (Table 6).

Table 6. Chlorine values in the 4 sampling points (mg/l)

Sampling point	15.05.2019	18.06.2019	09.08.2019	10.09.2019	Mean	Maximum
Manoleasa Village	24.00	22.85	24.50	22.15	23.38	24.50
Middle of the lake	21.50	21.00	22.10	20.90	21.38	22.10
Lake reservoir	22.40	22.10	23.20	21.50	22.30	23.20
Dam outlet	25.30	21.30	23.30	21.60	22.88	25.30

The values obtained in the 4 sampling points in the case of chlorine content ranged between 21.50 and 25.30 mg/l in May, between 21.00 and 22.85 mg/l in June, between 22.10 and 24.50 mg/l in August, respectively between 20.90 and 22.15 mg/l in September.

The average values obtained allow the classification of water from Stanca-Costești accumulation in the 1st class of quality.

Sulphates (SO_4^{2-}) - the sulphate content of the water from Stânca-Costești accumulation was determined in accordance with STAS 8601-70 (Table 7).

Table 7. Sulphates values in the 4 sampling points (mg/l)

Sampling point	15.05.2019	18.06.2019	09.08.2019	10.09.2019	Mean	Maximum
Manoleasa Village	55.60	54.50	44.40	48.80	50.83	55.60
Middle of the lake	74.40	61.40	47.80	49.10	58.18	74.40
Lake reservoir	70.10	68.70	48.70	50.60	59.53	70.10
Dam outlet	78.70	61.70	41.70	45.80	56.98	78.70

The values for this indicator oscillated between 41.70 mg/l, as recorded in August and 78.70 mg/l, the value recorded in May both values being recorded at the same sampling point located at the dam outlet.
The average values (50.83-59.83 mg/l) allow the classification of water from Stanca-Costeşti accumulation in the 1st class of quality.

The main specific (toxic) chemical indicators that were analyzed were the ions of cadmium (Cd^{2+}), lead (Pb^{2+}), nickel (Ni^{2+}), copper (Cu^{2+}), zinc (Zn^{2+}) and chromium (Cr^{3+} and Cr^{6+}).
The average concentrations were calculated for the values recorded in the 4 monitoring periods and presented in table 8.

Tabelul 8. Heavy metal values in the 4 sampling periods (µg/l)

Hevy metals	15.05.2019	18.06.2019	09.08.2019	10.09.2019	Mean	Maximum
Cd total (µg/l)	0.07	0.07	0.07	0.05	0.06	0.07
Pb total (µg/l)	1.08	1.19	3.58	1.07	1.73	3.58
Ni total (µg/l)	7.66	2.50	0.99	5.49	4.16	7.66
Cu total (µg/l)	2.18	1.01	2.98	1.81	1.99	2.98
Zn total (µg/l)	7.24	9.13	4.35	1.40	5.53	9.13
Cr total (µg/l)	1.94	0.82	3.31	1.59	1.91	3.31

Analyzes for the determination of heavy metals were performed using modern methods of spectrometry and atomic absorption spectrophotometry.
The heavy metal concentrations determined ranged from 0.05 to 0.07 µg/l for cadmium, between 1.08 and 3.58 µg/l for lead, between 0.99 and 7.66 µg/l for nickel, between 1.01 and 2.98 µg/l for copper, between 1.40 and 9.13 µg/l for zinc, respectively between 0.82 and 3.31 µg/l for chromium. None of the heavy metals analyzed exceeded the maximum allowable concentration for first class of quality.

CONCLUSIONS

The average values of the pH, were, in all studied periods and in all 4 sections taken into control of over 8, which indicates the alkaline nature of water from Stânca-Costeşti accumulation.
Analyzing the water quality from the Stânca-Costeşti accumulation through the prism of dissolved oxygen (average values over the entire analyzed period between 6.78 and 7.21 mg O_2/l) we find that it falls in the second class of quality.
The sulphate content of the Stânca-Costeşti accumulation water had average values between 50.83-59.83 mg/l, which allows the classification in the 1st class of quality.
The average concentrations of analyzed heavy metals are below maximum permissible concentration for first class of quality.
As a general conclusion based on these results, we can say that water from Stânca-Costeşti accumulation fall within the II class of quality in terms of the dissolved oxygen and in the first class of quality in terms of nutrients, salinity and heavy metals.

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FISH SPECIES DISTRIBUTION AND DIVERSITY INDICES FROM IARA RIVER – TRANSYLVANIA, ROMANIA

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Abstract

In the present study we analyzed fish species composition and diversity indices of Iara River (Cluj County). A total number of 189 specimens belonging to 9 species and 4 families were caught, using single pass electrofishing techniques. A total number of 11 sampling sectors were established. Our results were compared to previous studies and show changes in species distribution and diversity indices, due to environmental changes caused by climate changes and anthropic activities. We noticed the absence of the Grayling (Thymallus thymallus) and Stone loach (Barbatula barbatula) mentioned in previous studies, and the first record of Prussian carp (Carassius gibelio) in Iara River catchment. Also, the Caps dam (Iara – Fântânele deviation) blocks the upstream migration of Common bullhead (Cottus gobio). The calculated diversity indices (Shannon H', Simpson 1-D, Margalef Md, Berger-Parker d and Evenness J') show a low species diversity. This is the result of intense anthropic activities such as deforestation, dam construction and habitat fragmentation, even though the upper sector of Iara River is integrated in Natura 2000 protection areas.

Key Words: *electrofishing, habitat fragmentation, ichthyofauna, Natura 2000, Salmonidae.*

INTRODUCTION

In Romania there are two categories of Nature 2000 sites: 435 SCI sites (Sites of Communitary Importance) and 171 SPA sites (Sites of Avifaunistic Special Protection).
The superior sector of Iara River is integrated in ROSCI0263 Iara Valley. Iara River is a left side tributary of Arieș River, located in Cluj County and has a total length of 54 km.
The river crosses eight villages: Caps, Valea Ierii, Frăsinet, Moara de Pădure, Băișoara, Iara, Surduc, and Buru, where it flows in Arieș River. On Iara River, there is one fragmentation point, the Caps dam (Iara-Fântânele deviation). The dam blocks downstream-upstream migration of fish because there is no fish pass construction (Bâdiliţă et al., 2013; Danalache et al., 2017).
Habitat fragmentation causes genetic isolation and inbreeding (Curtean-Bănăduc et al., 2019). Fish communities from mountain water bodies are heavily influenced by several anthropic factors such as climate change, deforestation, agriculture, overfishing, poaching pollution, micro-hydropower plants and dam constructions (Holcík, 2003; Almodóvar et al., 2004; Hu et al., 2019).
Integrative monitoring programmes are required to prevent species and habitat losses (Colette et al., 2018).
Measuring biological diversity (Mohebban et al., 2019) is indeed challenging (Magurran, 2004) especially in areas where anthropic disturbances are frequent.
Distribution and diversity of fish are altered when constant changes to environment occur.
In order to evaluate the dynamics in fish community, a regular ichthyological survey is required in order to have a global view regarding fish species distribution (Oţel, 2007; Kottelat and Freyhof, 2007).

MATERIALS AND METHODS

Sampling method

Fish specimens were captured using single-pass electrofishing techniques. Samus 725 MP electrofisher apparatus was used, powered by a 22 Ah rechargeable battery. The frequency of the electrofisher was set to 45Hz. In general single pass electrofishing techniques are efficient when it comes to species composition (Benejam et al., 2012). Water temperature, conductivity and pH were determined in each sector using Hanna HI-9828 multi-parameter apparatus.

Geographical data

GPS coordinates were collected using a Garmin eTrex 20x device, and mapping (station length, station altitude) of the river catchment were generating using GIS software (Nemec and Raudsepp-Hearne, 2013; Nicula et al., 2017). Also river structure parameters (mean sector depth, mean sector width) were determined using a 20 m tape measure. At the starting point and at the end point of each sampling sector, GPS coordinates, water depth and river width were measured. The collected fish specimens were identified, measured and photographed.

Data analysis

The data was processed using MS Excel and GraphPad Prism. Diversity indices were calculated based on field counts and determinations: Species Richness (R), Relative Abundance (%Abd), Shannon's Index (H'), Simpson's Index (D), Evenness (J'), Margalef Index (M_d), and Berger-Parker Index.

Diversity analysis

Species Richness

Species Richness (R) refers to the number of species present in an ecosystem, area or region.

Relative Abundance:

$$pi = Ni \,/\, N$$

Where *pi* is the proportion of individuals of *i-th* species, *Ni* is the number of individuals of that species and *N* is the total number of individuals of all species and *S* is the total number of species (or Richness).

$$N = \sum_{i=1}^{S} Ni$$

Shannon's Index H':

$$H' = -\sum pi \ln (pi)$$

Where *pi* is is the proportion of individuals of *i-th* species (Shannon and Wiener, 1949).

Simpson's Index 1-D:

$$1 - D = 1 - \frac{\Sigma_{ni}(ni-1)}{N(N-1)}$$

Where n is the total number of specimens of a particular species and N is the total number of specimens of all species (Simpson, 1949).

Evenness J':

$$\mathrm{J}' = \frac{\mathrm{H}'}{\mathrm{H}'_{\max}}$$

Where H' is Shannon's Index and H'_{max} is the maximum possible value of Shannon's Index H' in the context of equality of species (Pielou, 1966).

Margalef Index Md:

$$M_d = \frac{S-1}{\ln N}$$

Where S is total number of species and N is the total number of individuals from all species (Margalef, 1958).

Berger Parker Index d:

$$d = \frac{N_{max}}{N}$$

Where N_{max} is the number of individuals of the most abundant species and N is the total number of individuals from all species (Berger and Parker, 1970).

RESULTS AND DISCUSSIONS

Geographical data

Our research was conducted on the main course of Iara River, located in the Apuseni Mountains (Western Carpathians) (Figure 1). The geographic location of Iara River hydrographic microbazine related to the main course is found between the coordinates 46° 29 '00' 'N 23° 14' 00 " E (Muntele Mare Peak) and 46° 30 '30' 'N 23° 35' 38 " E (at the confluence with Aries River). The topoclimate is of sub-mountain type, with foehnal processes that decrease in intensity from West to East. The average annual temperature is around 8°C and the quantitative values of annual average rainfall do not exceed 750 mm / year (Pop, 2001). According to our GIS calculations, the studied location covers an area of 165.7 km^2 with a perimeter of 125.168 km.

The altimetric upstream – downstream ecart is 1468 m, with the maximum altitude of 1826 m in the Muntele Mare Peak and the minimum altitude of 381.5 m at the confluence of the main course of Iara River with Arieş River, near Buru village. The average slope is 30 ‰ and the sinuosity coefficient is 2.06 (The atlas of the Romanian water cadastre, 1992).

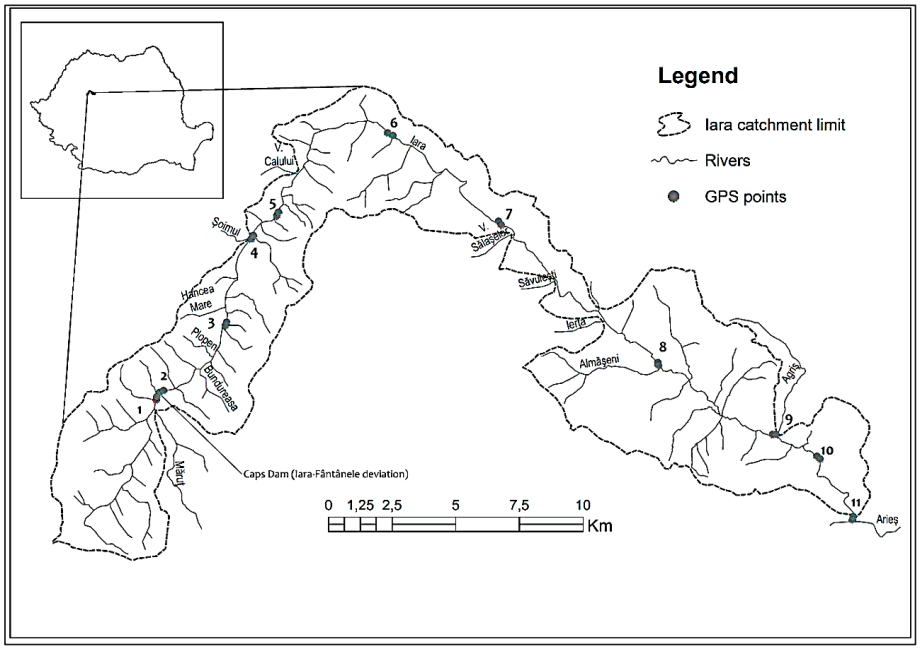

Figure 1. Iara River Catchement (Source: the authors)

Table 1. GPS coordinates of the sampling stations from Iara River

Sector	Upstream limit	Downstream limit	Mean altitude of sector (m)
1	N46° 32.768' E23° 14.206'	N46° 32.831' E23° 14.220'	1100
2	N46° 32.924' E23° 14.298'	N46° 32.966' E23° 14.429'	1093.5
3	N46° 34.374' E23° 16.276'	N46° 34.447' E23° 16.320'	940
4	N46° 36.225' E23° 17.021'	N46° 36.306' E23° 17.082'	847
5	N46° 36.733' E23° 17.790'	N46° 36.812' E23° 17.838'	843
6	N46° 38.541' E23° 21.152'	N46° 38.486' E23° 21.316'	708
7	N46° 36.714' E23° 24.613'	N46° 36.639' E23° 24.695'	599.5
8	N46° 33.779' E23° 29.600'	N46° 33.724' E23° 29.613'	475.5
9	N46° 32.296' E23° 33.162	N46° 32.294' E23° 33.268'	430.5
10	N46° 31.849' E23° 34.544'	N46° 31.792' E23° 34.642'	423
11	N46° 30.582' E23° 35.680'	N46° 30.512' E23° 35.658'	381.5

The length of Iara River is 53.304 km and runs through eight localities (Caps, Valea Ierii, Frăsinet, Moara de Pădure, Băișoara, Iara, Surduc and Buru). In the lower sector, the river forms the Surduc-Buru Defile (Pop, 2001), carved in hard formations mainly composed of crystalline shales.

The altitude and GPS coordinates of the sampling sectors are showed in Table 1. The lowest altitude (381.5 m) was recorded in sector 11. The highest sampling sector was situated at 1100 m. The mean length of the sampling sectors was 172.47 m (min=129.7m; max=266.7 m). The width of Iara River ranged from 2.5 m in sector 1 to 6.6 m in sector 11 (mean water width=4.75 m). Water depth ranged from 0.29 m in sector 2 to 0.45 m in sector 11 (Figure 2).

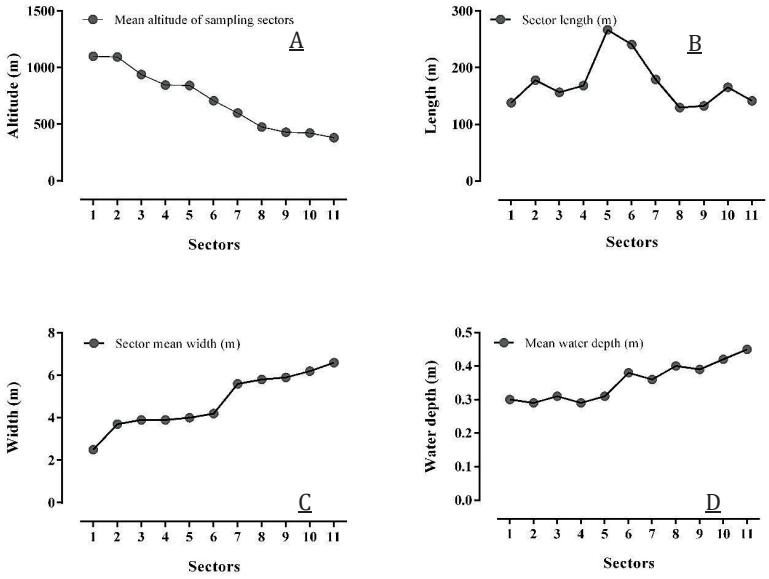

Figure 2. Water depth: A-altitude of studied sectors; B-length of sectors; C- width of sectors; D- mean water depth

Species composition structure and species abundance

Electrofishing and data analysis were performed from June 15th to June 27th 2019 on 11 sampling sectors. A total number of 189 fish specimens belonging 9 species (*Salmo trutta, Barbus petenyi, Squalius cephalus, Alburnoides bipunctatus, Phoxinus phoxinus, Carassius gibelio, Gobio obtusirostris, Cottus gobio* and *Eudontomyzon danfordi*), 4 families and 7 subfamilies, were caught (Table 2).

The most abundant species was *Salmo trutta* (77 specimens), representing 40.7407% from all the fishes caught, followed by *Barbus petenyi* (59 specimens), representing 31.2169%. Abundance percentages with lower values were registered for *Alburinoides bipunctatus* (15 specimens-7.9365%), *Cottus gobio* (14 specimens-7.4074%) and *Gobio obtusirostris* (12 specimens-6.3492%). The lowest abundance percentages were registered for *Squalius cephalus* (7 specimens-3.70375), *Carassius gibelio* (3 specimens-1.5873%), *Phoxinus phoxinus* and *Eudontomyzon danfordi* (1 specimen-0.5291%, for each species) (Table 3).

The presence of Brown trout *Salmo trutta* was signaled in the upper sectors of Iara River (Sectors 1 to 7). The distribution of *Salmo trutta* based on altitude ranged from 599.5 m to 1100 m.

The presence of European bullhead *Cottus gobio* was signaled in the upper sectors of the river (Sectors 3, 4, 5 and 7) and ranged from 599.5 m to 940 m. *Cottus gobio* was not signaled upstream the Caps Dam, in sectors 1 and 2 (Iara-Fântânele deviation).

The distribution of *Phoxinus phoxinus* in Iara River based on field data shows that it has a point presence (1 specimen in sector 4 at 847 m).

The Carpathian brook lamprey *Eudontomyzon danfordi* was present only in sector 6 at 708 m (1 specimen).

The Romanian barbel *Barbus petenyi* was present in the lower section of Iara River (sectors 8 to 10) at altitudes ranging from 423 m to 475 m. *Gobio obtusirostris,* was captured in two sectors from the lower section of the river (sectors 9 and 11) at altitudes ranging from 381.5 m to 430.5 m.

The Schnieder *Alburnoides bipunctatus* was present only in sector 11, very close to the confluence of Iara River with Arieș River (381.5 m).

The presence of Prussian carp *Carassius gibelio* is less common in this type of water. Three specimens were caught in sector 11 at 381.5 m altitude. The European chub *Squalius cephalus* was present only in sector 11, at the confluence with Arieș River (Table 4).

Table 2. Taxonomic table of fish species found in Iara River

Order	Family	Subfamily	Species
Salmoniformes	Salmonidae	Salmoninae	*Salmo trutta*
Cypriniformes	Cyprinidae	Barbinae	*Barbus petenyi*
		Leuciscinae	*Squalius cephalus*
			Alburnoides bipunctatus
			Phoxinus phoxinus
		Cyprininae	*Carassius gibelio*
		Gobioninae	*Gobio obtusirostris*
Scorpaeniformes	Cottidae	Cottidae	*Cottus gobio*
Petromyzontiformes	Petromyzontidae	Lampetrinae	*Eudontomyzon danfordi*

Table 3. Relative abundance percent of fish species found in Iara River

Species	n	Relative Abundance (%)	IUCN Status
Salmo trutta	77	40.7407	LC
Barbus petenyi	59	31.2169	LC
Alburnoides bipunctatus	15	7.9365	LC
Cottus gobio	14	7.4074	LC
Gobio obtusirostris	12	6.3492	LC
Squalius cephalus	7	3.7037	LC
Carassius gibelio	3	1.5873	LC
Phoxinus phoxinus	1	0.5291	LC
Eudontomyzon danfordi	1	0.5291	LC

LC – Least Concern

Table 4. Fish species distribution in the sampled sectors from Iara River

	Upstream→Downstream sectors											
	1	2	3	4	5	6	7	8	9	10	11	Total
Salmo trutta	19	13	22	6	9	4	4					77
Cottus gobio			4	5	4		1					14
Phoxinus phoxinus				1								1
Eudontomyzon danfordi						1						1
Barbus petenyi								4	37	18		59
Romanogobio vladykovi									6		6	12
Alburnoides bipunctatus											15	15
Carassius gibelio											3	3
Squalius cephalus											7	7
Total Number of Individuals/sector	19	13	26	12	13	5	5	4	43	18	31	189
Total Number of species/sector	1	1	2	3	2	2	2	1	3	2	4	9

Diversity Indices

The calculated value for Shannon Index (H') was 1.5414 and it shows low diversity. The same situation is encountered in the case of Simpson (1-D) index, 0.7229, Margalef Index (Md), 1.5262 and Berger-Parker Index (d), 1.5262. The calculated value of Evenness (J') is 0.7016 (Table 5).

This reflects the fact that Iara River fish community has dominant species (*Salmo trutta* in the superior section of the river and *Barbus peteniy* in the lower section of the river).

Table 5. Diversity indices of Iara River

Index	Value
Shannon (H')	1.5414
Simpson (1-D)	0.7229
Margalef (Md)	1.5262
Berger-Parker (d)	0.4074
Evenness (J')	0.7016

Fish communities distribution are affected by latitude and altitude (Parra et al., 2009) but also by climate change (Junker et al., 2014) and human activities (Ruppert et al., 2017; Nicolae et al., 2017).

The imprinted effects of deforestation, agriculture and urbanization are noticed in fish communities and may have destructive consequences (Petrișor, 2016).
The degradation of riparian vegetation caused by deforestation and ancillary activities (sawmills, wood processing and loading, log yards, storage of sawdust) (Adhikari and Ozarska, 2018) have negative impact on fish species diversity.
Fish species identification and historical evaluation of fish stocks represent scientific approaches for the evaluation of aquatic ecosystems (Clausen and York, 2007).
LeRoy Poff et al. (2001), stated that fish respond in three ways to climate change and land use changes: they may adapt to new conditions, change their distribution pattern in available coridors and extinct if environmental parameters and conditions change under tolerance levels of species. As Iara River fish community passes through severe landscape pressure caused by anthropic activities (Mulk et al., 2016) and climate change (Wabnitz et al., 2018), we noticed two out of three responses to environmental changes. First, fish adapted to the new conditions (*Salmo trutta, Cottus gobio*) even though their number is low.
It was found the same response in the case of *Carassius gibelio*, which has found a new coridor from Arieș River to Iara River. The second response was the absence of *Thymallus thymallus*, first signaled by Petru Bănărescu (1964).
A similar study to ours was conducted by Imecs and Nagy (2012) did not confirm the presence of *Thymallus thymallus.* Also in the same study the presence of *Barbabtula barbatula* was mentioned. In our study this species was not present. The small number of fish caught (189 specimens) is a consequence of water quality degradation over time due to logging activities, overfishing, poaching, household pollution and climate change.

CONCLUSIONS

The distribution of fish species in Iara River is influenced by anthropic activities. The most abundant area was at the confluence of Iara River and Arieș River (4 species). The presence of *Carassius gibelio* in sub-mountain fast flowing water is very unusual, but it is explained by the short distance to Arieș River. In four out of eleven sectors we found only one fish species (Sector 1-*Salmo trutta*; Sector 2-*Salmo trutta*, Sector 8-*Barbus petenyi*, Sector 10-*Barbus petenyi*). Fish species composition changes based on altitude. Fish species specific to mountain area are found from sector 1 to sector 7 (altitude ranging from 1100 m to 599.5 m). In the next sectors (8 to 11, altitude ranging from 475.5 m to 381.5) are found fish species corresponding to sub-mountainous area belong to Cyprinidae family.

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NEW DATA ON THE HELMINTH FAUNA OF *ABRAMIS BRAMA* FROM THE DANUBE RIVER, BULGARIA

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Abstract

During the summer of 2017, 10 specimens of freshwater bream (Abramis brama (Linnaeus, 1758)) from the Bulgarian part of the Lower Danube River were examined with standard techniques for parasites. Helminth parasites were recorded in 9 freshwater bream specimens (90.00%) from the Danube River. Five species of parasites were identified: one cestode species Caryophyllaeus laticeps (Pallas, 1781), two trematode species from family Monorchiidae (Asymphylodora imitans (Mühling, 1898); Palaeorchis incognitus (Szidat, 1943)), one acanthocephalan Pomphorhynchus laevis (Zoega in Müller, 1776) and one nematode species Rhabdochona denudata (Dujardin, 1845). All established helminth species are autogenic species, matured in fish. In the component community of Abramis brama from Danube River P. laevis, P. incognitus and R. denudata are core species. A. imitans and C. laticeps are component parasite species for the helminth communities of A. brama. The established in this study parasite species are discussed and compared with previous researches of parasite communities of A. brama from River Danube in Bulgaria. As a result of this study is presented new data for helminths and helminth communities of A. brama.

Key words*:Abramis brama, Bulgaria, Danube,helminth communities, helminths.*

INTRODUCTION

River Danube is the Europe's second-longest river. The River is a habitat to many fish species and has an important place in the European ecological network. The shoreline zone of the Danube River in Bulgaria was presented by 44 fish species (Polačik et al., 2008). Fish parasites communities of *A.brama* from the Bulgarian part of River Danube were studied relatively often during the past few years (Margaritov, 1959; Kakacheva-Avramova, 1977; Atanasov, 2012; Kirin et al., 2013; Kirin et al., 2014; Chunchukova et al., 2016; Chunchukova et al., 2017). Freshwater bream can serve as a final and intermediate host to a wide range of parasite species (see Moravec, 2001). Parasites with their complex life cycles can be used to determine the food web structure and are useful bioindicators of ecosystem stability and biodiversity (Marcogliese and Cone, 1997; Marcogliese, 2003; Marcogliese, 2004).

This study aims to present the diversity and communities of parasites of freshwater bream from the Bulgarian part of the Lower Danube River (town of Silistra). As a result of this survey, new data for helminths and helminth communities of *A. brama* is presented.

MATERIALS AND METHODS

In the summer of 2017 fish and fish parasites were collected and examined from the Danube River (town of Silistra, Bulgarian part). The town of Silistra (44^0117'N, 27^0267'E) is situated on the riverside, in the north eastern part of the Danube Valley, on the last Bulgarian part of the Danube River.

A total of 10 specimens of freshwater bream (*Abramis brama* (Linnaeus, 1758)) from the Danube River were collected and examined in 2017. The 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. Trematodes were fixed as permanent slides after their colouring with acetic carmine, differentiation in 70% acid ethanol, dehydrating in increasing ethanol

series, clarifying in eugenol and mounting in Canada balsam (Bykhovskaya-Pavlovskaya, 1985; Georgiev et al., 1986). The samples were counted and identified using keys of Bauer et al. (1981), Bauer (1987) and Bykhovskaya-Pavlovskaya (1985). Cestodes were stained with acetic carmine and mounted as permanent slides in a Canada balsam, according to Georgiev et al. (1986) and Scholz and Hanzelová (1998). Acanthocephalans were examined as temporary slides in ethanol-glycerin and identified (Petrochenko, 1956; Ergens and Lom, 1970; Bykhovskaya-Pavlovskaya, 1985). Nematodes were examined as temporary microscopic preparations in glycerin (Moravec, 1994; 2013). The dominant structure of the component helminth communities was determined according to the criteria proposed by Kennedy (1993) based on the prevalence (P%): accidental (P% < 10), component (P% < 20) and core (P% >20) species. The ecological terms prevalence, mean intensity (MI) and mean abundance (MA) were used and calculated, based on Bush et al. (1997). Analyses of helminth community structure were carried out in both levels: infracommunity and component community. The component data is used to determine the total number of species, Shannon diversity index (H'), Pielou evenness index (E), Berger-Parker dominance index (d) according to Magurran (2004). The infracommunity data is used to calculate the mean number of species, the mean number of helminth specimens, Brillouin diversity index (HB) (Kennedy, 1993, 1997; Magurran, 2004).

RESULTS AND DISCUSSIONS

A total of 10 specimens of freshwater bream (*Abramis brama* (Linnaeus, 1758)) are collected and examined from the Danube River. The freshwater bream is not included in the Red Data Book of the Republic of Bulgaria (Golemanski (Ed.), 2011). *Abramis brama* is estimated as least concern species (LC=Least Concern; IUCN Red List Status). Freshwater bream is freshwater, brackish, benthopelagic, potamodromous fish species. Adults of this fish species inhabit a wide variety of lakes and large to medium-sized rivers. The diet of freshwater bream includes insects, particularly chironomids, small crustaceans, molluscs and plants. Larger specimens of *A. brama* may feed on small fish. Larvae and juveniles of freshwater bream live in still water bodies, feeding on plankton (Fröse and Pauly, 2020).

A total of 10 specimens of freshwater bream (*Abramis brama* (Linnaeus, 1758)) are collected and examined from the Danube River. Helminth parasites are recorded in 9 freshwater bream specimens (90.00%) from the Danube River. Five species of parasites are identified: one cestode species (*Caryophyllaeus laticeps* (Pallas, 1781)), two trematode species from family Monorchiidae (*Asymphylodora imitans* (Mühling, 1898); *Palaeorchis incognitus* (Szidat, 1943)), one acanthocephalan (*Pomphorhynchus laevis* (Zoega in Müller, 1776)) and one nematode species (*Rhabdochona denudata* (Dujardin, 1845)) (Table 1). All helminth species occurred as adults. They are all autogenic species, matured in fish.

Table 1. Species diversity of helminth parasites of *Abramis brama* from Danube River (N – number of examined fish specimens, n – number of infected hosts, p – number of parasites, P% – prevalence, MA – mean abundance, MI – mean intensity)

Helminth species	N=10					
	n	p	P%	MA±SD	MI±SD	Range
Caryophyllaeus laticeps (Pallas, 1781)	1	4	10.00	0.4±1.2	4.0±0	4
Asymphylodora imitans (Mühling, 1898)	2	13	20.00	1.3±2.69	4.33±3.30	2-9
Palaeorchis incognitus (Szidat, 1943)	3	8	30.00	0.8±1.78	2.67±2.36	1-6
Pomphorhynchus laevis (Zoega in Müller, 1776).	4	32	40.00	3.2±5.79	8.0±6.75	1-17
Rhabdochana denudata (Dujardin, 1845)	3	9	30.00	0.9±2.07	3.0±2.83	1-7

Component community

In the component community of *Abramis brama* from Danube River *P. laevis* (P%=40.00), *P. incognitus* (P%=30.00) and *R. denudata* (P%=30.00) are core species. *A. imitans* (P%=20.00) and *C. laticeps* (P%=10.00) are component parasite species for the helminth communities of *A. brama* (Table 1). In the component community of freshwater bream from Danube River acanthocephalans are presented with the highest number of specimens, with one species and 32 specimens. Trematodes are presented with two species and 21 specimens. Nematodes are represented by one species and nine specimens. Cestodes are represented by one species and four specimens.

In general, the parasite communities of *A. brama* are represented by five species of parasites belonging to four classes, four orders and four families. The total number of isolated and studied specimens is 66. The obtained results are related to Shannon diversity index H'= 1.368, Pielou evenness index E = 0.850 and Berger-Parker Dominance Index d=0.485 (Table 2).

Table 2. Basic indices of helminth community of *Abramis brama* from Danube River

Number of helminth species	5
Number of helminth specimens	66
H' (Shannon, diversity)	1.368
E (Pielou, evenness)	0.850
d (Berger-Parker Dominance Index)	0.485
Dominant species	*P. laevis*

Infracommunity

Species richness in infracommunity of freshwater bream ranges from 1 to 3 species. With one helminth species were infected six fishes (60.00 %), with two helminth species - 2 fishes (20.00%) and with three species - only one specimen of fish (10.00%). The largest number of helminth specimens established in a single host specimen is 24. The average species richness (mean number of species for fish specimen) in infracommunity of freshwater bream is 1.4±0.8 species (Table 3). The parasite communities of *A. brama* from the Danube River showed Brillouin's diversity index, HB=0.453± 0.162 (Table 3).

Caryophyllaeus laticeps is a parasite of fish from Cyprinidae family, but for more specific hosts are considered the species of the genus *Abramis* (Kakacheva-Avramova, 1983).

Table 3. Parameters of the infracommunities of *Abramis brama* from Danube River

Abramis brama	Number of endohelminth species				
	0	1	2	3	Mean±SD Range
	1	5	3	1	1.4±0.8 0-3
Abramis brama	Number of endohelminthspecimens				
	Total number		Brillouin's index HB		Mean± SD Range
	66		0.453±0.162		6.7±6.9 31-24

It was found in *Vimba vimba carinata*, *Abramis brama* and *Abrami sapa* from Bulgarian section of River Danube (Kakacheva-Avramova, 1977). The development of *C. laticeps* is done through an intermediate host – *Tubifex tubifex, Psammoryctides barbatus* and *Limnodrilus claparedianus* (Kakacheva-Avramova, 1983).

Asymphylodora imitans was found in *Abramis brama* and *Blicca bjoerkna* from the Bulgarian section of the Danube River (Kakacheva-Avramova, 1977; Chunchukova et al., 2017). According to Zhytova and Kornyushyn (2017) *Contectiana contecta* (Millet, 1813) =*Viviparus contectus* (Millet, 1813) is the main host of *A. imitans* in Ukrainian polissya waters, while *Planorbis planorbis* (Linnaeus, 1758) is an accidental host. Akimova (2015) reported 4 Gastropoda species from the water bodies of Belarus as intermediate hosts for *A. imitans* (*Radix balthica* (Linnaeus, 1758), *Radix ampla* (W. Hartmann, 1821), *Stagnicola corvus* (Gmelin, 1791) and *Radix auricularia* (Linnaeus, 1758) from the water bodies of Belarus.

*Palaeorchis incognitus*was reported for Danube River basin from barbel (Moravec et al., 1997). *P. incognitus* was reported from *A. brama* for Danube River and Latorica River (see Moravec, 2001), but so far has not been reported for Bulgaria. According to Akimova (2015) the gastropod *Bithynia tentaculata* is involved in the digenean life cycle as the first intermediate host by the 20 or more species of parasites, one of which is *P.incognitus*.

Pomphorhynchus laevis is an intestinal parasite of many freshwater fish, most often by a family Cyprinidae and less frequently by families Salmonidae, Percidae, Siluridae and others (Kakacheva-Avramova, 1983). *P. laevis* was

found in *Abramis brama* and other fish hosts from the Bulgarian section of the Danube River (Atanasov, 2012). This acanthocephalan develops with the participation of an intermediate host – *Gamarus pulex* (Amphipoda) (Petrochenko, 1956).

P. laevis was found in *A. brama* from Danube River (Kakacheva-Avramova, 1977; Atanasov, 2012). The intermediate host of *P. laevis* is *Gammarus pulex*, and definitive hosts are fish (Kakacheva-Avramova, 1983).

Rhabdochona denudata is an intestinal parasite of many species of Cyprinidae family (Moravec, 2013). As parasite of freshwater bream *R. denudata* was reported for Danube River basin (see Moravec, 2001). It is the first report of *R. denudata* as a parasite of *Abramis brama* from the Danube River, Bulgaria. Moravec (2013) suggested that in addition to mayflies also some other aquatic arthropods may serve as intermediate hosts of *R. denudata.*

For the Bulgarian section of River Danube, 29 species were reported as parasites of parasite communities of *A. brama* (Table. 4)

Table 4. Overview of helminth species of *Abramis brama* registered in the lower section of the Danube River, Bulgaria

Authority / Helminth species	Margaritov (1959)	Kakacheva-Avramova (1977)	Atanasov (2012)	Kirin et al. (2013)	Kirin et al. (2014)	Chunchukova et al. (2016)	Chunchukova et al. (2017)	This study
Cestoda								
Caryophyllaeus laticeps		•						•
Caryophyllaeides fennica	•	•						
Caryophyllaeus fimbriceps			•			•		
Trematoda								
Nicolla skrjabini		•						
Asymphylodora imitans							•	•
Asymphylodora tincae						•		
Palaeorchis incognitus								•
Dactylogyrus auriculatus	•							
Dactylogyrus distinguendus		•						
Dactylogyrus sphyrna		•						
Dactylogyrus zandti	•	•						
Gyrodactylus elegans	•			•	•			
Diplostomum spathaceum larv.	•		•					
Diplostomum pseudospathaceum larv			•					
Paradiplozoon homoion						•		
Dactylogyrus yinwenyingae						•		
Diplozoon paradoxum		•		•	•			
Metagonimus yokogawai			•					
Nematoda								
Raphidascaris acus larv.							•	
Rhabdochona denudata								•
Contracaecum microcephalum larv.						•		
Acanthocephala								
Acanthocephalus lucii						•	•	
Acanthocephalus anguillae						•	•	
Metechinorhynchus salmonis		•						
Pomphorhynchus laevis		•	•					•
Pomphorhynchus tereticollis				•	•		•	

In this study, the fixed five species of helminths present only 17.24% of those established for the freshwater bream from the Danube River in the Bulgarian section. In this study of helminth communities of freshwater bream from the Bulgarian part of River Danube, the prevalence (P%) was 90.00%. It is close to the established prevalence from Atanasov (2012) - 85.37% but differs from some more recent studies of *A. brama* from Bulgarian section of River Danube. Chunchukova et al. (2016) and Chunchukova et al. (2017) establish a lower prevalence - respectively 55.3% and 64.44%.

Summary data on Table 4 show that cestode species that refer *to A. brama* from Bulgarian part of Danube River all belong to order *Caryophyllidea.* The numbers of trematode species vary in different studies from one species (Chunchukova et al., 2017) to five species (Kakacheva-Avramova, 1977). It is due probably not only to various ecological factors in individual years but also to the fact that monogeneans were most probably rarely checked. The acanthocephalan *Pomphorhynchus laevis* is a euryxenous parasite having a wide host range, which includes at least 48 fish species (Moravec, 2001). For Bulgarian section of Danube River, this thorny-headed warm has been often reported from various fish host including freshwater bream (Margaritov, 1959, 1966; Kakacheva-Avramova, 1977; Nedeva et al., 2003; Nachev and Sures, 2009; Atanasov, 2012; Chunchukova et al., 2019; etc.). Nematode species were not referred in *A. brama* from Bulgarian part of Danube River in earlier investigations. Only in the most recent studies had been reported two nematode larvae, which also belong to order Rabditida, as the reported *Rhabdochana denudata* in this study (Table 4).

CONCLUSIONS

As a result of the investigation of 10 specimens of freshwater bream from the Danube River, five gastrointestinal parasite species were established: *Caryophyllaeus laticeps*, *Asymphylodora imitans*, *Palaeorchis incognitus*, *Pomphorhynchus laevis* and *Rhabdochona denudata*. The establishment of *P. incognitus* in *A. brama* represents a new host record for the Bulgarian section of River Danube. It is the first report of *R. denudata* as a parasite of *A.brama* from the Danube River, Bulgaria.

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THE INFLUENCE OF STOCKING DENSITY ON GROWTH PERFORMANCE OF JUVENILE JAPANESE ORNAMENTAL CARP (KOI, *CYPRINUS CARPIO* L.)

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Abstract

The identification of suitable fish stocking density proves to be essential in order to elaborate a sustainable fish rearing technology. The aim of present study is to identify a proper stocking density for juvenile ornamental carp, that maximizes the productivity of this specie, in semi-intensive aquaculture production systems. Thus, two ornamental carp stocking densities were tested, in duplicate (V_1 – 6.3 kg/m^3, respectively V_2 – 7.01 kg/m^3). The results indicate a better feed conversion ratio (FCR) in case of V_1, compared to V_2. Also, V_1 registered higher specific growth rate (SGR) values and a better protein efficiency ratio (PER). However, in order to maintain the technological water quality within the optimum range for rearing ornamental carp, a higher water exchange rate was applied at V_2, compared to V_1, especially in the first part of the experimental period. The FCR registered better values in the first part (1.5% feeding ratio was applied), compared to the second part of the experimental period (1% feeding ratio). However, if production maximization is required, higher ornamental carp stocking densities and feeding ratio can be applied if water quality module will be improved.

Key words*: density, growth, ornamental carp.*

INTRODUCTION

Across the world, aquaculture is growing rapidly due to the action of two important factors: the growing demand for seafood and the low fish stocks in the world's oceans.

Japanese colored carp, called Nishikigoi in Japan (koi for short), originated from the common carp, specie raised for human consumption by the rice farmers in Niigata Prefecture. Carp were imported from China into Japan around AD 1500, although the Chinese had been raising carp for food as early as 2000 BC.

The ornamental carp, *Cyprinus carpio* L. (koi carp) is the king of pond fish, and it is currently enjoying the attention of many aquaculturists around the world. Growing valuable varieties of ornamental carp is by far the most profitable of the branches of ornamental aquaculture that can be successfully implemented in our country.

The production of the colored carp - the Japanese "nishikigoi" - presently exceeds in monetary value the production of carp as human food. The nishikigoi as "swimming flowers" delight modern people as much as the taste of carp delighted the Romans at the beginning of carp domestication (Eugene, 1995).

Because of their large size (up to 1 meter in length), longevity (60 years or more), beautiful colours, friendly personalities and high value (some koi exemplars have sold for over a hundred thousand dollars), koi are one of the most likely fish species to be seen by veterinarians.

However, since koi carp is growth in various aquaculture production systems, their growth and physiological performance are affected by various environmental and technological conditions. Stocking density, an important technological factor, generally negatively affects fish growth and welfare (Quan et al., 2020). In aquaculture, stocking density describes the number of fish that are stocked initially per unit area; however, it is generally

used to refer to the density of fish at any point of time (Eduardo et al., 2020).
Therefore, in order to be cost-efficient, aquaculture facilities must optimize their rearing technologies in terms of stocking densities. However, several types of production facilities, based on recirculating aquaculture systems (RAS) or partial RAS are forced to practice high stocking densities since they register significant high value of operational costs.
Thus, the identification of proper fish stocking densities for certain type of production systems is essential in order to maximize system economic efficiency and productivity.

MATERIALS AND METHODS

The trials were carried out at the Aquaculture Research Centre of Food Science and Engineering Faculty during a 31 days experimental period. The aquaculture equipment was a 180 L circulating aquaculture glass tank. The experimental design was made in duplicate, by using four rearing units equipped with independent water conditioning modules. Thus, for biological, chemical and mechanical filtration, each rearing unit was connected to a Hagen AquaClear power filter (500 L/hour flow capacity), 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 filtering module provides superior biological, mechanical and chemical filtration. The area of the filter sponge and the carbon particles increases the filtering capacity. The sponges can be easily washed and reused, allowing the conservation of colonies of biological bacteria. The carbon area of the filter removes dissolved organic compounds, while the ceramic components provide a favourable environment for growing colonies of bacteria that convert ammonia and nitrites into nitrates. It should be mentioned that the sponge area washing was done with dechlorinated water, in order not to affect the colonies of bacteria.
The biological material consists in koi carp exemplars, reared by applying two stocking densities: V_1 – 6.3 kg/m^3, respectively V_2 – 7.01 kg/m^3, in duplicate.
The temperature, pH and dissolved oxygen (DO) were determined daily, using a pH meter WTW - pH 340, respectively a WTW Oxi 315 I temperature and DO meter.
In both experimental variants the biological material was fed with NUTRA pellets which contain fish meal, cereal and cereal by-products, oils, antioxidants (BHT). The biochemical composition of pellets is presented in Table 1.

Table 1. Biochemical composition of NUTRA pellets

Biochemical composition	UM	Concentration
Crude protein	%	54
Crude fat	%	18
Cellulose	%	0.6
Ash	%	10
Phosphorus	%	1.45
Vit. A	U.I./kg	18000
Vit. D3	U.I./kg	1800
Vit. E	mg	500
Cu (CuSO4)	mg	4.5

For the calculation of feeding rate, it is necessary to know the relations between the nutritional requirements of the fish and the environmental conditions. However, it is recommended that the determination of feed quantities and their correction be based not only on the data of the feeding schedule, but also on the actual information and observations obtained during the growing period (Oprea and Georgescu, 2000).
Thus, fish were fed 3 times per day, using an average daily feeding rate of 1.25% from total fish body weight (BW), as follows: 1% BW in the first 14 days of the experimental period and 1.5% in the next 16 days, till the end of the trial. The total fish biomass was determined every 2 weeks in order to adjust the amount of administrated feed. Under growth conditions in the closed system, obtaining the maximum biomass production is essential in order to be profitable.
Thus, this requires high biomass growth rate, respectively a period of time as short as possible to reach the marketable size. To ensure this desideratum, fish are usually fed with granulated feed with high protein content. For these reasons, feed with 54% protein content was used in present experiment.
Artificial feeding is an integral part of managed fish culture practices, where the focus is on

maximizing fish production with minimum feed cost.
The analysed technological indicators were as follows: biomass gain (BG - g fish^{-1}), relative grow rate (RGR - g g^{-1} day^{-1}), specific grow rate (SGR - % fish biomass day^{-1}), feed conversion ratio (FCR) and protein efficiency ratio (PER).
These technological indicators were determined by using the following formulas: (Seyyed et al., 2020; Petrea et al., 2017):

1. Total biomass gain: TBG = TBf - TBi [g] where: TBf – total final fish biomass; TBi – total initial fish biomass;

2. Relative growth rate: RGR = ((TBf-TBi)/t)/TBi [g g^{-1} day^{-1}], where: t - duration of the experiment;

3. Specific growth rate: SGR = 100 * (ln TBf - ln TBi)/t [% fish biomass day^{-1}];

4. Feed conversion ratio: FCR = TF/TBG, where: TF – total feed intake, TBG – total biomass gain;

5. Protein efficiency ratio: PER = TBG/(TF * CP/100), where: CP - crude protein.

RESULTS AND DISCUSSIONS

The sale of ornamental carp is, from a financial point of view, superior to that of rainbow trout. The price for ornamental carp brood is 5-6 euros for a single fish. Viewed in our country with some suspicion, in other countries, such as the Czech Republic, ornamental aquaculture brings income to the fisheries sector twice as high as the production of fish for consumption, of all species together.
The identification of suitable fish stocking density proves to be essential in order to elaborate a sustainable fish rearing technology.
The growth of numerous varieties of koi carp may be a profitable activity if the eco-biological requirements are properly known. In Romania, koi carp is starting to be more appreciated, being preferred by aquarium entrepreneurs due to good growth rate and high sell price. However, maintaining water quality parameters within an optimal range is essential for obtaining the maximum growth performance, associated to a certain fish rearing technology.
Fish being poikilotherms, has the levels of their metabolism directly affected by the ambient temperature which can alter their levels of routine metabolism, food utilization and growth (Aleksander et al., 2010).
Different environmental factors play an important role in the growth and survival of fish. Temperature is probably the most important abiotic factor affecting life (Brett, 1979).
The activities of feeding, digestion and conversion of food are strongly influenced by the ambient temperature, which is ultimately reflected in the variation of the growth rate. Temperature affects the rate of food digestion by influencing the activity of digestive enzymes (Scerbina and Kazlauskiene, 1971).
The optimum temperature required for growth and other physiological activities varies greatly depending on the species. Each species has an optimum temperature for growth which is probably determined by the optimum temperature for growth which is probably determined by the optimum temperature for the specific activity of the enzymes (Davis and Parker, 1990).
The ornamental carp tolerates a fairly large temperature range, between 3-32^{o}C, but the optimal temperature is 23-27^{o}C.
At the optimum temperature, fish grow faster, efficiently convert food and are relatively more resistant to disease.
Thus, for present study, the technological water temperature registered values within optimum limits (20-23.5^{o}C) for koi carp growth, with no significant differences ($p>0.05$) recorded between the experimental variants.
In both experimental variants, the fish showed an active feeding behaviour and a good state of health.
The pH registered values between 6.1–8.2 pH values. However, the pH registered an up/down trend, most probably due to daily water exchange rate (40%). Thus, the added water had a pH which varied between 7.8–8.5 upH (fig. 1, 2), generating therefore high pH values during the experimental period, an advantage for the technological water biological filtration process.
The dissolved oxygen (DO) concentration in water registered mostly values within 4.4 and 6.5 mg L^{-1}, with significant decreases after the feed administration (Figures 1 and 2).

However, it can be observed that DO values are lower in V_2 experimental variant, compared to V_1, fact that can be explained by the applied stocking density, since a high stocking density will generate more metabolic fish wastes (Figures 1 and 2).

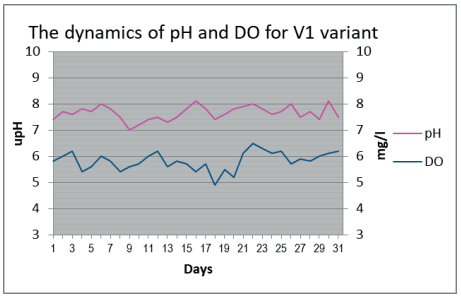

Figure 1. The dynamics of pH and DO for V_1 experimental variant

Stocking density is a major factor determining fish production and farm profitability because it directly influences fish survival, growth, behaviour, health, water quality and feeding (Vincent & Neill, 2019).

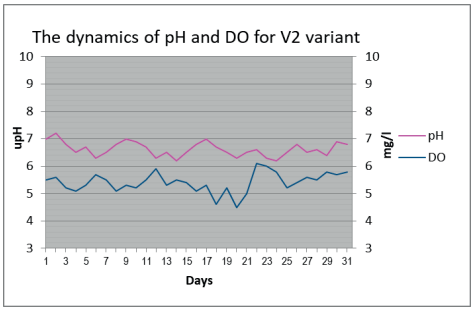

Figure 2. The dynamics of pH and DO for V_2 experimental variant

Increased stocking density causes stress and water quality deterioration. Stressful conditions lead to cortisol release, which chronically threaten the fish health and welfare and cause disease outbreak and fish loss; thus, it is necessary to find methods to suppress such a stress (Seyyed et al., 2020).

Technological performance plays an important role in the operation of a recirculation system, depending on a multitude of factors including meeting the nutritional requirements of the crop species and maximizing feeding efficiency, and optimizing the feed conversion coefficient.

The capacity of a recirculation system is not determined by the volume of water contained, which has the role of ensuring only a minimum dilution of the residues produced, or the density of fish fillings, but by the ability of aeration and filtration systems to maintain water quality in the optimal range during the most intense feeding.

Usually, the measure of the intensity of a closed growth system is expressed by the population density, i.e. the amount of biomass per unit volume (Cristea et al., 2002).

Popular density, expressed in this way, is not however, the most suggestive measure of expressing the production capacity of a closed production system.

The performance of a system is assessed, first of all, depending on the level of feeding intensity that can be sustained by the system, a level expressed as a percentage of the crop biomass.

The time required for the fish to grow to marketable size is also an important criterion for assessing the performance of such a system. Reducing the time required to obtain a marketable size of fish requires the administration of an optimal level of food.

The fish growth performance parameters registered at both experimental variants are presented (Table 2 and Table 3).

The average specific growth rate indicates a superior fish production at V1 (6.3 kg/m^3), compared to V2 (7.01 kg/m^3) experimental variant (Tables 2 and 3). Also, from the perspective of feeding strategy efficiency, the average food conversion ratio (FCR) indicates better values for V1 (6.3 kg/m^3) experimental variant, compared to V2 (7.01 kg/m^3) (Figure 3).

Table 2. The growth performance parameters for V_1 experimental variant

Indicator	Recorded values
Experimental period (days)	31
Total administrated feed quantity (g)	510
Total biomass gain (g)	435
Survival (%)	100
FCR	1.17
RGR (g g-1 day-1)	0.012
SGR (%/day)	1.06
PER	1.28

The protein efficiency ratio (PER) has registered higher values at V1 (6.3 kg/m^3) experimental

variant, compared to V2 (7.01 kg/m^3), revealing the ability of fish organism to utilize proteins, which positively affects growth rate.

Table 3. The growth performance parameters for V_2 experimental variant

Indicator	Recorded values
Experimental period (days)	31
Total administrated feed quantity (g)	375
Total biomass gain (g)	183
Survival (%)	100
FCR	2.04
RGR (g g-1 day-1)	0.009
SGR (%/day)	0.43
PER	1.13

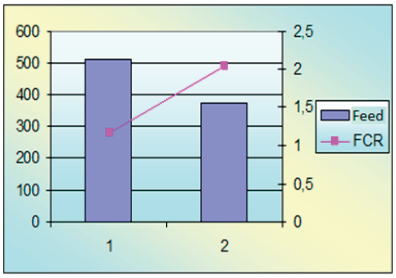

Figure 3. The FCR for both variants

The relative growth rate registered significant better values at V_1 (6.3 kg/m^3), compared to V_2 (7.01 kg/m^3) (Figure 4).

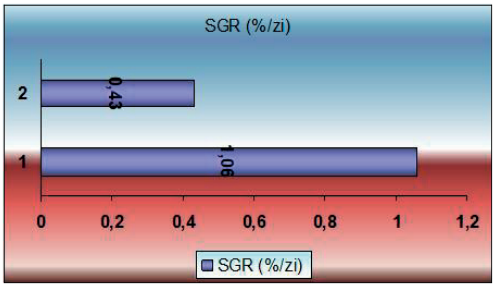

Figure 4. The SGR for both variants

In accordance with our findings, Hussain et al., (2014) have reported that the SGR of Koi carp cultured in intensive systems was strongly reduced with increasing density of fish from 1.4 kg m^3 to 2.1 kg m^3, respectively to 2.8 kg m^3. Also, according to Hussain et al. (2014) findings, the mortality of Koi carp increased (from 0% to 2%) when the density of fish increased from 2.1 kg m^3 to 2.8 kg m^3.

Also, according to Bahremand and Soleimanirad, 2017, a stocking density more than 150 fish per m^3, leads to growth reduction and incidence of stressful conditions in koi carp biomass. Also, same study he pointed out that the optimal stocking density, was 150 fish per m^3.

Considering the modern aquaculture sector, different types of aquaculture systems such as recirculating aquaculture systems (RAS), aquaponics and biofloc systems were established to conserve and minimize the water usage and to optimize the production. In a RAS, wastewater is reused after appropriate treatment and conditioning. It is one of the most intriguing strategies for intensifying aquaculture production while simultaneously reducing wastes (Nuwansi et al., 2019).

CONCLUSIONS

It can be concluded that stocking density of koi carp had a major impact on fish growth performance parameters (FCR, SGR, RGR, PER), since better values were registered at V_1 experimental variant.

Therefore, it can be stated that, in present research, during the analyzed koi carp development stage, a better cost efficiency as well as production maximization is registered if applying a 6.3 kg/m^3 stocking density. However, if water conditioning units which are integrated in the production system are upgraded, higher ornamental carp stocking densities may be possible to be applied, therefore improving the production technology and its performance.

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EFFECT OF FEEDING RATE ON MEAT BIOCHEMICAL COMPOSITION OF *ACIPENSER STELLATUS* (PALLAS, 1771) REARED IN A RECIRCULATING AQUACULTURE SYSTEM

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Abstract

The meat nutritional value is an important factor that influence consumer preferences for certain fish species. Thus, the aim of this study is to identify the influence of feeding rate on stellate sturgeon meat biochemical composition, assessed by determining the percentage of dry matter, crude fat, crude protein and ash. Two feeding rates were tested (F1 - 1% BW, respectively F2 - 2% BW), in replicate. The biochemical evaluation of stellate sturgeon meat revealed better values in terms of crude protein content at F2 duplicate (17.86±0.14 %, respectively 17.70±0.17%), compared to F1 experimental variant duplicate (16.71±0.2%, respectively 16.78±0.18%). Also, F2 registered a higher crude fat (1.32±0.04%, respectively 1.36±0.03) and ash content (1.69±0.06%, respectively 1.77±0.09), compared to F1 (crude fat: 1.21±0.04%, respectively 1.27±0.05%; ash: 1.33±0.10%, respectively 1.39±0.11%). The water-to-protein ratio indicate a high nutritional value for meat of stellate sturgeon exemplars, reared by applying a feeding rate of 2% BW (4.31±0.15, respectively 4.35±0.17 at F2 duplicate, compared to 4.71±0.11, respectively 4.67±0.10 at F1). As a conclusion, the use of 2% BW feeding rate for rearing stellate sturgeons in a recirculating aquaculture system (RAS) assures a superior protein and fat retention and improves protein use efficiency (PUE).

Key words*: biochemical composition, crude protein, feeding rate, RAS, stellate sturgeon.*

INTRODUCTION

Aquaculture has a key contribution in maintaining the balance of global economy, considering the present worldwide status, which is characterized in Cao and Li (2013) study by rapid population expansion, urban development, improved quality of life in most parts of the world and continuous increase of demand for animal protein. Thus, intensive production fish farms may represent a solution for above mentioned challenges, only if proper technical and technological solutions are applied, in order to maximize their economic performance.

In order to be able to assure continuous intensive production over the entire year, aquaculture farmers must adopt recirculating aquaculture systems (RAS). Several studies, characterized RAS as high capital and operating costs, compared to other systems such as cage culture in natural waters and raceway and/or pond culture systems. Therefore, as Petrea et al. (2019) stated, most aquaculture facilities based on RAS are focusing on rearing high economic value fish species as sturgeons, turbot, salmon, rainbow trout or tilapia, in order to maintain the profitability (Cristea et al., 2002; Engle et al., 2010; Timmons et al., 2018).

The fish rearing technologies applied in RAS are based both on high stocking densities and feeding rates, in order to maximize the productivity and, therefore, the profitability of aquaculture facilities. However, beside assuring a high productivity, the main goal of fish rearing technologies is to maintain or even improve the nutritional value of fish meat. According to Dorojan et al. (2014), the biochemical composition of fish can be significantly influenced by the composition of administrated feed, fish development stage, or rearing

conditions. Antache et al. (2013) revealed that fish body composition is largely influenced by feed composition. An increase in other parameters such as feeding rate and fish size also results in enhanced adipose deposition and decreases water content in the fish body (El-Zaeem et al., 2012). Therefore, the aim of present study is to identify the influence of feeding rate on stellate sturgeon (201.72±32.72 g) meat proximate composition, reared in RAS.

MATERIALS AND METHODS

The description of RAS Pilot Station

The present study was conducted in RAS pilot station of Food Science, Food Engineering, Biotechnology and Aquaculture Department, Food Science and Engineering Faculty - „Dunărea de Jos'' University of Galați, during a 28 days experimental period.

The configuration of the pilot RAS was sized according to specific technology described by Cristea (2008). The RAS pilot system consists in a number of four octagonal rearing units, connected to water conditioning modules, as described in Figure 1.

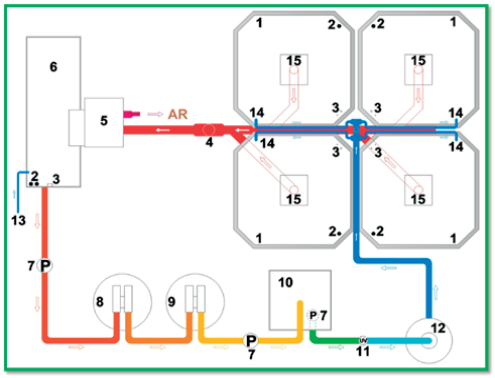

Figure 1. The design of RAS pilot station: rearing units - No.1, nitrogen compounds sensors - No.2; water level sensors – No.3; RAS outlet structure – No.4; mechanical drum filter – No.5; sump – No.6; pumps–No.7; sand filter – No.8; activated charcoal filter – No. 9; biological trickling filtration unit – 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)

Biological material and experimental design

The fish biomass composed of 92 specimens of stellate sturgeon (201.72±32.72 g), which are the subject of the present study, was equally distributed within the four rearing units B1, B2, B3, B4 (4 rearing units x 23 specimens). Two feeding rates were tested (F1 - 1% biomass weight - BW, respectively F2 - 2% BW), in replicate (F1 in B1, B2, respectively F2 in B3, B4).

Feed biochemical composition

During the experimental period, Classic extra 1 P pellets were administrated by using automatic feeders. The feed biochemical composition is presented in Table 1.

Table 1. The biochemical composition of administrated feed

Crt.no.	Composition	Quantity
1	Crude Protein %	41.0
2	Crude fat%	12.0
3	Crude cellulose %	3.0
4	Crude ash %	6.5
5	Phosphorus %	0.9
6	Digestible energy (MJ/kg)	14.2
7	Vitamin A (UI)	10000
8	Vitamin D3 (UI)	1250
9	Vitamin E (mg)	150
10	Vitamin C (mg)	75
11	Cystine%	0.6
12	Lysine %	2.4
13	Methionine %	0.75
Note:	Fish meal. fish oil. haemoglobin. full fat soybean. soybean oil. wheat gluten. sunflower flour. wheat and wheat products. BHT.	

Water quality assessment

In order to determine the water quality parameters throughout the experimental period, temperature, dissolved oxygen (DO) and pH were measured daily. Also, the rest of water quality parameters, presented in Table 2, were determined twice per week. The water quality evaluation methods and equipment are presented in Table 2.

Table. 2. Water quality evaluation methods and equipment

Analysed Parameter	Method	Equipment
DO pH Temperature	Sensor method	HQ40d Portable Multi-Parameter (HACH)
NO_3 NO_2 NH_4 PO4 COD	Spectrophotometric method using Merk kits	Spectroquant photometer, Nova 400
Percentage removal of BOD_5	Winkler`s method	Velp IP54 analyzer
Turbidity	Spectrophotometric method	Turbidometer VELP, TB1.

Stellate sturgeon meat proximate composition assessment

The determination of stellate sturgeon meat proximate composition was made both in the initial and final stage of experimental period, from fresh meat tissue. During sampling process, ensuring the uniformity of the analysed exemplars was targeted, in order to eliminate the possible errors due to biomass differences. The proximate composition analyses were performed on homogenized muscle tissue by using the Association of Analytical Chemists (AOAC) methods (AOAC, 2000).

Chemical composition of meat crude protein was determined according Kjeldahl method (N x 6.25). Crude lipids were determined according to Soxhlet solvent extraction method (petroleum ether). Dry matter was determined by heating the muscle tissue samples at a temperature of 105±2ºC, using Sterilizer Esac, while ash was evaluated by calcification at a temperature of 550±20ºC in a Nabertherm furnace.

Stellate sturgeon meat quality indicators

The main stellate sturgeon meat quality indicators were determined as follows:

1. $PUE = 100 (W_f \times P_f - W_i \times P_i)/(F \times P_b)$ (%), where: PUE - Protein utilization efficiency; P_f - muscle tissue protein at the end of experimental period (%), P_i - muscle tissue protein at the initial stage of experimental period (%); W_f – final biomass (kg); W_i – initial biomass (kg); F – total feed quantity consumed (kg); P_b – administrated feed protein concentration. (%).
2. $PR = W_f \times P_f - W_i \times P_i$ (g/specimen), where: *PR* - retained protein
3. $LR = W_f \times L_f - Wi \times L_i$ (g/specimen), where: LR- retained lipids; L_f = muscle tissue lipids at the end of experimental period (%), L_i = muscle tissue lipids at the initial stage of experimental period (%)

Statistical methods

Statistical analysis was performed using the IBM SPSS Statistics 20 for Windows. Statistical differences between treatments were tested using T test ($\alpha = 0.05$) after a normality test (Kolmogorov-Smirnov). Comparisons between variants were assessed using post-hoc Duncan test for multiple comparisons (ANOVA).

RESULTS AND DISCUSSIONS

Water quality parameter

During the experimental period, the technological water quality parameters registered proper concentrations for rearing stellate sturgeon in the analysed development stage. Thus, the nitrogen compounds ($N\text{-}NH_4$; $N\text{-}NO_2$; $N\text{-}NO_3$), as well as P_2O_5, registered a higher average concentration for both duplicate trials (B3: 0.28±0.08 mg L^{-1} for $N\text{-}NH_4$; 0.13±0.04 mg L^{-1} for $N\text{-}NO_2$; 91.83±11.6 mg L^{-1} for $N\text{-}NO_3$; 25.11±6.9 mg L^{-1} for P_2O_5, respectively B4: 0.30±0.11 mg L^{-1} for $N\text{-}NH_4$; 0.11±0.05 mg L^{-1} for $N\text{-}NO_2$; 87.39±16.1 mg L^{-1} for $N\text{-}NO_3$; 23.94±5.1 mg L^{-1} for P_2O_5) which composed the F2 experimental variant, compared to B1 (0.21±0.09 mg L^{-1} for $N\text{-}NH_4$; 0.09±0.03 mg L^{-1} for $N\text{-}NO_2$; 79.26±18.6 mg L^{-1} for $N\text{-}NO_3$; 21.32±6.2 mg L^{-1} for P_2O_5) and B2 (0.22±0.07 mg L^{-1} for $N\text{-}NH_4$; 0.08±0.02 mg L^{-1} for $N\text{-}NO_2$; 74.74±14.9 mg L^{-1} for $N\text{-}NO_3$; 19.77±5.3 mg L^{-1} for P_2O_5) duplicates, part of F1 experimental variant (Table 3). This may be due to high feed input, collaborated to low recirculation flow of the RAS, respectively low hydraulic retention time (HRT) recorded at the level of each of the four rearing units.

Also, this hypothesis is confirmed by low pH values of technological water recorded in F2 experimental variant rearing units (6.18±0.54 upH at B3, respectively 6.15±0.51 upH at B4), compared to F1 rearing units (6.32±0.43 upH at B1, respectively 6.37±0.48 upH at B2) (Table 3).

As well, high values of turbidity (5.33±0.52 NTU at B3 respectively, 5.19±0.42 NTU at B4), percentage removal of BOD5 (64.95±16.85% at B3, respectively 67.68±15.86% at B4) and COD concentration (81.07±22.79 mg L^{-1} at B3, respectively 79.81±24.93 mg L^{-1} at B4), correlated to low DO concentrations (7.42±0.88 mg L^{-1} at B3, respectively 7.49±1.09 mg L^{-1} at B4), recorded at F2 experimental variant rearing units, compared to F1 rearing units (turbidity 4.88±0.38 NTU at B1 respectively, 4.68±0.49 NTU at B2; BOD5 56.96±14.78% at B1, respectively 53.73±12.84% at B2; COD 68.45±14.84 mg L^{-1} at B1, respectively 73.12±16.04 mg L^{-1} at B2; DO 7.76±0.62 mg L^{-1} at B1, respectively 7.63±0.56 mg L^{-1} at B2), indicates a possible superior organic matter

accumulation rate due to feed rate (2% feeding rate applied at F2 vs. 1% feeding rate applied at F1 experimental variants).

Not significant differences ($p>0.05$) were recorded between the experimental variants in terms of technological water temperature.

Table 3. 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
Percentage removal of 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 (^oC)	22.82±0.44	22.83±0.46	22.90±0.39	23.87±0.41
COD (mg L^{-1})	68.45±14.84	73.12±16.04	81.07±22.79	79.81±24.93

Stellate sturgeon meat proximate composition assessment

The proximate composition of stellate sturgeon meat, reared in both experimental variants (F1 and F2), by applying different feeding rate conditions was determined both at the initial and final stage of the experimental period, in order to identify the ash, lipids, proteins and moisture content, as well as the moisture: protein (M/P) ratio (Table 4).

According to ANOVA test, not significant differences ($p>0.05$: $p=0.532$ for ash, $p = 0.278$ for lipid, $p=0.636$ for protein, $p=0.734$ for moisture and $p=0.324$ for M/P) were recorded between the exemplars reared in the rearing units corresponding to the same experimental variant. Thus, the duplicate experimental design is validated.

However, according to ANOVA test, statistically significant differences ($p<0.05$) were recorded between initial and final stage of experimental period, for both variants (F1 and F2) in terms of ash ($p=0.021$), lipids ($p=0.033$), proteins content ($p=0.048$), as well as for M/P ratio ($p=0.028$) (Table 4).

Also, by analysing the data, according to ANOVA test, it can be stated that statistically significant differences ($p<0.05$) were recorded, at the end of experimental period, between the two experimental variants (F1 and F2) in terms of moisture ($p=0.033$), protein ($p=0.045$), lipids ($p=0.042$) and ash content ($p=0.027$) (Table 4).

Thus, superior content of protein (17.86±0.14% - B3, respectively 17.70±0.17% - B4) and lipids (1.32±0.04% - B3, respectively 1.36±0.03 % - B4) are recorded at F2, were the highest feeding rate was applied, compared to F1 (protein: 16.71±0.2% - B1 and 16.78±0.18% - B2; lipid: 1.21±0.04% - B1 and 1.27±0.05% - B2) (Table 4).

However, the M/P ratio and moisture content indicates lower values for F2 variant (M/P: 4.31±0.15 at B3 and 4.35±0.17 at B4; moisture: 76.99±0.17% at B3 and 77.05±0.21% at B4), compared to F1 (M/P: 4.71±0.11 at B1 and 4.67±0.1 at B2; moisture: 78.72±0.33% at B1 and 78.38±0.27% at B4) (Table 4), situation which emphasizes a better condition status and meat nutritional quality for stellate sturgeon reared by using a 1% BW feeding ratio. The M/P ratio is considered an important indicator for the evaluation of meat quality, since it does not take into account the lipids content.

The PUE, PR and LR indicators were calculated in order to asses the nutrient retention efficiency of stellate sturgeon experimental biomass (Figure 2).

Therefore, results reveal a higher statistically significant ($p<0.05$) degree (ANOVA test) of both proteins ($p=0.008$; PR: 14.16 g/specimen at F1, respectively 30.17 g/specimen at F2) and lipids accumulation ($p=0,011$; LR: 0.76 g/specimen at F1, respectively 1.92 g/specimen at F2), for stellate sturgeons' specimens reared in F2, compared to F1 experimental variant (Figure 2).

Also, the evolution of PUE (Figure 2) emphasizes a better protein valorisation if stellate sturgeons' specimens are reared by

applying a 2 % BW feeding rate (51.18%), compared to 1 % BW (50.33%).

Thus, it can be stated that a higher feeding rate can led to an improvement of meat quality due to statistically significant ($p<0.05$, $p=0.041$) decrease of moisture (ANOVA test), collaborated with the statistically significant ($p<0.05$, $p=0,039$) increase of protein content (ANOVA test).

The findings recorded in present study confirm the statement of El-Zaeem et al. (2012) according to which the protein of the fish meat can range between 12.3 – 28%.

Also, according to Sikorski et al. (1994) classification, all the stellate sturgeons' specimens from present experiment can be included in second quality class (1st class - >20% protein content ; 2nd class – 15 – 20% protein content; 3rd class – 10 – 15% protein content; 4th class - <10% protein content), with meat protein content ranging between 16.71 – 17.86%.

According to Ackman et al. (1989) classification, all the stellate sturgeons' specimens from present experiment can be included in the first class (1st class - < 2% lipids content ; 2nd class – 2 – 4% lipids content; 3rd class – 4 – 8% lipids content; 4th class - >8% lipids content), with a lipid content ranging between 1.21 – 1.36%.

According to Ionescu et al. (2006), the percentage of lipids content in fish meat varies within 0.1 – 28 %. However, multiple studies which analysed lipids content variation in sturgeon's meat (Dorojan et al., 2014; Dicu et al., 2013; Paltenea et al., 2007; Vasilean et al., 2010) have reported values less than 4%, therefore characterizing sturgeons' species as low-fat fish.

The results recorded in present study, related to stellate sturgeon meat proximate composition assessment are have been compared to those reported by other authors, for stellate sturgeons' specimens reared in RAS condition during similar development stage (Table 5).

Thus, in terms of ash content, the registered results are higher compared to those reported by Dorojan et al. (2014) (1.14-1.17%), respectively Dorojan et al. (2015) (0.99 – 1.20%), (Table 5).

However, compared to present study, Dorojan et al (2015) recorded superior lipids content (2.58 – 5.50%), while Dorojan et al. (2015) and Dorojan et al. (2014) reported lower protein content (14.45 – 15.69%, respectively 12.48 – 14.80%), (Table 5).

The results in terms of moisture content and M/P ratio were similar to those reported by Dorojan et al. (2015) (moisture: 70.91 – 76.54%; M/P ratio: 4.55 – 5.30), (Table 5).

Table 4. Stellate sturgeon meat proximate composition assessment

Parameters	Experimental variant		Ash (%)	Lipid (%)	Protein (%)	Moisture (%)	M/P
Initial	*F1; F2*	*B1; B2; B3; B4*	1.22±0.03	1.09±0.02	16.10±0.12	79.02±0.45	4.91±0.03
Final	*F1*	*B1*	1.33±0.1	1.21±0.04	16.71±0.2	78.72±0.33	4.71±0.11
		B2	1.39±0.11	1.27±0.05	16.78±0.18	78.38±0.27	4.67±0.1
	F2	*B3*	1.69±0.06	1.32±0.04	17.86±0.14	76.99±0.17	4.31±0.15
		B4	1.77±0.09	1.36±0.03	17.70±0.17	77.05±0.21	4.35±0.17

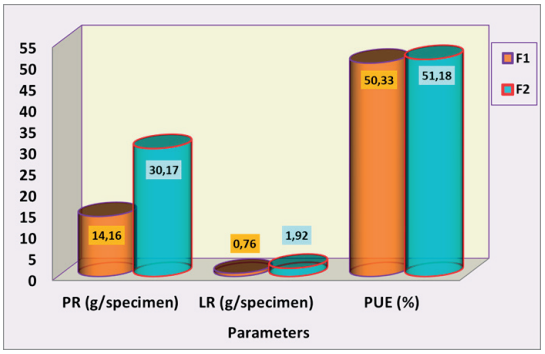

Figure 2. Protein utilization efficiency (PUE), retained protein (PR) and retained lipids (LR) for stellate sturgeon specimens reared in both experimental variants (F1 and F2)

Table 5. The meat proximate composition of stellate sturgeons reared in RAS, during similar development stage, reported by different authors

Reference	Stellate sturgeon average biomass (g)	Ash (%)	Lipid (%)	Protein (%)	Moisture (%)	M/P
Dorojan et al., 2015	188.33- 201.03	0.99 – 1.20	2.58 – 5.50	14.45 – 15.60	70.91 – 76.54	4.55 – 5.30
Dorojan et al., 2014	140 - 166	1.14 – 1.17	0.30 – 0.43	12.48 – 14.80	80.55 - 83.44	6.45 – 5.64
Dicu et al., 2013	204 ± 8	-	0.41 – 0.76	-	-	6.51 – 7.12

CONCLUSIONS

The results recorded in present study concludes that the use of 2% BW feeding rate for rearing stellate sturgeons, in RAS conditions, assures a superior protein and fat retention and improves protein use efficiency.

However, if analysing M/P ratio and moisture content, it can be concluded that a 1% BW feeding rate assures a better condition status for the biological material and superior stellate sturgeon meat quality.

However, the findings are influenced by the RAS capacity to maintain proper conditions for stellate sturgeon growth throughout the experimental period. For future studies, it is recommended to extend the experimental period in order to have a better view related to the influence of feeding rate on stellate sturgeon meat proximate composition.

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COULD STERLET (*ACIPENSER RUTHENUS*) AS POLYCULTURE SPECIES TO POSITIVELY INFLUENCE THE PIKEPERCH (*SANDER LUCIOPERCA*) FARMING IN RECIRCULATING AQUACULTURE SYSTEMS?

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Abstract

The pikeperch farming in recirculating aquaculture systems (RAS) has a relatively short history, and many ideas for production improvement in this species recently emerged. The polyculture is one of these. The aim of this study is to evaluate if sterlet as polyculture species reared in RAS with pikeperch could have a beneficial impact on tank's bioproductivity and growth dynamic of the juvenile pikeperch. One control (monoculture) (C) and two polyculture variants have been established in duplicate: 1335 pikeperch, 45 days old /m^3 with 10% (V_1), and 20% (V_2) sterlet. The experiment has been carried out during 35 days into a RAS with 6 tanks (1 m^3/tank). The fish were fed in all tanks with dry food, 10% of pikeperch biomass in each tank daily, assuring ad libitum feeding. A significant plus of fish biomass resulted by valorisation of the pellets unconsumed by the pikeperch, in both experimental variants (V_1, V_2). The pikeperch reared for 35 days in polyculture with 20% sterlet (V_2) had significantly higher body weight than the pikeperch reared with 10% sterlet (V_1) or in monoculture (C).

Key words*: pikeperch, polyculture, RAS, sterlet.*

INTRODUCTION

The potential of polyculture of two or more fish species and the polyculture of fish with other animals or plants for obtaining of multiple products with economical value has been already highlighted by many researchers (Dey et al., 2005; Elia et al., 2014; Nicolae et al., 2015; Stickney, 2015; Filep et al., 2016; Hisano et al., 2019). The polyculture in ponds is generally used for a better utilization of different trophic and spatial niches (Rahman et al., 1992), but the monoculture is the most popular stoking method in recirculating aquaculture systems (RAS). RAS is based on the water recycling using mechanical and biological filters which allows highly-intensive productions of various fish species (Grozea, 2002, 2007). Anyway, the polyculture in RAS, proven significant advantages in pikeperch farming (Kozłowski et al., 2014; Mihailov et al., 2017) due to the very selective feeding behaviour of this species, that eat pellets exclusively in the water column. The pellets reaching the bottom of the tank remain uneaten by pikeperch which have a very specific behaviour (Grozea, 2015; Grozea et al., 2016; Mihailov et al., 2017). Into a recent study carried out by our team we considered sterlet (*Acipenser ruthenus*) as a good candidate to be reared as additional species with pikeperch in RAS, eating pellets from the bottom of the tank (Mihailov et al., 2017). The preliminary results obtained by us corroborated with other information from specialty literature have been encouraging (Kozłowski et al., 2014). The aim of this study is to evaluate if sterlet (*Acipenser ruthenus*) as polyculture species (10 – 20%) with pikeperch (*Sander lucioperca*) reared in recirculating aquaculture system could have a beneficial impact on tank's bioproductivity and growth dynamic of the pikeperch.

MATERIALS AND METHODS

Forty-five days old pikeperch mixed with forty days old sterlet have been used in our polyculture variants.

Juvenile pikeperch was obtained by means of controlled reproduction in the recirculating aquaculture system (SAR) of the Banat's

University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, in April 2019. Juvenile sterlet was obtained from Nimb Fish farm - Giarmata. Both species have been previously farmed in RAS, in monoculture, being accommodated with the same dry feed.

Two polyculture variants have been tested in duplicate: pikeperch with 10% sterlet (V_1) (135 sterlet/tank), and pikeperch with 20% sterlet (V_2) (270 sterlet/tank). The control variant (C), meaning pikeperch in monoculture, was established with the same number of the pikeperch like in V_1 and V_2 (1335 individuals/m^3).

The average body weight of the pikeperch at the beginning of the experiments was 0.77±0.07, 0.82±0.08 and 0.85±0.09 g/individual for V_1, V_2 and C, respectively. The initial body weight of the sterlet used for polyculture varied between 2.57 and 3.48 g/individual.

The experiment has been carried out during 35 days into a RAS with 6 rectangular fiberglass tanks (1 m^3/tank) and a treatment unit which assured the water quality in the normal limits during all experimental period. The main physical-chemical parameters have been monitored two times per day and maintained in normal limits for pikeperch farming: temperature 22±1°C, dissolved oxygen 6-7.5 mg/l, nitrates <150 mg/l, ammonium <0.3 mg/l, nitrites <0.6 mg/l. In order to maintain the good water quality, 2 m^3 of water from RAS was daily replaced with fresh water.

The fish were fed during the study with 1.2- 1.5 mm ADVANCE and 2 mm STAR ALEVIN pellets (Alltech-Coppens, The Netherlands), according with the fish size, 10% of pikeperch biomass in each tank. This amount assured *ad libitum* feeding for pikeperch batch and was weekly adjusted according with the weight dynamic of the pikeperch. Feeding was done using 24-hours belt feeders (FIAP, Germany).

The unconsumed feed was siphoned once per day from the bottom of the tanks.

In order to evaluate growth dynamic of the fish, total length (TL) and the body weight (BW) were weekly measured for 15 pikeperches and 15 sterlets from each tank, meaning 30 fishes from each variant. The morphometry has been carried out after the fish were anesthetized with clove oil (Fares SA, Romania). The mean, standard deviation (SD), standard error (SE) and coefficient of variation (CV) have been calculated for each morphometric trait of both fish species.

Specific Growth Rate (SGR) for BW (SGR_{BW}) and TL (SGR_{TL}), Daily Growth Rate (DGR) and Feed Conversion Rate (FCR) were calculated using the following formula:

Specific growth rate (% day^{-1});

SGR_{BW} = [(ln final BW - ln initial BW) / ΔT] x 100

SGR_{TL} = [(ln final TL - ln initial TL) / ΔT] x 100

Daily growth rate (g d^{-1});

DGR = (final BW – initial BW) / ΔT

Feed conversion rate;

FCR = Feed distributed / (final Biomass – initial Biomass)

where: ΔT is the duration of the experiment, the other being described above

The data were analysed using STATISTICA10 software. Duncan post hoc test was used to assess the significance of differences. The data statistically processed are presented into the paper as Mean ± SD.

RESULTS AND DISCUSSIONS

As a result of the weekly measurements made for body weight and total length, of the juvenile pikeperch, during the experimental period, it was observed a constant and significant growth ($p \leq 0.05$) in both morphometric traits and polyculture variants. The Figures 1, 2 and Tables 1, 2 are suggestive in this regard.

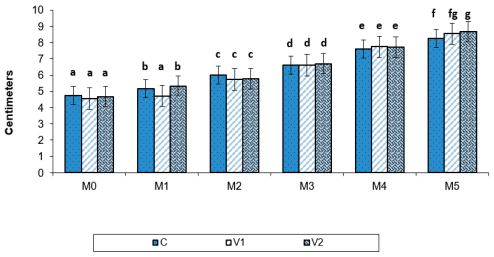

Figure 1. The weekly dynamic of the total length and the significance of the differences in pikeperch. M0 - M5 – weekly measurements. Same letter indicates not significant differences ($p>0.05$)

The growth of the TL and BW in pikeperch shown a quite similar dynamic which reflected a significantly better growth of the fish from V2 where the pikeperch were reared in polyculture

with sterlet 20%. The pikeperch from this trial were longer ($p \leq 0.05$) than the fish from control batch and also heavier ($p \leq 0.001$) than the fish from C and V1 variants. Therefore, BW of the pikeperch in V2 reached at the end of experimental period 5.86±1.67 g, significantly higher ($p \leq 0.001$) than the fish in C and V1 (4.81±1.36 g and 5.13±1.15 g, respectively). It was an interesting founding which could be due to the faster removal of the pellets from the bottom of the tank by the sterlet which lead to a local better water quality even at a higher fish biomass per tank.

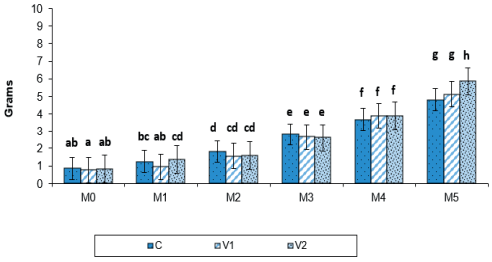

Figure 2. The weekly dynamic of the body weight and the significance of the differences in pikeperch. M0 - M5 – weekly measurements. Same letter indicates not significant differences ($p>0.05$)

Table 1. Growth dynamics of the total length - TL (cm) of the juvenile pikeperch during the 35 days (n = 30)

Specification	M0			M1			M2			M3			M4			M5		
	C	V1	V2	C	V1	V2	C	V1	V2	C	V1	V2	C	V1	V2	C	V1	V2
Mean (cm)	4.74	4.55	4.68	5.17	4.72	5.33	6.01	5.74	5.77	6.62	6.62	6.70	7.59	7.74	7.72	8.25	8.54	8.68
SD	0.57	0.11	0.42	0.48	0.46	0.42	0.59	0.72	0.47	0.60	0.57	0.7	0.86	0.62	0.85	0.70	0.66	0.79
SE	0.10	0.02	0.08	0.09	0.08	0.08	0.11	0.13	0.09	0.11	0.10	0.13	0.16	0.11	0.16	0.13	0.12	0.14
CV	12.10	2.35	8.96	9.22	9.68	7.96	9.75	12.58	8.17	9.00	8.58	10.68	11.28	7.99	11.06	8.54	7.73	9.07

M0 - M5 – the weekly measurements; SD – standard deviation; SE – standard error; CV – coefficient of variation; C – control; V1 and V2 – trials

Table 2. Growth dynamics of the body weight - BW (g) of the juvenile pikeperch during the 35 days (n = 30)

Specification	M0			M1			M2			M3			M4			M5		
	C	V1	V2	C	V1	V2	C	V1	V2	C	V1	V2	C	V1	V2	C	V1	V2
Mean (g)	0.85	0.77	0.82	1.25	0.94	1.37	1.81	1.56	1.59	2.81	2.66	2.62	3.68	3.90	3.90	4.81	5.13	5.86
SD	0.09	0.07	0.08	0.38	0.26	0.34	0.47	0.55	0.41	0.60	0.73	0.78	0.88	0.89	1.24	1.36	1.15	1.67
SE	0.02	0.01	0.02	0.07	0.05	0.06	0.09	0.10	0.07	0.11	0.13	0.14	0.16	0.16	0.23	0.25	0.21	0.31
CV	10.1	8.84	10.32	30.39	28.09	24.70	25.92	35.04	25.65	21.47	27.54	29.79	23.95	22.93	31.72	28.19	22.45	28.56

M0 - M5 – the weekly measurements; SD – standard deviation; SE – standard error; CV – coefficient of variation; C – control; V1 and V2 – trials

The growth of the TL and BW in sterlet shows the same trend like in pikeperch, with a better growth of the sterlet from V2 (Tables 3, 4 and Figures 3, 4). This dynamic is very well corelated with the observations of the pikeperch growth dynamic, and could be due to the same reason, upper specified. Therefore, the polyculture of the pikeperch with 20% sterlet could lead to the improving the growth of both species.

Table 3. Growth dynamics of the total length - TL (cm) of the juvenile sterlet during the 35 days (n = 30)

Specification	M0		M1		M2		M3		M4		M5	
	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2
Mean (cm)	7.46	8.59	9.81	10.38	12.01	12.01	13.69	13.89	15.07	15.89	16.23	16.61
SD	0.65	0.86	1.03	0.86	0.95	1.08	1.36	1.15	1.5	1.13	1.24	1.48
SE	0.12	0.16	0.19	0.16	0.17	0.20	0.25	0.21	0.27	0.21	0.23	0.27
CV	8.75	9.97	10.47	8.30	7.94	9.01	9.95	8.28	9.95	7.10	7.64	8.93

M0 - M5 – the weekly measurements; SD – standard deviation; SE – standard error; CV – coefficient of variation; C – control; V1 and V2 – trials

Table 4. Growth dynamics of the body weight - BW (g) of the juvenile sterlet during the 35 days (n = 30)

Specification	M0		M1		M2		M3		M4		M5	
	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2
Mean (g)	2.57	3.48	4.89	5.65	7.82	8.06	11.44	11.96	12.81	15.48	15.39	18.67
SD	0.27	0.30	1.52	1.17	1.64	1.89	3.42	2.34	3.31	3.13	3.76	4.77
SE	0.05	0.05	0.28	0.21	0.30	0.35	0.62	0.43	0.61	0.57	0.69	0.87
CV	10.38	8.57	30.47	20.68	20.96	23.44	29.91	19.54	25.88	20.23	24.46	25.56

M0 - M5 – the weekly measurements; SD – standard deviation; SE – standard error; CV – coefficient of variation; C – control; V1 and V2 – trials

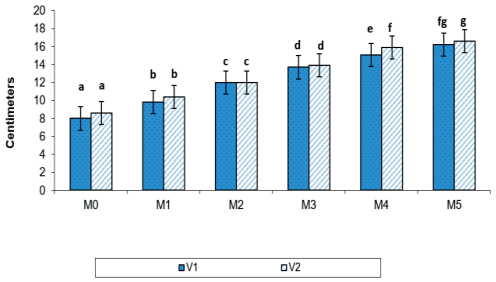

Figure 3. The weekly dynamic of the total length and the significance of the differences in sterlet. M0 - M5 – weekly measurements. Same letter indicates not significant differences (p>0.05)

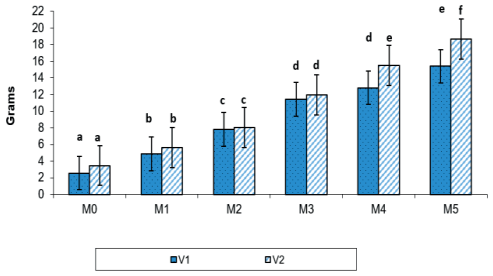

Figure 4. The weekly dynamic of the body weight and the significance of the differences in sterlet. M0 - M5 – weekly measurements. Same letter indicates not significant differences (p>0.05)

Comparing the total biomass per m^3 of the three trials the best yield was registered in the experimental variant V2 (Figure 5 and 6), where at the end of the experiment 12.095 kg of fish (7.054 kg pikeperch and 5.041 kg sterlet) were obtained. In the experimental variant V1, 8.261 kg of fish (6.184 kg pikeperch and 2.077 kg sterlet) were harvested.

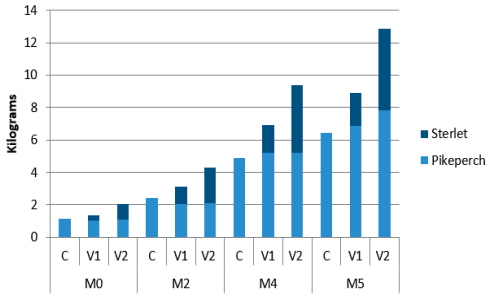

Figure 5. Graphical representation of fish biomass growth (kg/m^3) during experimental period

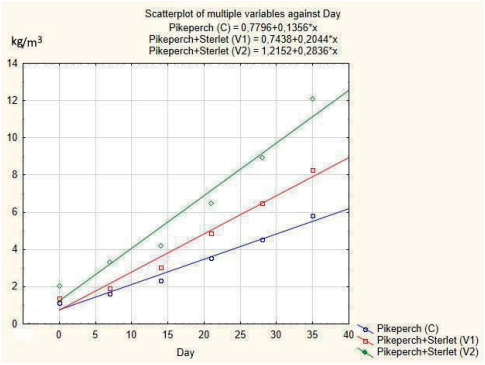

Figure 6. Fish biomass dynamic during 35 days, mathematically adjusted, in control and experimental variants

In both polyculture variants (V1 and V2) the fish biomass per m^3 was higher than in monoculture (variant C), but not only total fish biomass but pikeperch biomass as well (5.803 kg of pikeperch being obtained in variant C).
Bio-productive parameters are generally better for polyculture variants, the results being shown in Table 5.

Table 5. Bio-productive parameters of the pikeperch at the end of experimental period (35 days) in the experimental variants

Specification	Control	V1	V2
SGR_{BW} (% day^{-1})	4.952	5.418	5.618
SGR_{TL} (% day^{-1})	1.583	1.798	1.764
DGR (g)	0.113	0.124	0.144
FCR (Pikeperch)	2.039	1.843	1.597
FCR (pikeperch + sterlet)	-	1.381	0.946

(FCR) for the juvenile pikeperch may be noticed in variant V2 (1.597), compared to the variants V1 (1.843) and C (2.039) at the end of the experimental period. FCR for total biomass (pikeperch + sterlet) was also better in V2 (0.946) than in V1 (1.381).

CONCLUSIONS

The pikeperch reared for 35 days in polyculture with 20% sterlet (V2) had significantly higher body weight than the pikeperch reared with 10% sterlet (V1) or in monoculture (C).
A significant plus of fish biomass resulted by valorisation of the pellets unconsumed by the pikeperch, was obtained in both polyculture variants (V1, V2).

Bio-productive parameters were better for the polyculture variants.

Polyculture could be a good way to positively influence the pikeperch farming in RAS, having a beneficial impact on tank's bioproductivity and on the growth dynamic of the pikeperch.

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DETERMINATION OF THE PHYSICO-CHEMICAL, MICROBIOLOGICAL AND PARASITOLOGICAL PARAMETERS OF FROZEN FISH, MARKETED BY A COMMERCIAL COMPANY FROM THE NORTHEAST OF ROMANIA

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Abstract

The purpose of this paper is to identify the extent to which the veterinary legislation of the European Union is complied with by a company from North-Eastern Romania. The parasitological analysis of the fish had as reference the EC Regulation no. 2074/2005, and the test result was negative. To measure the level of radioactivity, a 500 gram sample of "Alaska Froock Pollock File Code" was sent to the laboratory, the detected values being compliant. The same range of frozen fish was analyzed from a microbiological and physico-chemical point of view. After self-monitoring on five samples of 900 grams, Listeria monocytogenes was not present in fish meat (absent / 25 grams), and sulfite-reducing bacteria were within the specified standard value <1.0 x 10 cfu g. In the case of easily hydrolyzable nitrogen, the value of 29.13 mg / 100g was obtained, so below the maximum value of 35 mg / 100g. As a result of the analysis samples, it is found that the fish assortments studied correspond both from a physico-chemical, microbiological, parasitological, but also radioactive point of view.

Key words: *analyzes, frozen fish, health, quality.*

INTRODUCTION

Fish is a product highly appreciated by educated consumers; its quality being given by the totality of organoleptic, physico-chemical, microbiological and parasitological properties. The significance of this product is increasing due to the high percentage of protein, polyunsaturated fatty acids, minerals represented by potassium, magnesium, iron, phosphorus and vitamins, especially vitamins A and D. The white-pink colour of the meat fish traced its inclusion in the group of white meats (Banu, 2009). In fish meat, proteins and lipids have a high biological value and provide digestibility (Oliveira et al., 2017). In the sphere of food quality is the consumer who occupies the central place. Production and trade must take place in such a way as to satisfy the consumer's requirements, the most important requirement being quality, more precisely this highlights the extent to which that product, the commodity satisfies the customer (Ionescu et al., 2006). Practically, in order to ensure consumer protection, it is necessary to combine all the elements related to the quality, safety and traceability of the food product, hence the idea that they form a whole (Rodriguez-Salvador and Dopico, 2020). Assessing the quality of fish meat is essential to ensure products compliance and customer satisfaction (Grassi et al., 2019). The aim of our research was to identify the extent to which the veterinary legislation of the European Union is complied with the commercial unit in Bacau in order to ensure the protection of the consumer of frozen fish.

MATERIALS AND METHODS

In order to assess the quality of frozen fish belonging to the commercial unit in Bacău County, a series of physico-chemical, microbiological, parasitological and radioactive analyzes were performed in May-June 2019. Also, certain varieties of fish were checked by

sampling (5 samples of frozen mackerel 250 g - parasitological examination; 1 sample "Alaska code file" 500 g - radioactive examination; 5 samples "Alaska code file" 900 g - microbiological and physical - chemical examination) to determine the extent to which consumer safety is not in danger. Due to the importance and veracity of the results of the analyzes, the sampling was performed in optimal conditions from a sanitary-veterinary point of view and thus all the previously mentioned parameters were analyzed, respectively the control of the work surfaces hygiene and the control of the packagings. Physico-chemical, microbiological, parasitological analyzes and sanitation tests were performed by the sanitary-veterinary laboratory S.C. Concordia Laboratory S.R.L. Bacău, the contamination with residues was evaluated within D.S.V.S.A. Suceava, while the identification of possible migratory substances from food packaging took place at the National Research-Development Institute for Food Bioresources of Bucharest (IBA). The parasitological analysis of the fish had as reference the EC Regulation no. 2074/2005, the analysis regarding the control of packaging had as reference the EU Regulation no. 10/2011 - (the method for identifying chemical contaminants in packaging that can migrate to food was ICP-MS spectrometry with inductively coupled plasma mass spectrum), this being a method that complies with the provisions of EU Regulation no. 10/2011, Annex II). The radioactivity of the two parameters (Cesium 134 and Cesium 137) is provided in Regulation no. 733/2008 in Article 2 and the determination of easily hydrolysable nitrogen was determined in accordance with SR 9065-7 / C91 / 2009.

RESULTS AND DISCUSSIONS

After taking the sanitation tests from the repackaging section of the frozen products on 10cm^2 surfaces on the floor, table, right side wall, calibration machine from the "Fish Packing Room", the following results on the enumeration of microorganisms were identified - total number of germs (NTG) but also on coliform bacteria, the tests being satisfactory, within the maximum allowed limits (Table 1).

Table 1. Work surfaces hygiene control

Sanitation tests	Characteristics	Results	
FISH PACKAGING ROOM - FLOOR -number of units / sample: 1 swab -sampled from 10 cm2 -sealed: insured -receipt status: Corresponding	Enumeration of microorganisms - NTG through SR EN ISO 4833-1/2014 (AR)(PS-CL M-04), CM	3 ufc/cm^2	The test result/ Satisfactorily (Corresponds to the provisions Ord. MS no. 976/98)
	Coliform bacteria through SR ISO 4831/2009 (NR)	abs/10 cm^2	
FISH PACKAGING ROOM - RIGHT SIDE WALL -number of units / sample: 1 swab -sampled from 10 cm2 -sealed: insured -receipt status: Corresponding	Enumeration of microorganisms - NTG through SR EN ISO 4833-1/2014 (AR)(PS-CL M-04), CM	4 ufc/cm^2	The test result/ Satisfactorily (Corresponds to the provisions Ord. MS no. 976/98)
	Coliform bacteria through SR ISO 4831/2009 (NR)	abs/10 cm^2	
FISH PACKAGING ROOM - FISH CALIBRATION TAPE -number of units / sample: 1 swab -sampled from 10 cm2 -sealed: insured -receipt status: Corresponding	Enumeration of microorganisms - NTG through SR EN ISO 4833-1/2014 (AR)(PS-CL M-04), CM	5 ufc/cm^2	The test result/ Satisfactorily (Corresponds to the provisions Ord. MS no. 976/98)
	Coliform bacteria through SR ISO 4831/2009 (NR)	abs/10 cm^2	

Sanitation tests	Characteristics	Results	
FISH PACKAGING ROOM - PACKING ROOM TABLE -number of units / sample: 1 swab -sampled from 10 cm2 -sealed: insured -receipt status: Corresponding	Enumeration of microorganisms - NTG through SR EN ISO 4833-1/2014 (AR)(PS-CL M-04), CM	2 ufc/cm^2	The test result/ Satisfactorily (Corresponds to the provisions Ord. MS no. 976/98)
	Coliform bacteria through SR ISO 4831/2009 (NR)	abs/10 cm^2	

* SR EN ISO 4833-1/2014
** SR ISO 4831/2009
*** Ord. MS nr.976/98

Article 84 of ch. VI of the Order no. 976/1998 allows the presence on the work surfaces in the food sector of 20/cmp NTG, only insofar as the coliform bacteria are absent on 10 cmp.
Given the frequent consumer complaints to the A.N.P.C. (National Authority for Consumer Protection) regarding the presence of the Anisakis parasite in the frozen mackerel and the controls carried out by the D.S.V.S.A. (Sanitary-Veterinary and Food Safety Directorate) on the quality of fish meat on the Romanian market through actions to verify and withdraw the amount of fish in which parasitic formations were discovered in the viscera and muscles, we sent to the laboratory five (5) samples of frozen mackerel of 250 grams each, finally the total relating to 1,25 kg to verify its conformity and to guarantee the safety of the consumer within the company from Bacău.
The mackerel came from the Netherlands, was valid and the lot was 30155.
The samples were collected in polyethylene bags for food use, stored and transported according to the rules in force. Also, the test result was negative, because the visual control did not identify Anisakis spp. in the viscera and muscles, which is a good thing (Table 2).

Table 2. Parasitological examination of frozen mackerel fish

Sample description	Test, method of analysis, referential	Sample no./ Values obtained	Conclusions
FROZEN MACKEREL 5 pcs x 250 g each Whole fish with head and viscera	Fish parasitological examination Reg. EC no. 2074/2005	At the parasitological examination performed by visual control, no specific parasitic formations Anisakis spp. in the viscera and musculature	The test result According to the results

* Reg. EC no. 2074/2005

It is known that mackerel is one of the most commonly involved fishery products in anisakidosis. Anisakis is a 2-3 cm worm and is positioned in the intestines; the negative hypostasis is when it migrates to the muscles and viscera, which makes it unsuitable for consumption.
Therefore, in financially developed countries with a food education, of course at a much higher price, it is preferable to trade and consume a gutted fish, because this parasite is removed, with no risk.
It should be noted that the parasite Anisakis spp. once reached the muscles affects the safety of today's consumer. Regarding the control of packagings, I asked the National Research and Development Institute for Food Bioresources (IBA) Bucharest to perform a set of analyzes on printed bags of various sizes and I wanted: to investigate the components of packaging in terms of their migration, examination of organoleptic changes and verification of specific migration of metals.
The standard sets a maximum value of 10 mg/dm^2 in terms of global migration of components, our values in the issued bulletin falling within the limit specified above.
For the organoleptic assessment of packaging, according to EU Regulation no. 10/2011, no changes of samples and extraction liquids are allowed, the result of our test being compliant.

The maximum permissible limits set by the standards for heavy metals are: barium (Ba) <1 mg/kg, cobalt (Co) <0.05 mg/kg, copper (Cu) <5 mg/kg, manganese (Mn) <0.6 mg/kg, zinc (Zn) <25 mg/kg and iron (Fe) <48 mg/kg. The values obtained after the test report were within the normal range, being small deviations in the case of metals: barium, cobalt and manganese (Table 3).

Table 3. Identification of chemical contaminants in packaging

Features	U.M.	Method of analysis	Results
Global migration of components -Extraction 10 days at 40ºC in ✓ 10% ethyl alcohol solution ✓ 3% acetic acid solution -Extraction 10 days at 40ºC in ✓ olive oil	mg/dm^2	PS-AB-01 SR EN 1186-9:2003 PS-AB-01 SR EN 1186-2:2003	 0.83 1.08 0.95
Organoleptic changes - Extraction 10 days at 40ºC in - 10% ethyl alcohol solution ✓ sample ✓ extract - 3% acetic acid solution ✓ sample ✓ extract		PS-AB-04	unchanged
Specific migration of metals - Extraction 10 days at 40ºC in - sol.acetic acid 3% ✓ Barium ✓ Cobalt ✓ Copper ✓ Zinc ✓ Mangan ✓ Iron	µg/kg	ICP-MS spectrometry	 4.2 0.19 2.1 12.19 0.67 11.93

* SR EN 1186-9:2003
** SR EN 1186-2:2003

For the measurement of the level of radioactivity, which consists in determining the chemical elements Cesium 137 and Cesium 134 in fish meat, we sent a sample of 500 grams of "Alaska Frozen Pollock Code File" (lot 0029935; valid) to the Sanitary-Veterinary and Food Safety Directorate Suceava (the only accredited laboratory in the area of Moldova), the transport being carried out in conditions of maximum safety, at a temperature of -18ºC, in order to ensure the verticality of food safety at the company in Bacău. The value obtained for Cesium 137 from fish meat was 10.9 Bq/kg and Cesium 134 was not detected (Table 4).
The maximum foreseen is 370 Bq/kg in the case of dairy products and baby food and 600 Bq/kg for other food groups (fish).

Table 4 Determination of the radioactivity of frozen fish

Sample details	Test, method of analysis / Performance parameters		Results	Conclusions
Frozen Alaska Pollock cod file	Specific activity radionuclides by Sp. Range with det. Pan flute (PS-CLR-RAD-01)	30744-1.1: Cs 137 Cs 134	 =10.09 (AMD=0,14) Bq/kg undetectable (AMD=2.06) Bq/kg	The test result corresponds to Order No. 1805 / 29.12.2006 to the M.P.S .; 286 / 08.12.2006 to the A.N.S.V.S.A .; 314 / 06.11.2006 to the C.N.C.A.N. and Reg. (Eurotom) no. 52 / 15.01.2016 of the Council

* Order No. 1805 / 29.12.2006

The same assortment of frozen fish (Frozen Alaska Pollock cod file) was analyzed microbiologically and physico-chemically at the laboratory of S.C. Concordia Laboratory S.R.L. Bacau. Following self-monitoring on five sample samples of 900 grams each, Listeria monocytogenes was not present in fish meat (absent/25g) and sulfite-reducing bacteria were within the standard value <1.0 x 10 cfu/g.

Regarding the determination of easily hydrolyzable nitrogen that indicates the freshness of the meat, the value of 29.13 mg/100g was obtained, so below the maximum of 35 mg/100 g (Figure 1).

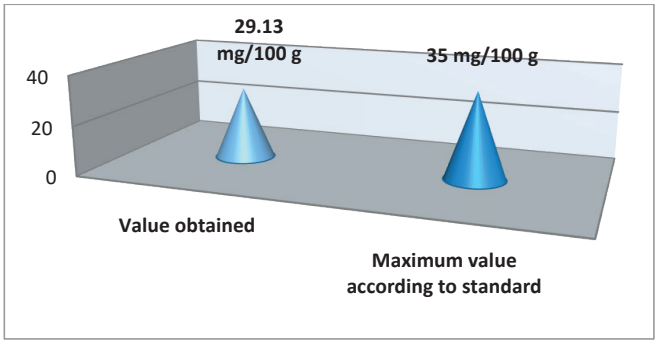

Figure 1. Easily hydrolyzable nitrogen for Alaska cod fillets

The assortments of fish belonging to the company from Bacău and appear on the list of Romanian consumers meet the food safety measures, because both the results of sanitation tests within the production unit and others such as: packaging compliance, parasitological, microbiological, physical examination - chemical but also the radioactivity test are compliant from a sanitary-veterinary point of view. The veracity of the conformity of the goods is given by the analysis bulletins issued by authorized laboratories. Although fish is not in the first place in terms of consumption for the population, especially ocean fish, it is appreciated for its taste and price. However, the research has achieved its goal, so we continued with the desire to capture several varieties of frozen fish in the network of stores in our country to see what stage it is at the level of presentation. The fish that was the subject of our study looked very good at first sight, the packaging was in a proper, compliant condition, the labels were visible, clean, with all the necessary information. We did not find any deviations from the above, nor in terms of price. The refrigerator provides the right temperature for the food product and its image had a strong impact in attracting the consumer (Figure 2).

Figure 2. Presentation of frozen fish in the commercial space

CONCLUSIONS

As a result of the analysis samples, it is precisely stated that the fish assortments within the Bacau Company correspond both from a physical-chemical, microbiological, parasitological, but also radioactive point of view. The control of the hygiene of the surfaces in the work unit resulted in the qualification "satisfactory", and regarding the global migration of components from the packaging, the values obtained were within the normal range, with very small deviations in the case of metals: barium, cobalt and manganese. Also, frozen fish retains its quality characteristics during storage, all parameters being met, especially climatic ones; the packaging appears with all the identifying elements and maintains the temperature of the product, the labelling is carried out according to the legislation and aims to ensure traceability, starting from the origin of the product with the aim of correctly informing the consumer; the transport gives safety to the goods, the refrigeration chain is not interrupted, so the frozen fish is kept at a temperature of -18ºC.
At the end of the conformity assessment of the frozen fish, in the commercial space, the form of its presentation was within the appropriate parameters, there were no deviations or deficiencies.

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Regulation (EC) No 2074/2005 of 5 December 2005 laying down implementing measures for certain products covered by Regulation (EC) No. 853/2004 of the European Parliament and of the Council and the organization of official controls provided for in Regulations (EC) no. 854/2004 of the European Parliament and of the Council and (EC) no. Regulation (EC) No 882/2004 of the European Parliament and of the Council derogating from Regulation (EC) No Regulation (EC) No 852/2004 of the European Parliament and of the Council and amending Regulations (EC) No 853/2004 and (EC) no. 854/2004.

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CONTRIBUTION OF SPRING AND EARLY AUTUMN ZOOPLANKTON POPULATIONS TO THE ROMANIAN BLACK SEA WATERS PRODUCTIVITY, IN 2019

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Abstract

Based on 53 vertical samples collected in May, August and October 2019 in the waters of the Romanian Black Sea shelf, we assessed the seasonal dynamic of abundances and diversity of zooplankton within the surface and certain depths layers of the pelagic system. Along with the control exerted on the primary production as main grazers, zooplankton species constitute a significant part of most commercially exploited pelagic fish diet (e.g. anchovy, sprat, horse mackerel juveniles). As result of increased global and regional climatic variability, we detected a seasonal shift in the occurrence of key copepod species as well as significant fluctuations in abundances of total fodder zooplankton. In May 2019, the sudden warming of water column led to proliferation of Noctiluca scintillans and therefore, to reduction of fodder zooplankton stocks. With the changing of thermal regime, a rapid shift of zooplankton composition was detected in August comparative with October, mainly seen in explosive development of thermophilic copepod Centropages ponticus in summer and of eurythermic species Paracalanus parvus in early autumn.

Key words*: NW Black Sea, production, zooplankton.*

INTRODUCTION

Strong evidences on the Black Sea mesozooplankton spatial and temporal changes in the last decades have been brought by numerous authors, which detected the main natural and/or anthropogenic causes of its dynamic (Kideys et al., 2000; Shiganova and Bulgakova, 2000; Kovalev et al., 2001; Gubanova et al., 2001; Oguz and Gilbert, 2007; Stefanova et al., 2010; Shiganova et al., 2018; Vereshchaka et al., 2019, Opdal et al., 2019).

Major cornerstone events have occurred at the level of pelagic system pointing out to the outbreaks of invasive species *Mnemiopsis leidyi* and *Beroe ovata* at the late of 90s, following an intense eutrophication and pollution period and surged by a global temperature climatic variability and extreme cyclic phenomena such as the ENSO (Martı, 2014) and NAO (Llope et al., 2011).

All these concurred to a decrease of general zooplankton stock production and diversity (Oguz and Gilbert, 2007) and thus to alteration of food chain, with large implications in fish productivity (Daskalov et al., 2017; Mihneva, 2018; Hidalgo et al., 2018).

Long term monitoring studies of zooplankton production at the Romanian littoral were conducted mainly in the 70s – 80s (Porumb, 1972, 1974, 1979, 1982; Petran, 1985), when a method integrating data on abundance of each zooplankton species life stage, its lifespan, female fecundity and water temperature was proposed for assessing. In accordance, in the period 1970 - 1975, the total production of main copepod species was estimated at 502.72 $mg.m^{-3}$, with large seasonal variations between cold and warm months, and between species (Porumb, 1994-1995). In summer, the major species contributor turned out to be *Acartia clausi* with an average daily production of 1.55 $mg.m^3$ (about 60% of the whole copepods daily production), while *Pseudocalanus elongatus* and *Oithona nana* reached about 0.723 $mg.m^{-3}day^{-1}$ in winter-spring, and 0.54 $mg.m^{-3}day^{-1}$ in autumn, respectively. Later, during 1978 – 1980, the same author estimated a global production of zooplankton on the Romanian shelf at 191,308.86 tones, of which *Noctilluca scintillans* represented more than 80%. A recent review study evaluated a zooplankton production at the Black Sea level after 2000s up to the present at $230\pm87*10^{12}$ kJ $year^{-1}$ as

comparing with the after and before 1976's production of 303±75*10^{12} kJ $year^{-1}$ and 193±72*10^{12} kJ $year^{-1}$, respectively (Opdal et al., 2019). More than 20% of daily zooplankton production is consumed by sprat and anchovy during spring - summer periods (Porumb, 1972, 1973; Bulgakova, 1996).

MATERIALS AND METHODS

The mesozooplankton was collected in three seasonal periods of 2019 (24 – 29 May, 15 – 20 August and 14 – 23 October) by vertical hauls using a "Juday" plankton net (equipped with a flowmeter), with a 36 cm diameter opening and mesh size of 150 µm, at discrete water column depths depending of thermocline, and of maximum chlorophyll and salinity gradient position. In laboratory, a total number of 53 samples were processed according to methodology for zooplankton studies in the Black Sea (Alexandrov et al., 2014). The samples locations were mainly confined to the north-western part of the shelf under direct influence of Danube (Figure 1).

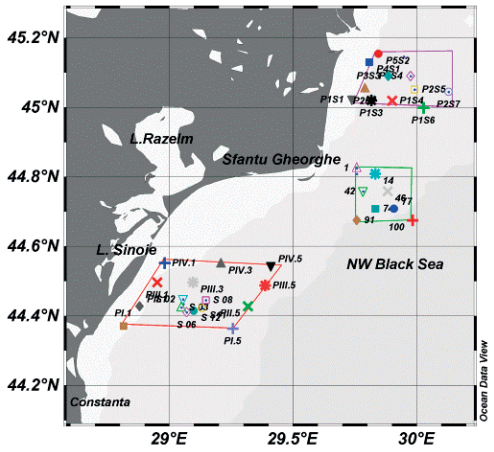

Figure 1. The stations map location within the perimeters and periods analysed in the present study (green polygon (L-35-120-D): 22 -28 May 2019 – 321.45 km^2; purple polygon: 15 – 20 August 2019 -701.18 km^2; red polygon: 14 -23 October 2019 ~ 800 km^2)

The wet weight mass (Petipa, 1957) of each species was converted to body carbon weight (mg C), according to several authors as given in Alexandrov et al. (2014). The body size of copepods was retrieved from Brun et al. (2017).

The specific daily growth rates of each species were calculated using the regression equations as showed in Uye and Shimazzu (1997). The seawater temperature value at each station (surface, thermocline and under thermocline) were taken into calculus, as this parameter was proved as determinant in relationship with weight–specific growth rate of zooplankton (Huntley and Lopez, 1992). The specific growth rates of benthic larvae (bivalves, gastropods and polychaetes) were estimated from regressions given by Hirst et al. (2003).The production rates of zooplankton species were calculated from the species- and stage-specific body masses (copepods) and their respective size-specific daily growth rates according to Hayashi and Uye (2008), Pi = Bi × Gi, where i, Bi and Gi are production rate (µg C $m^{-3}d^{-1}$), biomass (µg C m^{-3}) and specific growth rate (d^{-1}) of the species or taxonomic group i, respectively. The production rate of total net zooplankton community (P, mg C $m^{-3}d^{-1}$) was calculated as the sum of all individual taxon production rates.

Physical parameters

In May, the surface water temperature and salinity in the study area varied within 15.10°C to 20.68°C range, and 12.92 PSU and 16.14 PSU, respectively. Underneath (5 – 6 m depth), a thermocline was formed, a gradual decreasing of temperature, dissolved oxygen and increasing of salinity being recorded (7.74 – 9.90°C, 82 – 96%, 7.97 – 9.64 mg/l and 18.01 – 18.28 PSU up to 20 m depth followed by a slightly decreasing of temperature and a salinity increasing downward the bottom.

In August, the surface temperature varied within 24.66ºC and 26.2ºC range, with a sharp decrease at the level of thermocline to 13 -15ºC (upper part generally set at depths between 8 and 20 m), and further on under it up to a constant temperature of 8.6-10.72ºC. Salinity vertical profile was similar, slightly influenced by the Danube freshwater (average surface PSU: 17.45 and 18.40 PSU under thermocline).

In October, a mixed surface layer of 2 to 10 – 15 m thickness, with an average temperature and salinity of 18.27ºC and 17 PSU, and beginning of a gentle thermo- and halocline formation characteristic to the early autumn season has been noticed. Under it, an average temperature

of 11.6°C and a higher salinity (> 18 PSU) were recorded.

RESULTS AND DISCUSSIONS

Zooplankton population structure and abundance

In May, a relative low diversity formed of 6 copepod species (*Acartia clausi, Pseudocalanus elongatus, Calanus euxinus, Centropages ponticus, Paracalanus parvus, Oithona spp.*) and a small number of meroplankton representatives (bivalves, gastropods, polychaetes) were identified. The accentuated thermal leap at the end of May as compared with the previous couple of weeks (in average 7ºC), combined with a predominant northern cyclonic circulation and Danube freshwater input led to a rapid blooming of *N. scintillans*, and phytoplankton (surface Chl *a* 4.32 – 8.38 µg/l). In 70% of the stations, *Noctilluca* densities in the upper layer, dominated the overall zooplankton abundance, with values varying at the surface between 1352.73 ind.m^{-3} and 119.04 mg C.m^{-3} and 16,081.63 ind.m^{-3} and 1415.18 mg C.m^{-3}, respectively, with a high spatial heterogeneity (CV%=105.9). Its abundances decreased significantly under the thermocline (more than 20 times) (in average: 494.05±82.13 ind.m^{-3}) (Figure 2).

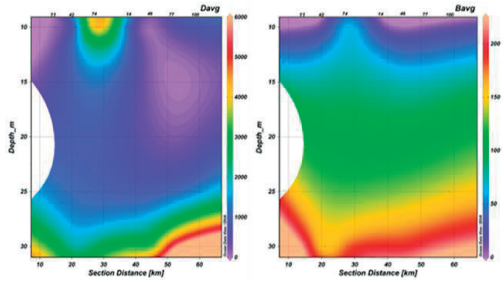

Figure 2. Spatial horizontal and vertical densities ($D_{avg.}$ - indv.m^{-3}) and biomasses (B_{avg} - mg.m^{-3}) of zooplankton in May (within the area L-35-120-D)

At that time, less than 15% of zooplankton abundance was constituted of typical thermophilic species *C. ponticus*, *Pleopis polyphemoides,* prefiguring an early development as result of sudden warming. Under the thermocline, the cryophilic copepods *P. elongatus* and *C. euxinus* with about 53% of population structure constituted of 3, 4 and 5 copepodite stages, dominated as abundance. The biomasses of ctenophore *Pleurobrachia pileus* and of chaetognat *Parasagitta setosa*, showed a proportional proliferation with those of copepodes development at a rate of 3 to 1. The meroplancton/holoplancton proportion was 22%.

In August, 21 taxa belonging to holoplankton and meroplankton and two jelly ones (*P. pileus* and *A. aurita*) were identified. The copepods with 10 species and cladocerans, with 4 species made the highest contribution to general diversity. Over 70% frequencies in the study area were given by *A. clausi*, *O. similis*, *O. davisae, C. ponticus, Evadne spinifera, P. setosa, Oikopleura (Vexillaria) dioica,* and bivalve larvae, while an incidental occurrence recorded *C. euxinus*, *P. polyphemoides*, and *Amphibalanus improvisus* larvae. *C. ponticus* attained its maximum abundances, with a maximal development of adult stages (copepodite stage 5 and females (females: male biomass ratio: 1.24) during this period. Up to 52% of total copepods abundances in some stations was formed of *C. ponticus*, while only 2 -5% by the species such as *Paracalanus parvus.* The abundances of zooplankton yielded general significant higher average abundances in the upper layer (F=18.71, df =18.15, p<0.05) (6730.9 ± 3390 ind.m^{-3}) than in thermocline (2902.4 ±1870.25 indv.m^{-3}), largely varying (CV% 50.37) between a maximum density in upper layer of 13678.25 indv.m^{-3} and a minimum of 639.7 ind.m^{-3}. In thermocline, it varied between 924.3 and 6797 indv.m^{-3} (CV% 64.4) (Figure 3).

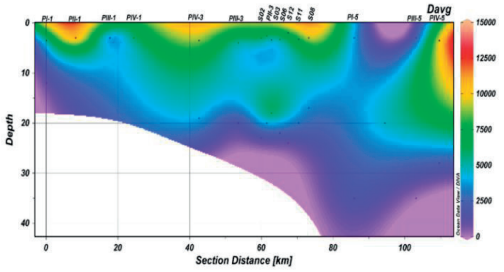

Figure 3. Spatial horizontal and vertical densities ($D_{avg.}$ - indv.m^{-3}) of zooplankton, in August 2019

The biomasses within the two layers exhibit a similar distribution, reaching an average of 126.46±73 mg.m^{-3} in the upper layer (16 - 311

mg.m^{-3}) and 55 ±44,9 mg.m^{-3} (9 – 145 mg.m^{-3}) within the thermocline (Figure 4).

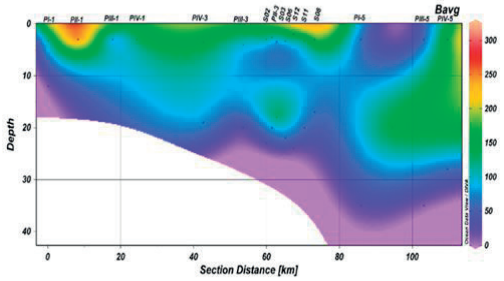

Figure 4. Spatial horizontal and vertical biomasses (B_{avg} - mg.m^{-3}) of zooplankton, in August 2019

In October, 13 holoplankton species (most abundant being: *Paracalanus parvus, Acartia clausi, Oithona davisae*) and 5 jelly ones (*N. scintilans, P. pileus, Beroe ovata, Obelia longissima, Clytia hemisphaerica*) were found in the study area.

The abundances and biomasses of the latter varied between 669 ind.m^{-3} and 58. 91 mg.m^{-3} to more than 10000 indv.m^{-3} and 940 mg.m^{-3}, respectively. The fodder zooplankton reached abundances comprised within 1, 401.70 indv.m^{-3}, and 84.40 mg.m^{-3} and 24,525.92 indv.m^{-3}, 1541.16 mg.m^{-3}, respectively (Figure 5).

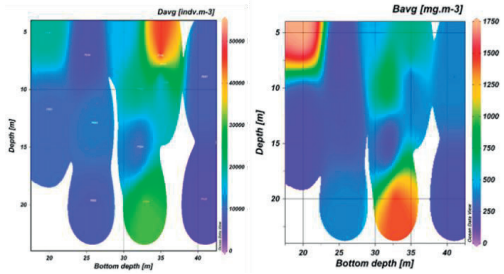

Figure 5. Spatial horizontal and vertical densities ($D_{avg.}$ -indv.m^{-3}) and biomasses (B_{avg} - mg.m^{-3}) of zooplankton, in October 2019

As result of presence of thick mixed surface water layer and lowering of thermocline at 15 m depth or more in most of the stations (except the shallower and those under direct influence of Danube stations), the zooplankton vertical distribution was relatively uniform, with marking differences both in the upper layer and thermocline in only three stations (Figure 4).

N. scintillans and jelly species contributed with 10 to 80% of total as biomass. On board observations noted an average abundance of *Beroe ovata*, *Mnemiopsis leidyi* and *Aurelia aurita,* within the neustonal layer of 5 – 32 ind.m^{-2} in average.

Species growth rates and production

The growth rates analysed as function of taxa body weight change at given temperature revealed significant (though, not statistically relevant) differences between species and seasons. Thus, considering a temperature range of 8.82ºC – 26.2ºC, specific growth rates of copepods species varied between 0.17 and 0.72 day^{-1}. *C. ponticus* among all reached the highest specific growth rate (0.65 – 0.71 day^{-1}) in August (the highest temperature), while in May this dropped at 0.44 day^{-1} and to 0.36 - 0.42 day^{-1} in October at similar temperature.

A. clausi, the first dominant species in all seasons, showed a variation of specific growth rates in the range of 0.12 - 0.29 d^{-1} in May, at 0.12 - 0.5 d^{-1} in August and within 0.15 – 0.28 d^{-1} in October. *P. parvus* showed comparable specific growth rates with *A. clausi,* but slightly lower. In May, the species recorded 0.17 – 0.34 d^{-1}, in August 0.51 d^{-1} (26ºC) and 0.20 – 0.33 d^{-1} in October (11.6-18.73ºC). In turn, *Oithona* spp. reached in May contrasting lower specific growth rates (0.06 – 0.16 d^{-1} within 8.82 - 19^{0}C range temperatures) as compared with the other copepods. However, in August, at higher surface temperature, it almost doubled the growth rate reaching a maximum of 0.4 d^{-1}.

In October, it maintained the rate in the limits of 0.09 – 0.19 d^{-1}. As for the cryophilic species *P. elongatus* and *C. euxinus* (assuming the same growth rate regression equation), it ranged between 0.17 – 0.26 d^{-1} in May and October, and between 0.47 – 0.69 d^{-1} in August. The filter feeding *O. dioica* out of all species had the highest growth rate in August, with an average of 2.05 d^{-1}, while the carnivorous *P. setosa*, 0.35 – 0.39 d^{-1} in May, 0.51 – 0.53 in August and 0.37 – 0.44 d^{-1} in October. The meroplankton taxa (bivalves, gastropods, polychaetes larvae, and barnacle) estimated growth rates varied between 0.44 – 0.55 d^{-1}, assuming a dependence of C body weights and not of temperature (Hirst et al., 2003).

The averaged total and surface layer zooplankton production was highest in August (87.22 and 78.10 mg Cm^{-3}d^{-1}, respectively). In

May and October, on the other hand, it yielded as much as 39 and 8 times lower surface production but comparable with the values from August was within the under thermocline layer (9.12 mg C $m^{-3}d^{-1}$) (Figure 6). To calculate the seasonal total production within the perimeters analysed, the duration of each season as shown in Porumb (1994 – 1995) (spring - 92 days, summer - 122 days and autumn - 61 days), and the water volumes within each perimeter (km^3), (calculated based on the area and average water column length within the perimeter) were considered. Thus, the average total production was as following: 13,538*103 tonnes for the May perimeter, 238,744*10^6 tonnes for the August perimeter and 21,476*10^3 for the October perimeter.

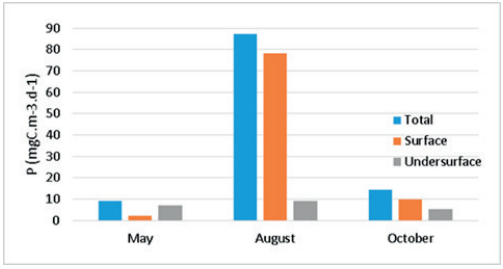

Figure 6. The zooplankton daily mean production (mgC.$m^{-3}d^{-1}$) of zooplankton in May, August and October 2019 on the Romanian shelf

This variability is mainly explained through the contribution of various species to zooplankton community within the periods analysed. The cladocerans (*Penilia avirostris*, *Evadne spinifera P. polyphemoides*, and *Pseudevadne tergestina*) contributed significantly to surface production (75%, 38.57 mg C $m^{-3}d^{-1}$).

The second important species, *O. dioica* 18.20%, 9.35 mg C$m^{-3}d^{-1}$) followed by *C. ponticus* (2.42%, 1.24 mg C$m^{-3}d^{-1}$) accounted for the difference to May and October surface production. During the latter, a shift in community production has been recorded, with the dominance of *P. parvus* (9.02%; 1.32 mg C $m^{-3}d^{-1}$) among copepods, followed by *A. clausi* (8.61%; 1.25 mg C $m^{-3}d^{-1}$). *O. dioica* still remained one of the most productive with 41.65%, respective 6.06 mg C $m^{-3}d^{-1}$. *C. euxinus*, in turn, formed in May the highest bulk of copepods production with about 9% and 1.03 mg C $m^{-3}d^{-1}$. However, the first ranked species was *P. pileus* with almost 60% (6.89 mg C $m^{-3}d^{-1}$) contribution, mostly confined within the under thermocline (Figure 7).

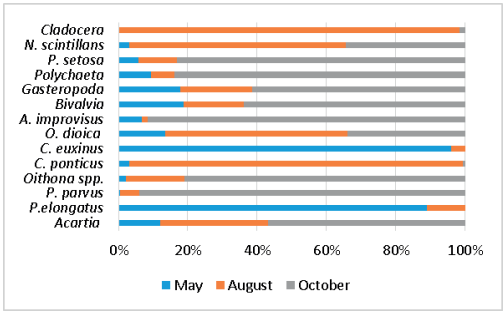

Figure 7. The contribution of zooplankton taxa to daily mean production (mgC.$m^{-3}d^{-1}$)

The north-western shelf of the Black Sea is known as one of the most productive part of the Pontic basin and among other world marine ecosystems (Bologa et al., 1999; Demidov, 2008; Llope et al, 2011; Moncheva et al., 2014). This is mainly due to strong nutrients input from tributary rivers (Cociaşu et al., 2008; Bondar, 1989; Ragueneau et al., 2002) that trigger a high primary production almost all year round and seasonal secondary production peaks. Although several times less diverse than in other worldwide marine basins, the fodder zooplankton annually total production estimated in the early times of eutrophication (beginning of 70s) at the Romanian littoral managed to support sustainable stocks of commercially fishes. Thus, at the end of 70s, there was estimated a peak summer bulk production (without *Noctiluca*) of 2,067.90*10^3 tonnes, of 322.64*10^3 tonnes during spring, of 344.31*10^3 tonnes in winter, and about nine times lower in autumn than in summer (Porumb, 1994-1995). Yet, at the same time, the dinoflagellate *N. scintillans* has become one of the dominant species, frequently overwhelming the fodder zooplankton production (Porumb, 1980, 1994 - 1995).

Similar to our study, in 80s, the species with high contribution to daily production were represented by *Acartia clausi* (1.5 mg.m^{-3}), *C. ponticus* (0.15 mg.m^{-3}), and *P. parvus* (0.18 mg.m^{-3}) in summer, and by *P. elongatus* (0.6 mg.m^{-3}) in spring (Pasternak, 1983).

After invasions of *M. leidyi* and later of *B. ovata* at the beginning of 90s, a new state has installed that led to changing of pelagic trophic web

paradigm. Daskalov (2002) suggested that while the planktivorous fish reduced predominantly the stocks of bigger sized zooplankton (preferences consistent with the findings of Chouvelon et al., 2015) the new incomers took advantage of rapid development of small zooplankton. Indeed, Anninsky et al. (1998) suggested that the impact of *Mnemiopsis* is stronger on small zooplankton. Llope et al. (2011) inferred that this fact has led to a bottom –up effect, manifested through development of the new organizational trophic order at expense of the "classic" web trophic model (secondary carnivorous fish - planktivorous fish - big sized zooplankton, e.g., *C. ponticus*). The outcompeting preying capacity of jellyfish on zooplankton was also demonstrated by Opdal et al. (2019) who established a strong correlation between the food energy requirements of planktivorous fish and jellyfish before the 1976 (when jellyfish biomass started to increase), between 1977 and 2010 and after 2010. Their findings showed that in the first period, zooplankton accounted for ca. 12% of the combined fish-jellyfish energy consumption, for ca. 42% in the period after (1977–2010) and slightly declined in the recent time-period (2000–2010) to ca. 30%.

According to Vereshchaka et al. (2019), a new zooplankton evolution phase has been taking place since 2002, characterised by a strong 1-year signals (according to continuous wavelet transform analysis) of most taxa and of the total biomass and by a weak one for the *M. leidyi*. However, Stefanova et al. (2014, 2019) found a significant fluctuation of fodder zooplankton general stocks evolution in the period 2010 – 2016. Thus, by taking into consideration the proposed threshold values for the Good environmental status (280 - 550 $mg.m^{-3}$ at coast; 300 – 130 $mg.m^{-3}$ at shelf and 150 - 50 $mg.m^{-3}$ at open sea) for the biomasses of trophic zooplankton, one of the quality indicators of zooplankton set for the Black Sea pelagic habitats (coastal, shelf and open waters), it was found that it failed to attain these amounts in coastal habitats in over 60% of the cases. According to the 2018 evaluation (ANPM, 2019; Muresan et al., 2019) and the present study, the fodder mesozooplankton biomass varied within 250 – 700 $mg.m^{-3}$, in over 80% of the coastal area sites.

Zooplankton and especially marine copepods biochemical composition was largely documented by several authors. For example, high levels of certain fatty acids like 16:0, EPA, and DHA (Sørensen et al., 2007), indispensable free amino acids, proteins (about 50% of dry weight), astaxanthin, thiamine, riboflavin, vitamin C, and vitamin E (Hapette and Poulet, 1990) in copepods are deemed essential for normal fish larval development, survival and growth, and stress tolerance (Olsen et al., 1991; Coutteau et al., 1997; Shields et al., 1999; Bell et al., 2003; Cahu et al., 2003; Hadas et al., 2003). *Oikopleura dioica* also, an abundant food resource for the planktivorous fish in the Black Sea (up to 25% of total zooplankton abundances), has the capacity to store fatty acids as high-energy droplets (Cima et al., 2002).

High stocks and good quality zooplankton prey are needed to support daily growth, survival and recruitment success of fish (Porumb, 1972; Shlyahov and Shlyahova, 2011; Plounevez and Champalbert, 1999; Nikolioudakis et al., 2012). At the Romanian littoral, there was estimated for example a monthly consume on zooplankton (including meroplankton) by *Sprattus sprattus* varying between 1,450 tonnes in April (when the species forms schools in coastal waters) to 8,000 tonnes in August. The main food items during this period are constituted of *A. clausi, P. elongatus, C. euxinus*, *P. parvus*, *P. settosa, O. dioica,* larvae of barnacles, polychaetes, and decapods (Porumb and Porumb, 1985; Glushtenko, 2011; Bişinicu et al., 2017; Mihneva, 2018). *Engraulis encrassicholus* population is able to consume up to 20% of the daily fodder plankton production of the Black Sea, while the larvae and juveniles of *Trachurus mediterraneus* may consume crustaceans up to 33% of the total wet weight of ingested food (e.g., *P. parvus, C. ponticus*), *O. dioica* (about 33% after its importance in diet) and *P. setosa* (3% also after importance), in the autumn season (Bulgakova, 1996). Other species such as *Clupeonella cultriventris*, *Atherina boyeri, Merlangius merlangus* or *Pomatomus saltatrix* feed also on zooplankton in different life stages.

CONCLUSIONS

The present data on zooplankton production and species richness distribution in spring, summer

and autumn is in some respects in agreement with observations made in the 70s on the Romanian shelf. These characterized a period of ecological disruption caused by eutrophication and fish overexploitation but in the same time still productive, which is similar in many aspects with the current state. The seasonal daily growth rates of main zooplankton species along with the abundance data presented with this occasion showed a dynamic controlled by local and regional climatic variables as seen in May, August and October when the prolonged high seawater temperatures caused an enhanced primary production and blooms of *N. scintillans* and not ultimately a high secondary production of thermophilic species such as *C. ponticus, O. dioica* and cladocerans. The last decades' changes that affected the overall fodder zooplankton structure evinced its high resilient capacity so, soon after the disequilibrium occurred in the pelagic system of the Black Sea by arrival of *Mnemiopsis* and *Beroe,* has become a bottom-up regulator of trophic web. The recent literature and our own data show a slightly improvement of functional structure of trophic zooplankton, as result of decreasing or balancing of abundances of the two invasive species. As a main food source of commercially exploitable fishes in the Black Sea, the zooplankton itself should be regarded as a primary resource of the Black Sea and a good indicator of climatic changes. Keeping in mind its dietary value, the fishery productivity and management should have an integrative approach.

ACKNOWLEDGEMENTS

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STUDY REGARDING THE DIFFERENT REARING SYSTEMS FOR PHEASANT

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Abstract

This study is a part of an ample research regarding the pheasant exploitation systems. In Romania, the main game, for a long period, it was the hare. In the last decade the hare population has recorded a significant decreasing. In these conditions, the hunters will increase the pressure on pheasants. It is now very important, from economically point of view but not only, to produce more pheasants. Because of the Romanian politics regarding the protection of predatory game, an important increasing of pheasant population, in wild conditions, it is impossible. The pheasant farms from Romania were established between 1970 and 1980. The main purpose of these farms was to produce pheasant meat for export, and only secondary to produce individuals for population of hunting areas. More than that, the individuals obtained are less resistant, becoming an easy prey for predators. In these conditions, the knowledge of the other functional models from the western states and not only has a special importance. The literature cites record harvests of 10,000 pheasants annually on a plot of approximately 17,000 acres (approximately 6,883 ha).

Key words*: farm, hunt, pheasant, predators, wild game.*

INTRODUCTION

Globally, the pheasant was the most widespread and best-known bird of hunting interest in the world. Imported from Asia, the home of the pheasant, it developed explosively where it had a favourable environment. Colonisations have taken place in over 50 countries on all continents, except Antarctica, since antiquity, due to the sporting – hunting qualities but also due to the meat quality, adaptability, growth rate, etc. Pheasants have arrived in Europe since the time of the Roman Empire, multiplying in the wild, in England, since the 15th century. In countries like France or Italy they were raised as domestic birds.

In the new world, pheasants arrive relatively late, with evidence of pheasant populations dating back to 1730, the first success being recorded in 1882 (Hill and Robertson, 1988.

Not to be overlooked is the fact that colonization with this species is mentioned in Greek mythology, legend has it that around the 13th century BC, Argonauts looking for Golden Wool brought pheasants to Greece from the Colchis region of the Caucasus (now Georgia). exactly from the valley of the river Phasis, hence the name – *Phasianus cochicus*. Leaving aside these historical aspects, we must mention that this species maintains its leading place in the preferences of hunters for the same reasons: survival skills and quality and quantity of meat (Castiov, 2010).

MATERIALS AND METHODS

The present paper is a bibliographic study of the captive rearing systems of the pheasant, but also of the methods, of the efficient models for populate. It was taken into account the systems applied in Romania, in other European countries, and not also on the American continent. We must point out that the growth of the pheasant in captivity, but also its exploitation and capitalization is more difficult and demanding compared to domestic birds, due to the wild nature of the species.

RESULTS AND DISCUSSIONS

Two main pheasant breeding systems have been developed worldwide: an intensive system and a semi-intensive system. From these two systems derived in time a third, a semi-extensive system that developed rapidly in Europe but not only. Unfortunately, pheasant rearing technology has

not modernized as it has with the poultry industry.

In Romania, the semi-intensive system has a history of almost a century, if not better. It is also known as the semi-natural pheasant rearing system using hens.

Incubation and hatching of pheasant eggs is done in this system, naturally, using domestic hens. Eggs incubated and hatched come from pheasant farms or are harvested from endangered nests from the hunting areas.

In this system, are used boxes for incubation and for raising chicks (figure 1), these being simple "constructions", made of softwood board (the box) and wire mesh (for breeding pens).

It is very important to choose the right land where the incubation boxes will be placed. It must meet several conditions from which we mention: the soil must be permeable, must be raised to prevent water stagnation, must be protected from strong winds, provide shade especially at noon, be as far away from localities, busy roads, other poultry farms, and last but not least, to be suitable for efficient disinfection, disinfection and rodent control.

The most expensive investment in such a farm is given by the external fencing, this being an indispensable element for operating at optimal parameters.

On this incubation field, the boxes can be arranged in groups - in batteries, keeping a maximum distance of 0.5 meters between the boxes.

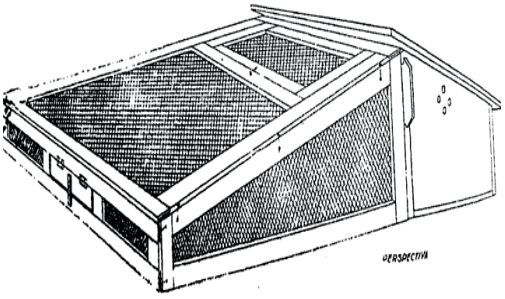

Figure 1. Incubation and raising boxes (Cristescu, 2003)

Another important step is choosing of the hens. It is very important that the hens chosen for hatching to be healthy, vigorous and gentle. Before introduction into the hatching boxes, the hens will be vaccinated and dewormed. Another operation prior to introduction into boxes is the hatching test, performed on chicken eggs, a test that lasts for a period of 3-5 days.

A maximum of 20 eggs are laid in each hatch, under each hen, and in exceptional cases, due to the lack of hens, 24 eggs (figure 2). In natural situation, the pheasant female have an average of only 12 eggs in each hitch (Comşia, 1963).

Figure 2. Pheasant eggs in incubation hatch

Hatching takes place 23 days after laying eggs under the nest. Various studies have shown that it is necessary to monitor the nests, as there is a possibility that they will kill their chickens in the first 24 hours after hatching, but also at a longer interval, this "killing" taking place mainly during night (Babuția, 1964).

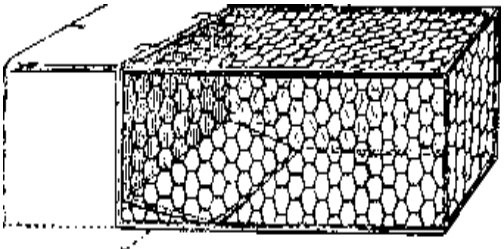

Figure 3. Pheasant growth box

The next step involves transferring of the hens and chickens to the raising area. This land, in addition to the similar conditions that the incubation land presents, must be cultivated in order to have permanent vegetation and implicitly animal food represented by insects.

Research has led to the conclusion that the best results are obtained when the land is located in or near the forest, in meadows and close to a

water source. Growth boxes are placed on this land (figure 3).

Almășan et al. (1987, 1989), analysing the different types of crops, observe that the most suitable culture for this action is the alfalfa, the lucerne.

The lucerne (alfalfa) culture ensures both the shelter of the chicks from the action of the sun's rays, and the contribution of food of animal origin due to the numerous species of insects that "visit" this culture. More than that this crop, being perennial, can be exploited between four to seven years.

Pheasants are then transferred from raising yards to storage lofts of different types from where will be then delivered either to hunters' associations and clubs (for the colonization of hunting funds) or to slaughterhouses (less often in the case of pheasants raised in this system).The types of lofts (large aviaries) do not differ from one system to another, but only in terms of size, having to provide 4-5 square meters for an adult pheasant.

In this system, but not only, the success of pheasant breeding starts from the correct and complete feeding of the chicks. If this was a major problem, mainly due to the nutritional requirements of the species, in the last half century specific combined feeds have been introduced in pheasant breeding. If in the past ants were moving and multiplying, at present we can only rely on this combined fodder. The multiplication of ants is still applied today, starting on the idea of accustoming individuals to natural food in the living environment, the main purpose being to obtaining more resistant, more adapted specimens. However, the studies did not reveal better results, in terms of viability, for the specimens fed in this way, in comparison with those in which the combined fodder was administered, as long as they came from the same breeding system.

This semi-intensive rearing system have a lot of advantages, like minimal investment in technology, good technological parameters (70-80% hatching, a relatively good average daily gain for young pheasants, obtaining pheasants are resistant to diseases and adapted to environmental conditions;, are good fliers, almost wild, and are wary of predators, and last but not least, the obtained individuals cumulate the qualities of the hunting pheasant. As disadvantages we specify the dependence for intensive pheasant farms in order to obtain the eggs for hitching, the use of relatively large area for hatching and rearing yards, and the low capacity of production that cannot meet market demands.

It is obvious that this semi-intensive system is a good alternative for the population of hunting fields but not for the recovery of pheasants for slaughterhouses.

Figure 4. Pheasants in a storage loft at Gherghița farm, Prahova County

The intensive pheasant breeding system has developed in response to market demand for pheasant meat but also for the increasing demand for the population of hunting areas with this species.

An intensive pheasant growing farm is structured in several compartments.

Reproduction compartment. Individuals used for reproduction are maintained in this compartment. The sex ratio is 1:6 (1 male for 6 females) – ”harem” reproduction system.

Figure 5. Male with his ”harem” in a reproduction pen

The compartment is divided into pens, made of wire mesh on a wooden resistance skeleton. In each pen, according to the aforementioned sex ratio, are introduced6 females and one male. (figure 4). Practically, each box corresponds to an egg compartment. Regarding the choice of breeders, healthy specimens are kept for reproduction, females that have produced at least 45 eggs and males with full plumage, and with a quiet behaviour. In addition to the breeders introduced in the pens, a percentage of 10% spare breeders will be retained. They are maintained in outdoor aviaries until their will be use (introduction into the boxes) when appropriate.

Cristescu (2003) shows that, if the laying compartments are insufficient to achieve production, the group reproduction system can be used. In this system, the individuals from the reproductive nucleus are introduced into storage or wintering aviaries, the sex ratio being 1:4, ensuring an area of 5 square meters for each individual.

Figure 6. Male's fight

Particular attention must be paid to male behaviour. During the period of accommodation between them, there are fights that can result in quite serious injuries. (figure 6). In this case, the roosters are replaced.

Table 3. Comparative overview of pheasant rearing systems

No.crr.	System	Advantages	Disadvantages
1	**Semi-intensive**	- minimal investment in both technology and labor; - good technological parameters (70% -80% hatching), a relatively good average daily gain, etc.; - obtaining pheasants are resistant to diseases and adapted to environmental conditions; - individuals are good fliers, almost wild, and are wary of predators - the obtained individuals cumulate the qualities of the hunting pheasant.	- dependence on intensive farms for the purchase of eggs for hatching; - the need for relatively large spaces for hatching and rearing yards; - low production capacity.
2	**Intensive**	- very good productivity; - ensuring of biological material from own farm, a fact that gives the certitude of quality of individuals. - Very good technological parameters.	- expensive investments; - the pheasants obtaining in this system are less adapted for surviving in wildlife.

CONCLUSIONS

It is obvious that this species is suitable for breeding in captivity. Given the fact that the pheasant has developed in areas where intensive agriculture is practiced, unlike partridges, pheasant colonization of game areas is required as a mandatory measure both economically and recreationally - sports.

The choice of the growth system depends only on the preferences of the breeder, of the beneficiary, on the financial power of the investor, but above all on the possibility of capitalizing on the production.

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AN ANALYSIS OF THE DYNAMICS OF FISHING CATCHES IN THE ROMANIAN DANUBE SECTOR

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Abstract

In this paper it analyzed the dynamics of fishing catches in the waters of Romania where commercial fishing is allowed and regulated, in the period 2008 - 2018. Data on fishing catches are taken from the official website of the National Agency of Fisheries and Aquaculture and processed. These data come from the commercial fishing reports, drawn up annually. From a quantitative point of view, in the period 2008 - 2018 the situation of recorded catches varied between a minimum of 2457 tons recorded in 2010 and a maximum of 3868.51 tons recorded in 2016. The main species caught in the inland waters of Romania in 2008 - 2018 were the following: Prussian carp (41.74%), Freshwater bream (11.66%), Pontic shad (10.53%), Roach (6.77%), Carp (5.46%), Wels catfish (5.01%), Pikeperch (4.29%), Northern pike (3.89%). The results of the paper are intended to be an overview of the dynamics of freshwater fisheries in Romania in the period 2008 – 2018.

Key words*: catch, Danube, fish.*

INTRODUCTION

Fisheries are an integral part of most societies and make a significant contribution to economic and social health and economic development in many countries and areas.

Despite this importance and the enormous value, or more correctly, due to this aspect, the world's fish resources are affected by the combined consequences of overexploitation and, in most cases, environmental degradation.

Freshwater fisheries supply only about one-fifth of the world's total fish catches. The global economic importance of freshwater fisheries is decreasing.

Fishery has a long tradition in Romania and is an important activity especially for riparian populations. The freshwater commercial fishery is allowed on the Danube River but also rivers such as: Prut River, accumulation lakes on the Siret River and Olt River. In Romania, commercial fishing is regulated and the right to fish resources is directly attributed to professional fishermen organized in professional associations based on commercial fishing permits, licenses and authorizations.

The Danube is, undoubtedly, the most important running water on the territory of our country not only from an economic point of view but also from a faunal point of view (Bacalbașa, 2002).

The Danube river basin included the richest ichthyofauna of all European rivers.

Antipa (1909) scientist rightly said, that the key ichthyological the whole of Europe is the Danube River and the Danube Delta. Due to the richness of fish species with economic value, the Danube fishery has always been varied and rich.

The ichthyofauna of the Danube is very diverse, containing over 100 species of fish, which represents about 20% of the freshwater ichthyofauna of Europe (Bănăduc et al., 2016 from Bănărescu, 1964; Balon, 1964; Kottelat and Freyhof, 2007; Freyhof and Brooks, 2011).

This diversity of fisheries resources is increasingly threatened in recent decades.

Pollution, increased commercial transport on the river, alien species introduced into the basin, illegal fishing and overfishing are some of the threats not only to ecosystems but also to the well-being of fishing communities.

Maintaining the diversity of the Danube fishery resource, through efficient fisheries management, is necessary to ensure living aquatic resources for both current and future generations.

MATERIALS AND METHODS

The analysis of the fish catches' dynamics in 2008 - 2018 was made using the official records of the National Agency for Fisheries and Aquaculture from Romania. These data come from the reports of economic operators authorized to engage in commercial fishing.
The tools and methods used to legally catch fish species depend on several factors, such as:
• extremely diverse type of ecosystems (running water and stagnant water ecosystem);
• varied size of fish species;
• the degree of their mobility;
• the water horizon in which the species carries out its main stages of life and therefore from where it can be captured.

RESULTS AND DISCUSSIONS

Between 2008 and 2018, a number of 21 species appear in the reported commercial catches (Table 1).
It should also be noted that in these official statistics there are two categories of catches "other species" and "other cyprinids" where have been registered the species caught accidentally, species with low economic value were recorded, whose percentage in multiannual catches varied between 0.29% and 0.52%.
In the period 2008 - 2018 the recorded commercial catches varied between a minimum of 2457 t (2010) and a maximum of 3868.51 t (2016) (Figure 1).

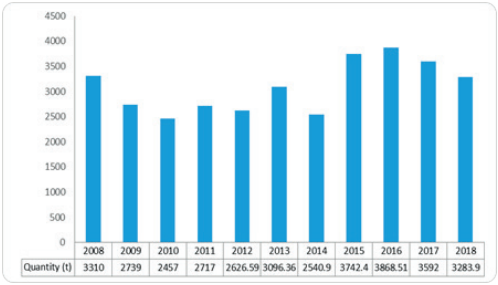

Figure 1. Fisheries catch dynamics (2008 – 2018)

Among freshwater species, the Prusian carp was the most abundant species in catches holding 41.74% of the total amount, followed by freshwater bream (11.66%), and among migratory species the Pontic shad held a percentage of 10.53% in catches multiannual (Figure 2).

Table 1. The commercial catch structure in Romania (2008 – 2018)

No.crt.	*Systematic group/species*	*English name*	*Commerial capture reported (t) (2008 – 2018)*	*Percentage of multiannual catches (%)*
	Clupeidae family			
1	*Alosa immaculata* (Bennett, 1835)	Pontic shad	3580	10.54
	Cyprinidae family			
2	*Cyprinus carpio* (Linnaeus, 1758)	Common carp	1857.14	5.466
3	*Carassius gibelio* (Bloch, 1782)	Prussian carp	14181.05	41.74
4	*Rutilus rutilus* (Linnaeus, 1758)	Roach	2298.79	6.767
5	*Tinca tinca* (Linnaeus, 1758)	Tench	216.92	0.638
6	*Scardinius erythrophthalmus* (Linnaeus, 1758)	Rudd	222.15	0.654
7	*Aspius aspius* (Linnaeus, 1758)	Asp	265.57	0.782
8	*Alburnus alburnus* (Linnaeus, 1758)	Bleak	131.23	0.386
9	*Blicca björkna* (Linnaeus, 1758)	White bream	352.23	1.036
10	*Abramis brama* (Linnaeus, 1758)	Freshwater bream	3962.58	11.66
11	*Vimba vimba (Linnaeus, 1758)*	Vimba bream	206.39	0.607
12	*Carassius carassius* (Linnaeus, 1758)	Crucian carp	49	0.144
13	*Barbus barbus* (Linnaeus, 1758)	Barbel	289.21	0.85
14	*Ctenopharyngodon idella* (Valenciennes, 1844)	Grass carp	53.2	0.15
15	*Hypophthalmichthys molitrix* (Valenciennes, 1844)	Silver carp	709.53	2.08
16	*Chondrostoma nasus* (Linnaeus, 1758)	Common nase	45.43	0.134
17	*Aristichthys nobilis* (Richardson, 1845)	Bighead carp	332.12	0.98
	Siluridae family			
18	*Silurus glanis (Linnaeus,1758)*	Wels catfish	1702.32	5.01
	Percidae family			
19	*Sander lucioperca (Linnaeus,1758)*	Pike perch	1456.38	4.29
20	*Perca fluviatilis (Linnaeus,1758)*	European perch	467.36	1.38
	Esocidae family			
21	*Esox lucius* (Linnaeus,1758)	Northern pike	1319.96	3.89

To make a more detailed analysis of industrial catches we will divide the ichthyofauna of economic importance into 5 groups as follows:

- Native cyprinids: Common carp, Prussian carp, Freshwater bream, Vimba bream, Barbel, Roach, Rudd, White bream, Tench, Bleak;
- Asian cyprinids: Grass carp, Silver carp, Bighead carp;
- Predatory species: Wels catfish, Pikeperch, Northern pike, European perch, Asp.
- Other species: Common nase;
- The Pontic shad.

The structure of multi-annual catches is dominated by native cyprinids 70.61% followed by predatory species 15.34% and the Pontic shad 10.54%.

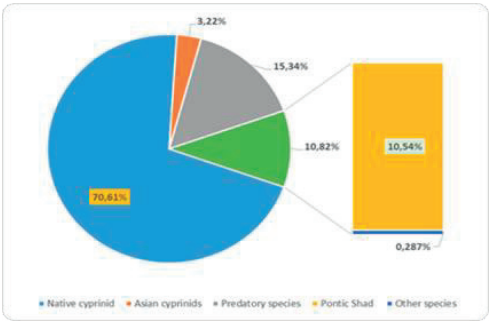

Figure 2. The structure of multiannual catches (2008 - 2018) by economic groups

Among the native cyprinids, the Prussian carp is the dominant species holding 59.11% of catches followed by Freshwater bream 16.52%, Roach 9.58% and Common carp 7.74%. The least abundant species are represented by Crucian carp 0.204%, Vimba bream 0.86%, Tench 0.904% and Rudd 0.92% (Figure 3).

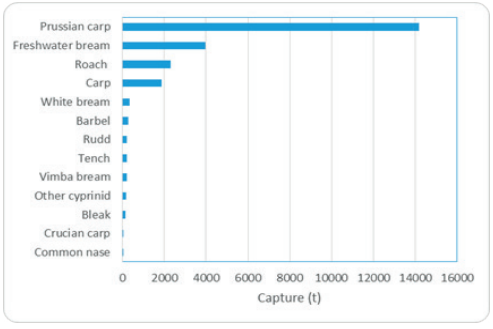

Figure 3. Evolution of native cyprinids

Multiannual commercial catches of Asian cyprinids are dominated by silver carp 64.8%, bighead carp 30.33% and the grass carp has the lowest percentages, only 4.86% (Figure 4).

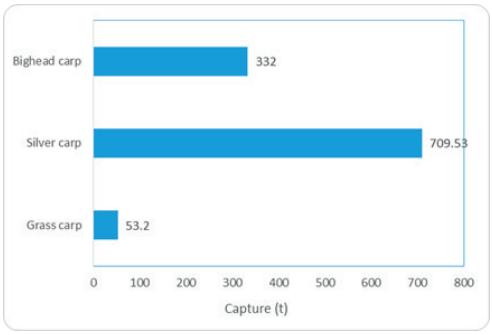

Figure 4. Dynamics of Asian cyprinids in multiannual catches

In the multi-annual commercial catches of predator species, the Wels catfish holds the highest percentages (32.66%) followed by the Pikeperch (27.95%) and on the third place is the Northern pike with 25.32% of the catches (Figure 5).

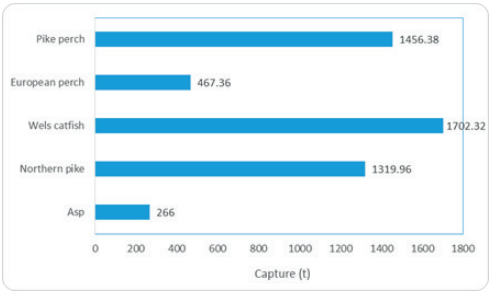

Figure 5. Evolution of multiannual catches of predator species

Due to its nutritional value, its gustatory qualities, the Pontic shad is a species highly appreciated by the population of the lower Danube area, which greatly increases its economic value. This can also be seen from the value it holds in multi-annual commercial catches of 10.54%.

The analysis between the annual data of the reported catches and those established by annual orders shows that there are significant differences between the reported and established data (Table 2).

Table 2. Situation of catches (reported and established) and fishing effort in 2008-2018

Year	The reported catch (t)	The catches established by TACs	Fishing effort (number of boats)	Reported percentage (%)
2008	3310	5523.632	1470	69.43
2009	2739	-		
2010	2457	4452.042	1497	55.2
2011	2717	3646.9	1647	74.5
2012	2626.59	3260.902	1589	80.54
2013	3096.36	6982.529	1714	44.34
2014	2540.9	5767.623	1602	44.05
2015	3742.4	5266.979	1560	71.05
2016	3868.51	5671.038	1602	68.21
2017	3592	5286.807	1549	67.94
2018	3283.9	5978.72	1854	54.92

It is noted that sometimes less than 50% of the quantity regulated by TAC (2013, 2014) was reported, which makes us believe that this is underreporting.

Regarding the regulated fishing effort, quantified only by the number of boats (according to the annual regulatory orders) it is observed that the differences are not significant in the analyzed period (Figure 6).

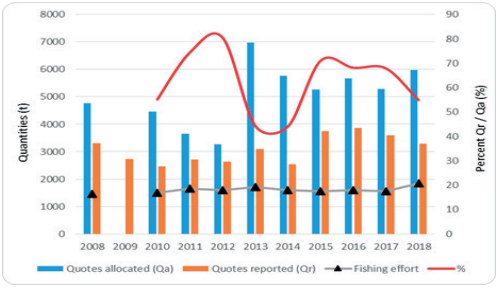

Figure 6. Evolution of TACs, reported catches and fishing effort (2008 - 2018)

CONCLUSIONS

This paper presents an analysis of commercial catches made between 2008 and 2018.

The processed data are statistical reports on the total catches of commercial fishing realized by economic agents authorized to practice commercial fishing in the waters under the jurisdiction of Romania, in the period 2008 - 2018, published on the official website of National Agency for Fisheries and Aquaculture from Romania.

The analysis of the dynamics of these catches in terms of qualitative and quantitative structure allows us to conclude:

The species structure of the catches only partially reflects the composition of the river's ichthyofauna because the type of gear conditions the report between the different species of fish caught. Thus, the number of species present in commercial catches is 21 species. These are the main species of great economic importance, because in these reports there are also 2 groups called “other cyprinids” and “other species”, which include non-important species that have accidentally appeared in catches.

Among the cyprinids (including the Asian ones), the best represented were: Prussian carp 41.74% and Freshwater bream (11.66%), and among the migratory species, the Pontic shad held a percentage of 10.54%.

The lowest percentages in the multiannual catches were held by: Crucian carp 0.144%, Vimba bream 0.607%, Tench 0.638% and Rudd 0.654%.

Among the dominant predatory species is the Wels catfish with 5.01% in the multi-annual catches, followed by the Pikeperch (4.29%) and in third place is the Northern pike with 3.89% of the catches.

In the period 2008 - 2018 the reported commercial catches varied between a minimum of 2457 t (2010) and a maximum of 3868.51 t (2016).

Also, according to the annual regulatory orders, the total quantity of fish allocated through TACs varied between a minimum of 3260.9 t in 2012 and 6982,529 t in 2013.

We consider that there are significant differences between the allocated and reported quotas, which indicate that there are under-reports.

The fishing effort quantified by the number of boats (allocated by annual orders) does not differ significantly between 2008 and 2018. We do not have data about the real fishing effort.

ACKNOWLEDGEMENTS

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DEGRADATION OF FISH FAUNA IN THE PONDS OF THE SOUTHERN REGION OF MOLDOVA

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Abstract

The paper proposes the presentation of research on the quantitative and qualitative characteristics of surface waters in the Southern Region of the Republic of Moldova. The main purpose of the research is the study of ponds with aquaculture potential, based on complex analyses of impact factors, natural and anthropogenic. The information was collected from two ponds with significant fishing potential, located in the villages of Pelinei and Libidenco. The basins are located near the national road (30 m and 200 m, respectively) and 16 km from the city of Cahul, a development node for the Southern Region of the Republic of Moldova. Research has shown that the direct impact of the population is smaller compared to that of agricultural technologies, based on the intensive use of chemical fertilizers. This study is part of a complex research on the implementation of an efficient monitoring and control system of surface water quality in the Republic of Moldova, with the main objective of reducing the impact of low anthropogenic factor by at least 10% for each pond of Southern Region.

Key words*: aquaculture, fisheries, pond, quality, water.*

INTRODUCTION

The polluted ponds contain waste, sand, soil particles, clay inclusions, mineral oils, as well as toxic chemical substances detected only in the laboratory (nitrites, nitrates, phenols) etc. The physical, chemical or biological components, as anthropic result, have a toxic effect upon the biota (live organisms), effect which can be considered pollutant (Pronina, 2013).

One of the acceptable and universal manners to control the pollution is the involvement of the pond in a man-controlled biological process, and a certain formation of some basic ideas regarding the metabolic adaptation plant and animal mechanism to the anthropic impact, the degradation degree of the pond, considering the regional conditions, nature and concentration of pollutants ending up in the pond (Munteanu Pila and Stanciu, 2019a).

Thus appears the need to use in a more rational manner of the areas surrounding the ponds, eliminating the possible cases which affects the quality of the ponds' water, as well as the waste cleaning, as required, leading to the massive overcrowding, worsening the self-cleaning system of the pond. Another extremely harmful factor is the use of the pond water as the agricultural land irrigation source representing a direct anthropic pollution, and is a combination between the supply sources and the used water evacuation sources, in a vicious circle because through a circuit, the water once again reaches the pond once again affecting the fish culture.

A special importance in the used water division is the analysis of the vital activity of the live organism din the pond (plant and animals), and of the aquatic biocoenosis, reported to the degree the pond was anthropically affected pond (nitrites, nitrates, metals, phenols).

A pond is considered bio-effectively when within it is found a variety of aquatic plants and vegetation, besides the fact that it represents the food for many species of herbivore fish, it can fulfil also oxidative functions, taking place the detoxification of the organic pollutants (the plants accumulate the toxic substances and transform them in non-toxic substances) – photosynthesis, as a result the water is enriched with oxygen (Munteanu Pila and Stanciu, 2018a).

Among the aquatic plants three groups are distinguished: plants floating on water surfaces, plants growing and floating in the water mass and fully submerged plants. If I were to compare

the entire food chain, aquatic plants are the most resistant in the anthropic pollutions.
Some research shows that using the water *Hyacinth* (*Eichornia*) helps the treatment of used household water. In US, populating the ponds with this plant established a purification degree up to 97-98 %; in China, the polluted waters during the purification decreased by 98.6%, and in Russia, in the aquatic basins, the nitrogen and ammonium concentration decreased from 30-50 mg/L to 4-5 mg/L, and the concentration of the dissolved oxygen increased from 0.5 to 2-5 mg O_2/L (Vetrov, 2013).
The pond pollution is divided in two groups: minerals, whose content is approximately 42% and organic up to 58%, including biological and bacterial (Novikov, 2013). Some microorganisms such as bacteria, aquatic mushrooms and, moreover, some algae can participate in the decomposition of some organic substances, using as supply source – self-cleaning process. For example, the protozoa (*Ciliate sp.*) feed on bacteria, and each can destroy up to 30,000 bacterial cells. Although of small dimensions, the filtrations organisms (molluscs, daphnia etc.), can filtrate through their body large water volumes, filtrating particles suspended in the pond, and leaving the lump-like particles dropping at the bottom of the pond – natural fertiliser (Korjagina, 2013).

MATERIALS AND METHODS

The work was carried out on the basis of research on the functional state of hydro-biota, as well as by establishing quantitative and qualitative analyses of surface waters in the Southern Region of the Republic of Moldova.
This study was based on the establishment of the causes leading to the degradation of the aquatic circuit – resulting in swamping, fishery mortality, damaged ponds.
Water was collected from the ponds in two localities (Pelinei and Libidenco), area with fishery growth potential and capitalisation in order to establish the anthropic degradation degree.
The analyses were carried out in the Aquatic Resources Research and Exploration laboratory at the "Dunărea de Jos" University, Faculty of Food Sciences and Engineering, by using kit Merck-type using the Spectroquant, NOVA 60 spectrophotometer.
Some data was collected from specialty books and works and compared with real data (personal research) for an adequate interpretation.
The collected data were sorted, processed by statistical methods and represented graphically.

RESULTS AND DISCUSSIONS

The intensive and continuous pollution of the water sources in the Republic of Moldova became a severe issue among the fishery capitalisation. Thus, it resulted only 1% of the waters (ponds) in the Southern region of the Republic of Moldova correspond to the 1st quality class, and 17% do not correspond even to the 3rd quality class for capitalisation and fish increase in the intensive system (polyculture) (Munteanu Pila and Stanciu, 2019b).
The household anthropic waste increases annually due to the agriculture practice around the pond, as well as the mass-scale usage of fertilisers, pesticides and other harmful products, endangering the pond.
At the same time with the annual rains, these substances wash from the land treated surface and leak in the pond, endangering the ichthyofauna, resulting in fish dying and those resisting endangering the human health through the accumulation of toxic substances in the fish meat. In various localities in the Southern Area of the Republic of Moldova, the water composition will be different.
There are 31 ponds in the southern area of the Republic of Moldova, of which 19 are not functioned (damaged), 3 ponds have dried up and were abandoned, and other 9 ponds are dedicated the growth of fish in the intensive system (polyculture) (Figure 1).
The cause of the pond aquatic fauna degradation in the Republic of Moldova is their placement near some metallurgic, machine building and petrol waste, in other countries mineral pollution of enterprises (e.g. Giurgiulesti, Moldova) (Munteanu Pila and Stanciu, 2018b).
Those having nearby animal raising farms, polluted with waste leaking in the pond, have a high nitrogen -organic pollution content.
In water, the nitrogen is present in the following forms: molecular nitrogen (N_2), absorbed from

the air; nitrogen organic compounds partially resulted from the decomposition of the organic material; ammonium nitrogen (NH_4, NH_4OH); nitrites (NO_2, HNO_2) and nitrates (NO_3 up to 10-15 kg/ha nitrogen per year), originating from the atmospheric precipitations.

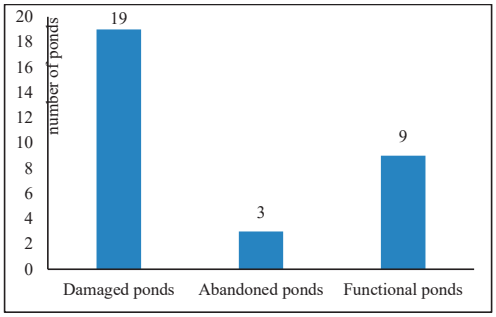

Figure 1. Ponds in the Southern Republic of Moldova
Source: Author research

The organic pollution takes place also following the drain of some chemical substances (pesticides), originated from the agricultural lands in the pond's surroundings – the chemical substance of this type of pollution is carbon.
After the disappearance of many fish species, and the partial swamping, the pond is fully populated only by microorganisms (Munteanu Pila and Stanciu, 2018a).
The pond can carry out the biotic activity only after a mechanical cleaning, and a later removal of the organic material absorbing all the nutritious substances in the pond: nitrogen, phosphor, potassium, calcium, magnesium, manganese, sulphur – mineralisation process.
Some plants such as the reed, a form of tall plants, with thick stalks found in many pounds in the region, can accumulate many mineral substances toxic for the fish, yet it is easy to renew after mowing.
In its turn, the used water contains two main groups of pollutants: conservatory which are not easily included in the chemical reactions and practically are not biodegradable (examples type are heavy metals, phenols, pesticides); and non-conservatory, pollutants which can suffer self-cleaning processes.
For this purpose, it was collected water from the pond in two localities located in the Southern Region in the Republic of Moldova, Pelinei (Pond 1) and Libidenco (Pond 2), in order to establish the status and quality of the ichthyofauna aquatic, the elimination of the fishery illness or death risk in the area – the development factor of the fishery productivity in the South Region.
The following physical and chemical parameters of water were analysed: phosphates, nitrates, nitrites, ammonium and organic substance (mg/L). Average values for results have been represented in Table 1 (Pond 1, Pelinei), respectively in Table 2 (Pond 2, Libidenco).

Table 1. Water parameters of Pond 1 (Pelinei)

Parameters / Station	pH (pH units)	$P\text{-}PO_4^{3-}$ (mg/L)	$N\text{-}NO_3^-$ (mg/L)	$N\text{-}NO_2^-$ (mg/L)	$N\text{-}NH_4^+$ (mg/L)	C-CoCr (mg/L org.comp.)
Supply	7.57	0.70	0.60	0.11	0.01	152.00
Evacuation	7.56	0.42	0.70	0.08	0.05	40.00
Middle basin	7.73	0.68	1.10	0.10	0.07	
Interior basin	7.66	0.33	0.60	-	-	
Surface basin	7.41	0.44	0.80	0.07	0.00	
Moldovan regulations	6.5-8.5	5.00	10.00	1.00	2.00	500.00
Romanian standards	SR ISO 10523-97	STAS 10064-1975	SR EN ISO 13395:2002	SR EN 26777:2002	SR ISO 5664: 2001	SR ISO 6060/96

Source: Author research

Results of laboratory analysis of water samples from pond 1 (Pelinei), are according to the Regulatory regarding the establishment of the industrial and urban used water loading polluting limits at the evacuation of the natural receptors, NTPA-001/2002; Regulatory regarding the used water evacuation conditions in the sewerage networks of the localities and directly in the treatment stations, NTPA-002/2002, namely the Resolution no. 352 dated April 21st 2005 regarding the amendment and fulfilling of the Government's Resolution no. 188/2002 for the approval of some norms regarding the used water unloading conditions in the aquatic environment (with the later amendments) for the indicators foreseen in the "control monitoring" section – except the supply source organic substances, C-CoCr – 152 mg/L (Table 1).
Results of laboratory analysis of water samples from Pond 2 (Libidenco), are according to the Regulatory regarding the establishment of the industrial and urban used water loading polluting limits at the evacuation of the natural receptors, NTPA-001/2002; Regulatory regarding the used water evacuation conditions in the sewerage networks of the localities and directly in the treatment stations, NTPA-002/2002, namely the Resolution no. 352 dated April 21st 2005 regarding the amendment and

fulfilling of the Government's Resolution no. 188/2002 for the approval of some norms regarding the used water unloading conditions in the aquatic environment (the last amendments) for the indicators foreseen in the "control monitoring" section- exception: Middle basic station - regarding the pH value (8.77) (Table 2).

Table 2. Water parameters of pond 2 (Libidenco)

Parameters / Station	pH (pH units)	P-PO_4^{3-} (mg/L)	N-NO_3^- (mg/L)	N-NO_2^- (mg/L)	N-NH_4^+ (mg/L)	C-CoCr (mg/L org. comp.)
Supply	7.38	0.38	7.6	0.14	0.25	31.0
Evacuation	7.43	0.34	1.1	0.08	0.03	49.0
Middle basin	**8.77**	0.46	2.1	0.07	0.01	
Interior basin	7.10	0.30	2.1	0.06	0.01	
Surface basin	7.38	0.60	2.1	0.09	0.04	
Moldovan regulations	6.5-8.5	5.00	10.00	1.00	2.00	500.00
Romanian standards	SR ISO 10523-97	STAS 10064-1975	SR EN ISO 13395:2002	SR EN 26777: 2002	SR ISO 5664: 2001	SR ISO 6060/96

Source: Author research

The mass development of the cyanobacteria of the *Microcystis, Anabaena, Nodularia, Nostoc, Aphanizomenon, Oscillatoria*, causes great damages to the eutrophic ponds, hindering the water supply process and the fish, when in pond there are more than 40-50% de cyanobacteria, fish populations and aquatic birds die as a result of toxin poisoning. The issue can be solved by adding, for example, nitrogen compounds, for the suppression of the cyanobacteria and microalgae blooming in the pond or the introduction of phytoplankton-eating fish, for example silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Hypophthalmichthys nobilis*) (Levich and Bulgakov, 1993). The algae absorb the nitrogen in the water mainly in the form of nitrates (NO_3) and ammonium compounds (NH_4). For the development of the algae, especially the phytoplankton – the main oxygen supplier dissolved in water – the best nitrogen concentration is of 2 mg/L and phosphor (P_2O_5) – 0.5mg/L (Figure 2). Without nitrogen in water the phosphorus would not be absorbed. A nitrogen deficit in the pond is much worse than the phosphorus deficit because this dramatically reduces the increase and maturation of the aquatic plants (food for herbivore fishes), and as a result, it lowers the capacity of the plants to consume the CO_2 in the water. The slow increase of the aquatic plants is a sign of the nitrogen lack in the pond (under normal illumination and CO2), the NO_3 level is zero and the presence of phosphates PO_4 is 0.1 mg/L, (PO_4> = 0.1 mg/L).

Generally, the fish nutrition is calculated according to the phosphorus quantity because it is considered the grow limitative factor. The optimal PO4 dosage depends on the intensity of the light permeating the pond, as well as on the quantity and the growth rate of the aquatic plants and can start at 0.2-5.0 mg/L (Figure 2). The phosphorus plays an important role in the photosynthesis process, being an energy regulator included in the componence of all the organisms. In the water, the phosphorus is found in the form of phosphates.

The aquatic vegetation cannot use the organic phosphorus PO_4 (fish excrements), without this being mineralized – decomposed by bacteria – KH_2PO_4 an inorganic compound used by plants - aquatic circuit – self-cleaning process.

And when the potassium is lacking in the water, the aquatic vegetation acquires a yellow-brown colour, and develops harder – the potassium deficit becomes the reason for slowing the photosynthesis process (affecting also the herbivore fish populations), the norm being of 0.90 mg/L (Figure 2).

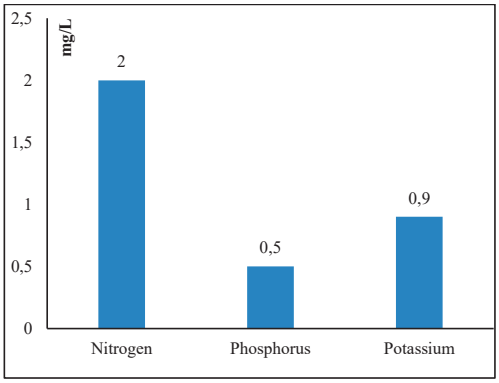

Figure 2. Physical and chemical concentration for phytoplankton in the pond
Source: Authors, by using Korjagina (2013)

In order to evaluate the physiological state of the surface waters (ponds) and of the organisms (aquatic organisms) living in the water: fish, crayfishes, some molluscs species, aquatic vegetation is necessary:

- Carrying out annually the water analyses and the control of the pond's damage degree;

- The control and improvement of the reproduction process of the valuable fish species;
- The possibility to adapt and acclimatize the area to the environment factors (drought, low temperatures);
- Early detection of the pathology (laboratory analyses).

CONCLUSIONS

Fishing basins face significant anthropogenic stress, which is also the cause of the environmental degradation of the various water bodies and thus takes place the destruction of their biota. It must be emphasised the fact that the ponds in this region, as well as other eutrophic ponds, face a large anthropic load. Carrying out an environmental survey regarding the aquatic circuit will establish the actual status of the pond and will allow for the development of some improvement measures in order to prevent the death of the fishery populations and of the aquatic vegetation. These locations were chosen because the impact of the anthropogenic factor is strong, due to the proximity to the populated area. Chemical analyses of water quality were performed and the quantity and quality of aquatic fauna was assessed. Water from Libidenco Pond has been characterized by a higher share of nitrite in the water, with a strong negative impact on aquatic fauna. The research highlighted the existence of differences between the two systems analysed, mainly due to the proximity of agricultural areas with different plant cultivation techniques. Thus, the intensive use of chemical fertilizers in neighbouring agricultural areas has led to different values of chemical parameters of surface water. In order to increase the use of the natural potential of ponds, it is necessary to optimize the growing conditions of different species of fish, adapting fishing technologies and reducing the action of factors that have a negative impact on surface water quality.

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THE INFLUENCE OF DAMMING ON THE DISTRIBUTION OF BROWN TROUT *SALMO TRUTTA* LINNAEUS, 1758 AND EUROPEAN GRAYLING *THYMALLUS THYMALLUS* LINNAEUS, 1758 (PISCES: SALMONIDAE) FROM SOMEȘUL CALD RIVER

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Abstract

Monitoring fish fauna in altered aquatic habitats may provide relevant information regarding the distribution, presence or absence, and dynamics of fish species. Someșul Cald River is the main tributary of Beliș-Fântânele Reservoir and Dam, build from 1970 to 1974. The presence of the dam could have affected fish fauna, especially potamodromous brown trout Salmo trutta and European grayling Thymallus thymallus because of habitat obstruction. Altitude, riverbed width, water depth, and water velocity are easy to determine aquatic environmental parameters. River altitude and riverbed width played an important role in European grayling preference regarding the auspicious environment. Correlation coefficients showed that increasing altitude is a limiting factor for European grayling ($r=-6024^*$, $p<0.05$). Wider riverbed is preferred by the same species ($r=0.672^*$, $p<0.05$). Beliș-Fântânele Dam blocked the migration of fish and made the grayling ecological zone framed by two trout ecological zone (upstream and downstream). The distribution range on the altitude of the brown trout in Someșul Cald River was from 518.5m to 1153.5m and 577.5m to 1068.5m for the European grayling.

Key words: *altered aquatic environment, electrofishing, endangered species, ichthyofauna.*

INTRODUCTION

Anthropic activities such as dam constructions, reservoirs, cascade dams, hydropower plants, and other hydro-technical constructions lead to major fragmentation of river systems and diversity loss (Kang et al., 2009; Danalache et al., 2017b; Jonsson et al., 2018; Kirin et al., 2019). Assessing the fish species contributes to the development of good management measures for ichthyofauna biodiversity conservation (Nicolae et al., 2018). Nilsson (2005) stated that dams have dramatically altered over half of the river systems in the world. The main habitat alteration mechanisms consist of: lotic habitats converted to lentic habitats (Martinez et al., 1994), construction of physical barriers (Watters, 1996) and constant change of water parameters regimes (pH, temperature, turbidity) (Magilligan and Nislow, 2005). Migration of fish is essential and artificial barriers limit or obstruct their movement (Danalache et al., 2017a). Also, fish species like brown trout, European grayling, Danube salmon, Black Sea trout, that are associated with fast-flowing waters tend to diminish in numbers and sometimes disappear (Jansson, 2006). Similar phenomenon is encountered on Someșul Cald River, located in Cluj County, North-Western Romania. The distribution of fish species belonging to the Salmonidae family could be affected by habitat alteration caused by

damming, in this case, brown trout *Salmo trutta* and European grayling *Thymallus thymallus* (Ombredane et al., 1987). Constant survey of the fragmented river systems fish fauna will provide data to generate the appropriate evolution and distribution of fishes, and also the long term effects of dams. The present study aimed to provide an up-to-date image of the anthropic barriers from the Someșul Cald River and how habitat alteration progresses.

MATERIALS AND METHODS

Sampling site

The study was conducted from August, 2018 to September, 2018 on Someșul Cald River (Someș-Tisa catchment, North-Western Romania). The springs of Someșul Cald River are located in Bihariei-Vlădeasa Masiff near Piatra Arsă Peak (Burned Stone Peak) at 1550 m altitude and has a total length of 66.5 km (Duma, 2016). On Someșul Cald River, four dams were constructed during 1970-1974: Beliș-Fântânele Dam, Tarnița Dam, Someșul Cald Dam and Gilău Dam. At Gilău Dam, Someșul Rece River flows into Someșul Cald River and form Someșul Mic River (Figure 1).

Sampling method

Fish sampling was performed by single-pass electrofishing techniques using a SAMUS 725 MP apparatus, powered by 12V and 24 A rechargeable battery (Cocan et al., 2020). The efficiency of freshwater fish sampling by electrofishing methods is highly effective especially in streams and small rivers (Kubečka et al., 2012). Water conductivity was tested before electrofishing to adjust the output current and non-lethal frequencies (www.fao.org) using Hannah HI9828 multi-parameter (Uiuiu et al., 2020).

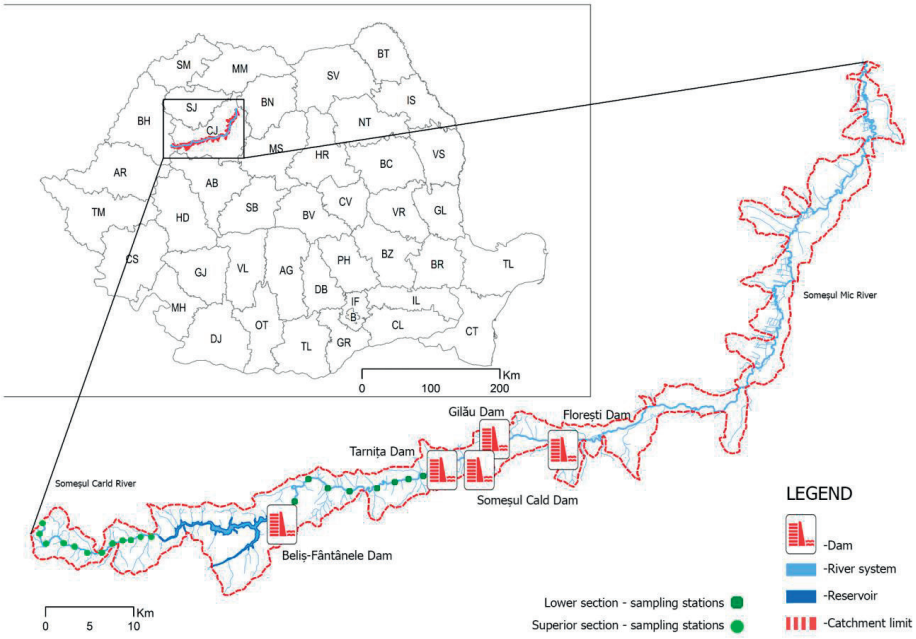

Figure 1. Someșul Cald River catchment and constructed dams

Habitat characterization

Fish sampling stations were marked using Garmin eTrex 20X GPS device. Each station's altitude, width, depth, water velocity was measured. The altitude of the stations was measured based on GPS data using Garmin BaseCamp software (ex-situ). Station riverbed width was measured with a 50m measuring tape. A total number of 5 measurements were performed inside each station (1 measurement at

the lowest point of the station and 1 measurement at the highest point of the station, and 3 intermediary measurements). Water depth and water velocity were measured in the same locations as riverbed width.

Data analysis

The data was processed using MS Excel, GraphPad Prism Ver. 8. Pearson correlations were performed to highlight the "links" between the amount of fish, presence or absence of fish and river configuration.

RESULTS AND DISCUSSION

GPS coordinates and altitude of the sampling stations

The sampling stations were grouped into two sections based on the Beliș-Fântânele Dam position as follows: the *lower section* (from station 1 to station 8) positioned between Tarnița Reservoir (downstream) and Beliș-Fântânele Dam (upstream) and the *superior section* (station 9 to station 20) positioned between Beliș-Fântânele Dam and the springs of Someșul Cald River. The altitude for each sampling station was determined in two points (1 point on the downstream limit of the station and 1 point on the upstream limit of the station). In Figure 2, the mean altitude recorded values of the sampling stations are presented. The lowest altitude was recorded in station 1 (518.5 m) and the highest altitude was recorded in station 20 (1153.5 m). Beliș-Fântânele Dam is situated between stations 8 and 9. GPS coordinates of each river section with the corresponding stations are presented in Table 1.

Altitude is one of the most important factors which influence fish species richness. At low altitudes, the trophic composition is more diverse (Pouilly et al., 2006) and ecological zones for fish species can be determined. Barradas et al., in 2012, concluded that the historical and spatial distribution of fish species can help to identify the potential impact of damming.

Table 1. GPS coordinates of the sampling stations

River section	Station	Downstream point	Upstream point
Lower section	1	N46° 42.413' E23° 12.932'	N46° 42.454' E23° 12.816'
	2	N46° 41.914' E23° 10.990'	N46° 42.005' E23° 10.902'
	3	N46° 41.892' E23° 09.538'	N46° 41.926' E23° 09.286'
	4	N46° 42.023' E23° 08.125'	N46° 42.013' E23° 07.991'
	5	N46° 42.080' E23° 07.781'	N46° 42.153' E23° 07.672'
	6	N46° 42.495' E23° 07.029'	N46° 42.578' E23° 06.906'
	7	N46° 42.609' E23° 06.465'	N46° 42.612' E23° 06.363'
	8	N46° 41.925' E23° 04.602'	N46° 41.959' E23° 04.825'
Superior section	9	N46° 38.766' E22° 52.112'	N46° 38.787' E22° 51.952'
	10	N46° 38.818' E22° 51.837'	N46° 38.832' E22° 51.622'
	11	N46° 38.834' E22° 51.457'	N46° 38.786' E22° 51.326'
	12	N46° 38.589' E22° 50.581'	N46° 38.498' E22° 50.221'
	13	N46° 38.429' E22° 49.409'	N46° 38.378' E22° 49.323'
	14	N46° 38.301' E22° 49.060'	N46° 38.216' E22° 48.755'
	15	N46° 37.955' E22° 48.284'	N46° 37.814' E22° 48.080'
	16	N46° 37.770' E22° 46.407'	N46° 37.813' E22° 46.327'
	17	N46° 38.092' E22° 45.022'	N46° 38.112' E22° 44.932'
	18	N46° 38.485' E22° 44.067'	N46° 38.532' E22° 43.995'
	19	N46° 38.653' E22° 43.712'	N46° 38.649' E22° 43.616'
	20	N46° 38.356' E22° 43.138'	N46° 38.310' E22° 43.131'

Based on altitude, brown trout is present in both sections of the river and in all the sampled stations. European grayling is also present in both sections of the river but in small numbers in the lower section. The highest point where European grayling was signalled was station 16

at the altitude of 1068.5 m. In this case, we can stipulate its limit of habitat based on altitude. The ecological zone of trout is above/upstream the grayling zone. The influence of damming clearly changed the distribution based on the altitude of the two species. The ecological zone of the grayling is "framed" by two ecological trout zones: one downstream the Beliș-Fântânele Dam and the second one upstream station 16. In an unaltered habitat, the grayling ecological zone is below (downstream) the trout zone (Aarts and Nienhuis, 2003; Lasne et al., 2007).

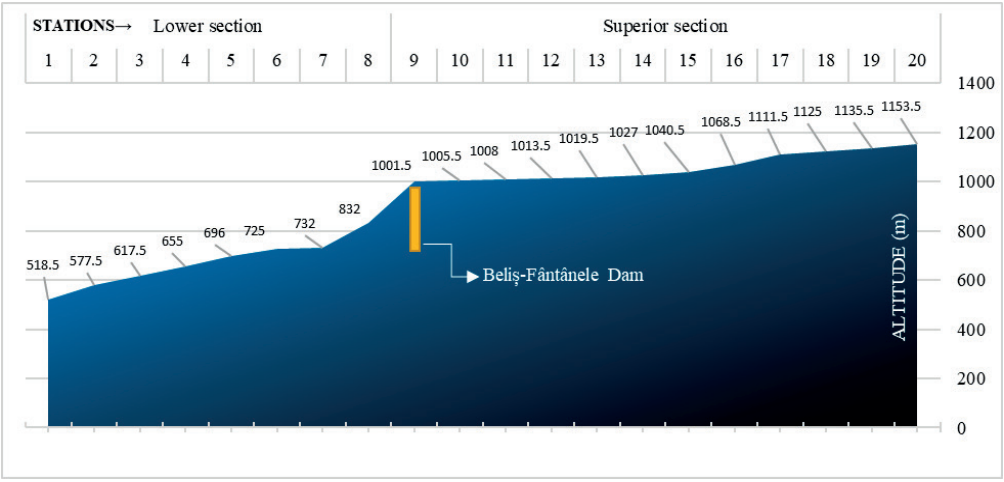

Figure 2. The altitude of sampling stations

Water depth

Water depth in the lower section of Someșul Cald River ranged from 20.4 cm (station 5) to 55.8 cm (station 1). The mean value of water depth was 37.2 cm. In the superior section of the river, water depth ranged from 11.9 (station 17) cm to 42 cm (station 16) (Figure 3).

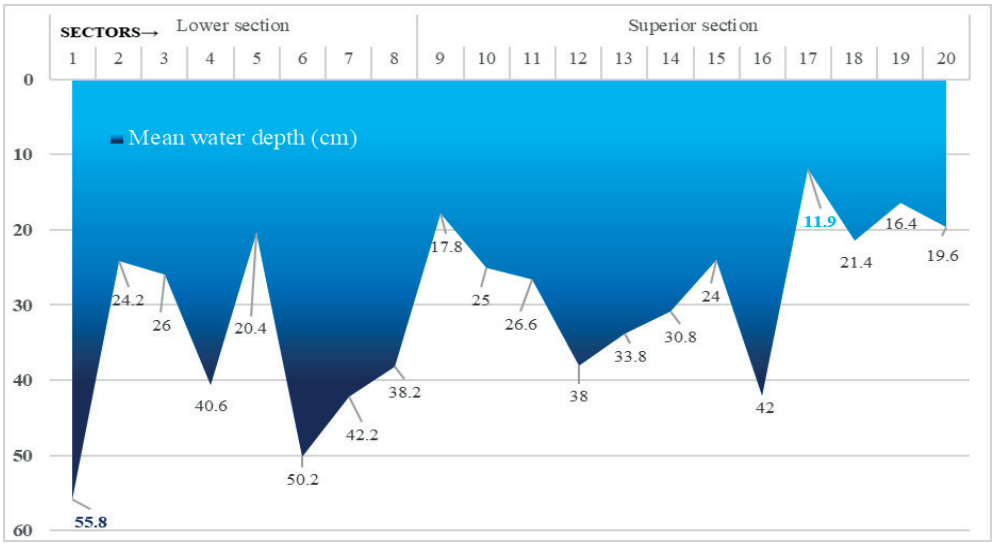

Figure 3. Mean water depth of the sampling stations

The mean value of water depth was 25.6 cm. The coefficient of variation (CV%) was highly similar for the two sections: 34.14% for the lower section and 35.47% for the superior section. In this case, water depth did not influence the presence or absence of both species. Large specimens of European grayling prefer deeper water than small specimens (Mallet et al., 2000) but in this case, in the deeper pools from the lower section of the river, we did not encounter any specimen. The two specimens caught in the lower section were caught in stations 2 and 3 where the mean water depth was 24.2 cm respectively 26 cm. The highest number of brown trout *Salmo trutta* was caught in station 4 (lower section) and station 12 (superior section). The mean water depth in both cases was similar (40.6 cm and 38 cm). Water depth is not related to the number of specimens but rather for their size, a fact confirmed by Vismara et al. (2001) who stated that adult brown trout prefer deeper water than smaller specimens.

Water width

Water width in the lower section of the Someșul Cald River ranged from 4.48 m (station 4) to 9.53 m (station 1). The mean value of the water width was 6.433 m. In the superior section of the river, water width ranged from 2.348 m (station 20) to 24.68 m (station 9). The mean value of water depth was 11.30 m. The coefficient of variation (CV%) was 23.45% for the lower section and 66.26% for the superior section. The alteration of the habitat can be best visualized in Figure 4.

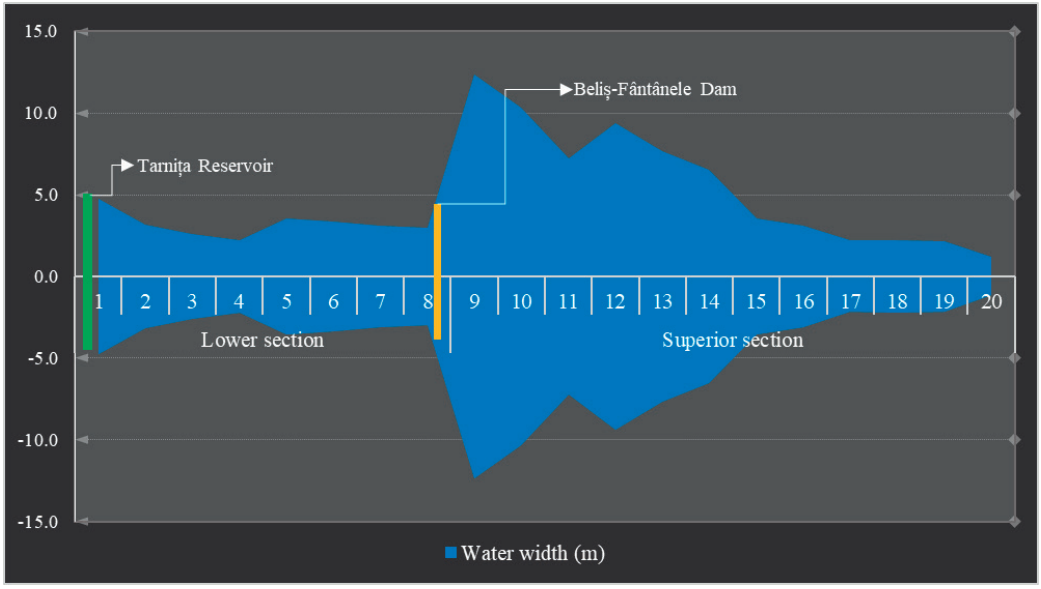

Figure 4. Mean water width of the sampling stations

Water width is constantly increasing from station 20 to station 9, where the Beliș-Fântânele Dam is positioned. Downstream of station 9, and implicitly the Beliș-Fântânele Dam the width of the river is much narrower. Fish migration is stopped by the dam and also the habitat continuity is altered. In figure 4 can be observed as a similarity in the lower section and the superior section of the river.

The mean width of stations from 2 to 8 (lower section) is almost identical to the mean width of stations from 14 to 20 (superior section) (2 to 8= 5.99m vs. 14 to 20= 5.97m). The number of brown trout specimens in stations from 2 to 8 is 483 and in stations from 14 to 20 is 312.

In the case of European grayling, in stations from 2 to 8 two specimens were caught and in stations from 14 to 20, 35 specimens were caught. In the superior section (stations 9 to 13) the total number of European graylings is higher than the number of brown trout (181 vs. 119).

In the same stations, the mean width of the river is 18.76 m, 3 times wider than the stations interval 2 to 8 and 14 to 20.

Water velocity

The mean recorded velocity in the lower section of the river was highly similar to the velocity recorded in the superior section (0.335 m/s vs. 0.363 m/s). The smallest value for the lower section was 0.14 m/s (station 1) and the highest value was 0.44 m/s (station 5). The superior section of the river showed more homogenous water velocity. The smallest value was recorded in station 16 (0.3 m/s) and the highest values were recorded in sectors 10 and 19 (0.42 m/s) (Figure 5).

Both brown trout and European grayling have great swimming capacity and their presence can be influenced by water velocity. For spawning, they use slow flow habitats (10-30 cm/s). The data presented in Figure 5 may indicate spawning sites since velocity is one of the most important variables together with substrate composition (Fukuda et al., 2013).

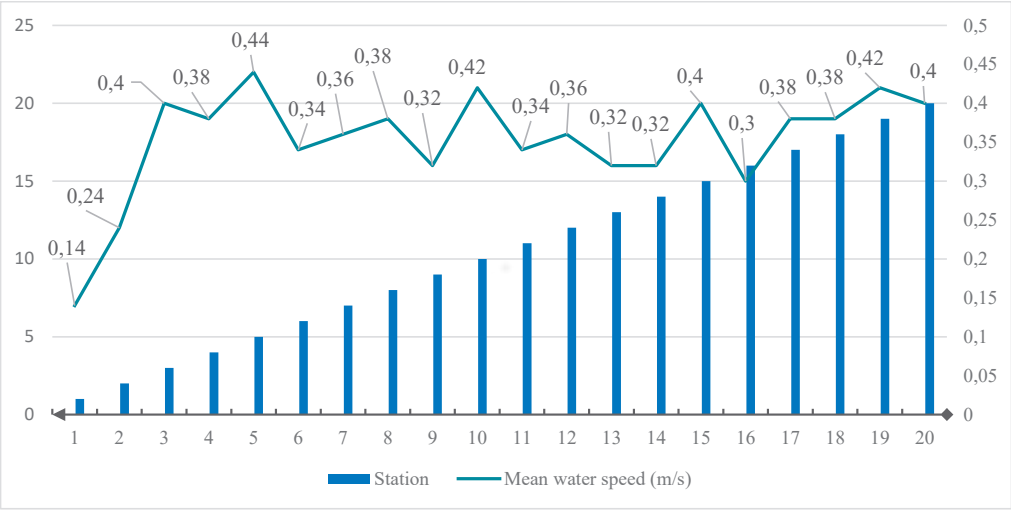

Figure 5. Mean water velocity of the sampling stations

Fish samples

A total number of 1235 fishes were caught from the Someșul Cald River of which 938 brown trout Salmo trutta specimens (488 in the lower section and 450 in the superior section) and 297 European Grayling Thymallus thymallus (2 in the lower section and 295 in the superior section) (Figure 6).

Brown trout represented 75.95% of the total number of fish caught and European grayling represented 24.05%. In the lower section of the river, brown trout represented 99.59% and European grayling represented 0.41%. A different situation was noticed in the case of the superior section of the river, where brown trout represented 60.40% and European grayling represented 39.60%. The largest number of brown trout specimens were caught in the lower section of the river in station 4 (174 specimens) while the smallest number was caught in the superior section in station 20 (1 specimen). In terms of numbers, in station 12 from the superior section of the river, 104 European grayling specimens were caught and the species was missing in stations: 1, 4, 5, 6, 7, 8 (lower section) and 17, 18, 19 and 20 (superior section).

Correlations of fish and habitat parameters

Pearson correlations were performed to characterize the relationship between the aquatic environment (in its current state) and the distribution of the two salmonid fish species under the present conditions (Figures 7 and 8). The brown trout was present in all the analyzed stations and it appears that altitude, water width, water depth and water velocity do not affect its distribution and all the correlations were not statistically significant (for $p<0.05$). The

correlation coefficient was weak and moderate in the case of brown trout. In biological terms, this may be explained by the plasticity of the species which adapts very well to different new environments with different morphology (Valiente et al., 2010; L'Abée-Lund and Vøllestad, 2017). The presence of European grayling is affected by two major parameters: altitude and water width (Table 2).

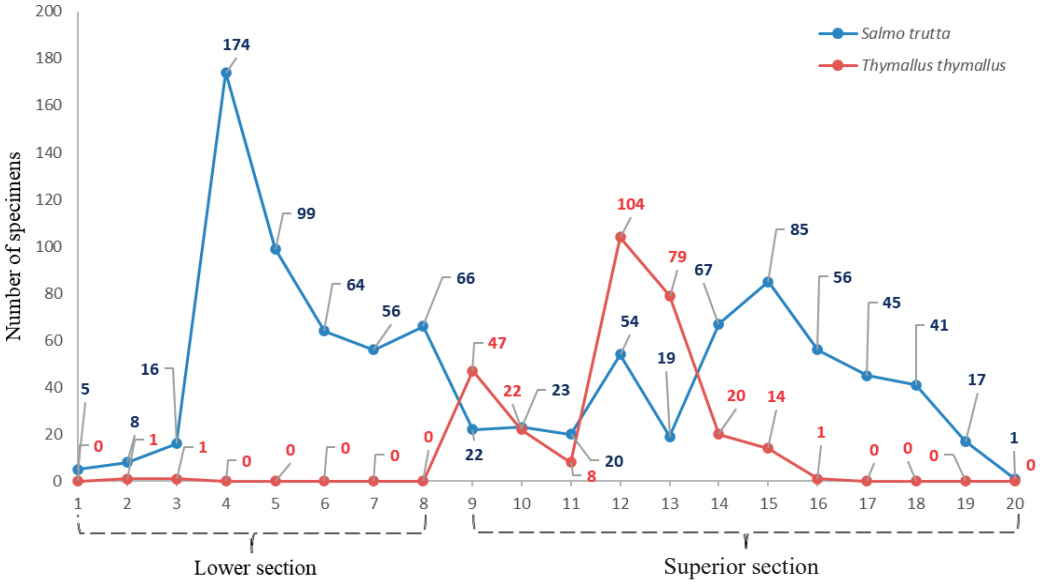

Figure 6. Brown trout and European Grayling specimens from the two sections of the river

Table 2. Correlation of species (*Salmo trutta* and *Thymallus thymallus*) with habitat parameters (*significant for p<0.05)

		Altitude (m)		Width (m)		Depth (cm)		Velocity (m/s)	
River Section	Species	r	p-value	R	p-value	r	p-value	r	p-value
L	*Salmo trutta*	0.4031	0.3221^{ns}	-0.5089	0.1978^{ns}	-0.01672	0.9687^{ns}	0.5756	0.1354^{ns}
S		-0.2268	0.4784^{ns}	-0.0980	0.7617^{ns}	0.34	0.2796^{ns}	-0.1756	0.5851^{ns}
L+S		-0.1959	0.4078^{ns}	-0.2165	0.3592^{ns}	0.2239	0.3426^{ns}	0.3178	0.1721^{ns}
L	*Thymallus thymallus*	-0.448	0.2656^{ns}	-0.2917	0.4832^{ns}	-0.588	0.1253^{ns}	-0.09463	0.8236^{ns}
S		**-0.6024**	**0.0382***	**0.672**	**0.0167***	0.4806	0.1137^{ns}	-0.3193	0.3116^{ns}
L+S		0.2799	0.2320^{ns}	**0.7181**	**0.0004*****	0.0432	0.8565^{ns}	-0.04945	0.8360^{ns}

Legend: L-lower section; S-superior section; L+S-both sections, r-correlation coefficient; p-statistical significance.

A strong negative correlation can be observed (r=-0.6024) in the case of European grayling and altitude, meaning that the number of fish decreases when altitude increases. An almost identical situation is encountered in the case of European grayling and water width (r=0.672). The strong and positive correlation between European grayling and river width was observed only for the superior section of the river. The presence of the Beliș-Fântânele Dam had two negative side effects: first, the dam is blocking the European grayling upstream and downstream migration and second, it is genetically isolating the upstream existing population (Curtean-Bănăduc and Bănăduc, 2016). Petru Bănărescu (1964) mentioned that European grayling was found in Someșul Cald River, at the confluence of Someșul Cald River

and Someșul Rece River and downstream, in Someșul Mic River (formed by Someșul Cald River and Someșul Rece River) close to Florești Village and Cluj-Napoca, Cluj County. The species was not signalled in the last 20 years in Someșul Mic River.

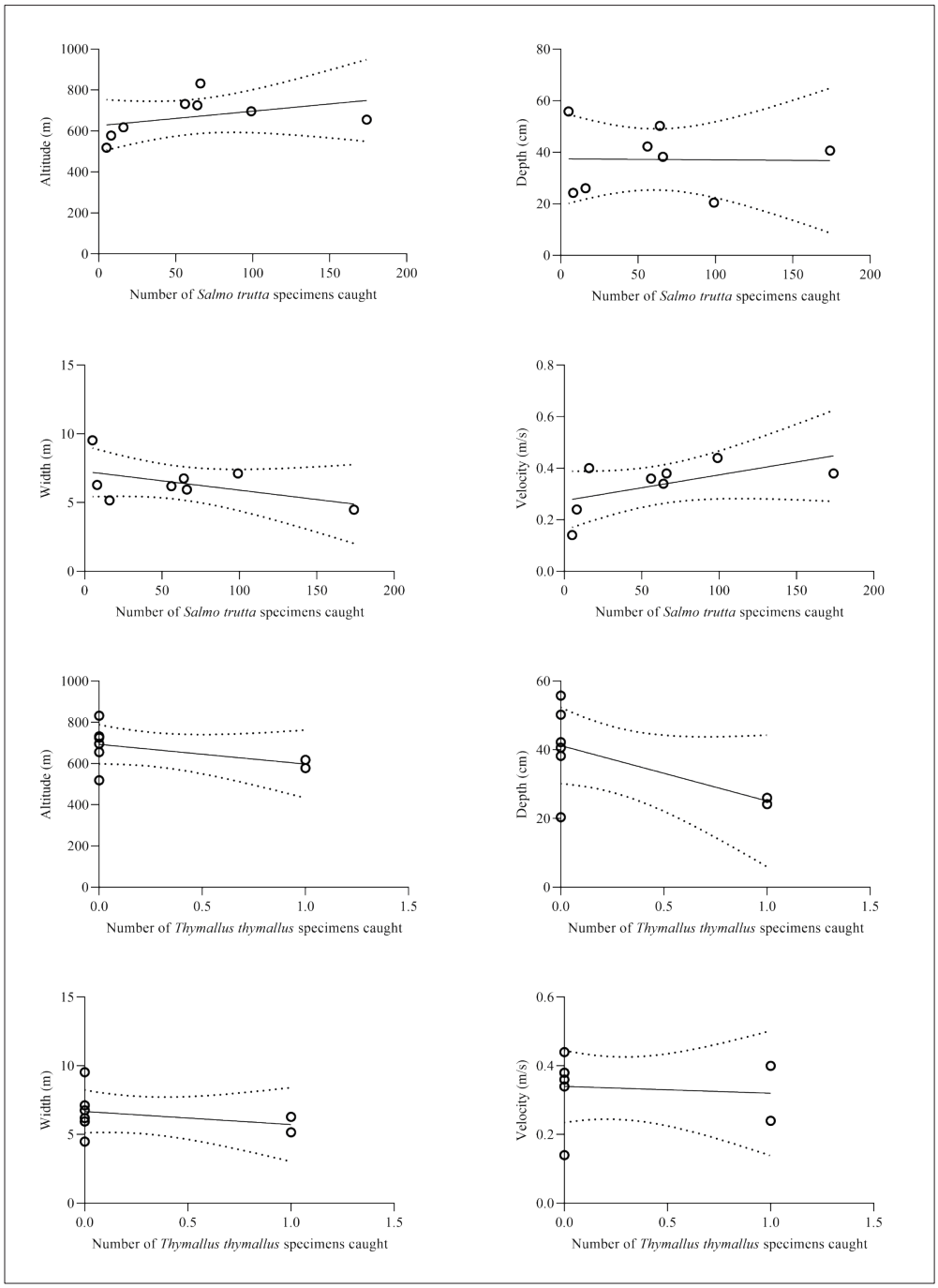

Figure 7. Correlations and 95% confidence intervals between fish number and aquatic habitat parameters - The lower section of Someșul Cald River

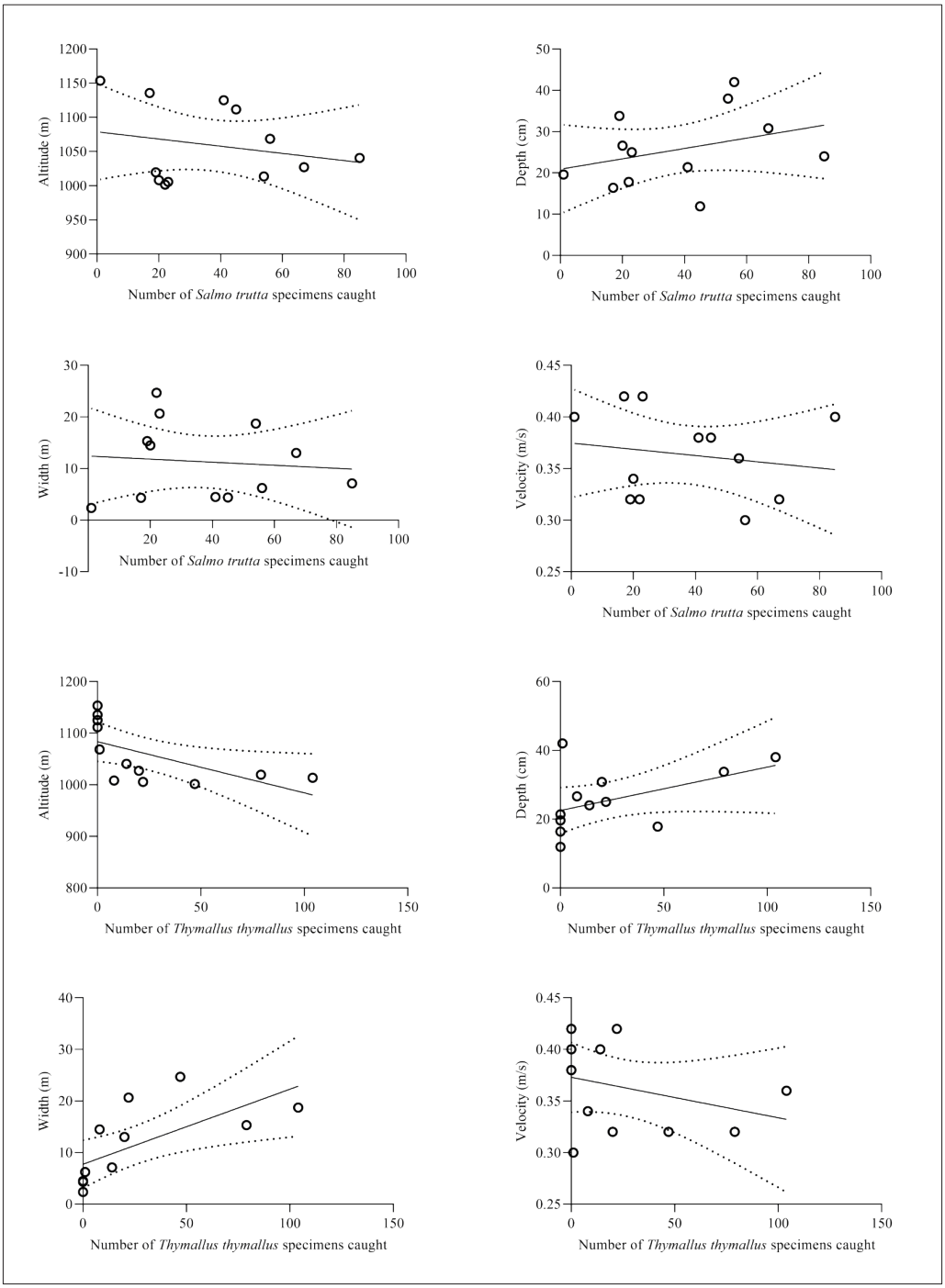

Figure 8. Correlations and 95% confidence intervals between fish number and aquatic habitat parameters -Superior section of Someșul Cald River

CONCLUSIONS

Brown trout (*Salmo trutta*) and European grayling (*Thymallus thymallus*) distribution is affected by the damming from the Someșul Cald River.

Someșul Cald River configuration is altered by the cascade dammings and as a consequence, fish ecological zones are abnormal. There is also the risk of genetic drift for the European grayling population located in the superior section of the river.

Riverbed width and altitude also influence the distribution of the studied species.

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INTER-RELATIONS BETWEEN THE MESOZOOPLANKTON COMMUNITY AND *SPRATTUS SPRATTUS* FROM THE ROMANIAN BLACK SEA AREA

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Abstract

Sprat is a small pelagic species, with a key-role in the marine ecosystem, acting as a link between plankton and production on higher trophic levels. Sprat individuals collected from stations along the Romanian Black Sea coast were measured and weighted and the food array was performed by analyzing the stomachal content. Sprat fed on a broad diversity of mesozooplankton components, such as copepods, cladocerans and meroplankton. Analyzing the mesozooplankton component, copepods and meroplankton represented the bulk of the community in the area. Variability in zooplankton abundance may create a pressure on the feeding conditions for sprat populations.

Key-words*: Black Sea, food array, mesozooplankton, sprat, stomachal content.*

INTRODUCTION

Marine pelagic species, sprat - *Sprattus sprattus* is among the main zooplankton consumers in the Black Sea, forming alone the trophic base for several fish species.
Sprat (Figure 1) competes for food with other planktivorous organisms such as small pelagic fishes, juveniles, and gelatinous zooplankters (Mihneva et al., 2015).

Figure 1. *Sprattus sprattus*

Sprattus sprattus is a keynote species in the Black Sea, and stock dynamics is highly affected by the fisheries and environmental condition (Nicolae et al., 2018; Raykov et al., 2019).
In the daytime, it keeps to bigger depths and in the night, comes to surface**.** It forms important agglomerations and performs unregulated migrations between nutrition areas and spawning places determined by temperature conditions.
In the spring there is a tendency of movement towards the coast and northwards and toward offing in the autumn, but there are not specific migrations of spawning or feeding. Mostly, adults tend to remain under the thermocline, penetrating above its only in the spring and autumn (Totoiu et al., 2017).
Research on the food component of the sprat has shown that it is a planktivorous fish and consumes copepods, cladocera, eggs of various crustaceans, Balanus nauplii and cypris, Mytilus veligers, and in the summer months it descends close to the bottom, consuming Myside (Porumb, 1977).
Sprat passes to exogenous feeding at a length of 6.7 mm. The larvae feed on diatoms, flagellates, eggs, and young stages of copepods. Large larvae of sprat feed only on zooplankton. Sprat

20.30 mm in length consume nauplii and copepodite stages of copepods, mollusk larvae and eggs of invertebrates. The most common food items of 30 mm and longer (adult inclusive) sprat are calanoids, *Pseudocalanus* mainly in winter, *Eurytemora* and *Acartia* mainly in summer and autumn. In summer, cladocerans (*Bosmina, Evadne* and *Podon*) also occur in the food (Ojaveer and Aps, 2003). Many studies document that sprat feeds preferably on large coldwater zooplankton such as *Calanus euxinus*, *Pseudocalanus elongatus* and *Parasagitta settosa* in the Black Sea. The eurytherm copepods (*Acartia spp*.) and meroplankton larvae can represent an important food component, especially in the young individuals' diet (Mihneva et al., 2015).

The mesozooplankton consists of pelagic organisms, mainly by copepods, cladocerans, meroplanktonic larvae of benthic invertebrates, and other groups, constituting the fodder component.

In the Black sea mesozooplankton includes *Noctiluca scintillans*, representing the non fodder component.

Despite of the fact that this prevalent species is classified as a genus of heterotrophic, pigment-less dinoflagellate alga, it has traditionally been monitored as a member of the mesozooplankton community. This is a consequence of its non-photosynthetic, heterotrophic lifestyle and its large size (200-700 µm), corresponding to the size/length of mesozooplankton organisms.

Knowledge of zooplankton dynamics is essential for understanding the pelagic ecosystem. For instance, availability of suitable zooplankton affects growth and survival of fish that feed on it as well as variations in zooplankton abundance and community composition may affect recruitment of important commercial fish species, in both open and coastal waters (Díaz-Gil et al., 2014, Nicolae et al., 2015).

Zooplankton communities are also of great interest as potential indicators of climate change in the marine environment (Hays et al., 2005).

Mesozooplankton have vital importance in the feeding of commercially valuable fish species and their larvae. Mesozooplanktonic organisms occupy, according to their size range and variety of feeding habits, a key position in pelagic food webs, representing the food source of small pelagic fishes (Alcaraz and Calbet, 2009)

Among the biotic factors, the mesozooplankton structure is closely linked to dynamics of sprat weight, condition factor, stomach fullness index and fat accumulation (Mihneva et al.,2015).

The zooplankton' s presence and abundance are the reason why fish species find in the Black Sea Romanian waters the most favorable area for feeding, reproduction and growth (Porumb F., 1986).

The aim of this paper was to record information of mesozooplankton's diversity and to identify the food composition of sprat, providing data regarding the diet description for this pelagic fish in the Romanian Black Sea area. Feeding behaviour of sprat in relation to size, as well as abundance, composition and distribution of potential prey could be established.

MATERIALS AND METHODS

Both mesozooplankton and sprat samples were collected from stations located along the Romanian Black Sea coast (Figure 2), during surveys organized by NIMRD in the warm season of 2019. The collected samples were preserved in formaldehyde for further analysis in the laboratory.

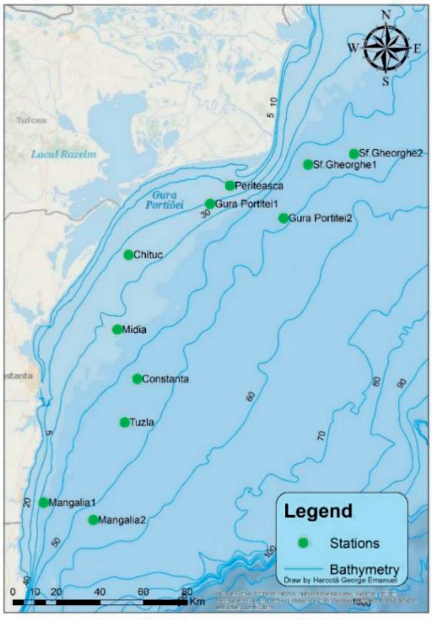

Figure 2. Map of sampling stations

Sampling of sprat was made using the pelagic trawl for juveniles by surface trawling (0-5m) at 1.5-2 Nd speed, the duration of the trawling being 15 minutes and the horizontal opening of the 14 m trawl (Figure 3).

Figure 3. Trawl for sprat sampling

Collecting of mesozooplankton samples was performed using a Juday net (0.1 m^2 mouth opening area, 150 μm mesh size) by vertical hauls (Figure 4).

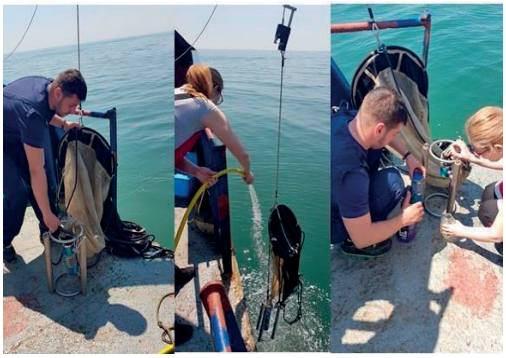

Figure 4. Mesozooplankton sampling

According to the methodology, the mesozooplankton sample was homogenised, and quantitative and qualitative processing was performed in the Bogorov chamber, under Olympus SZX10. In the subsample(s) all plankters were counted until each of the three dominant taxonomic groups reached 100 individuals. For estimation of large animals' numbers, the whole sample was examined in a Petri dish (Figure 5).

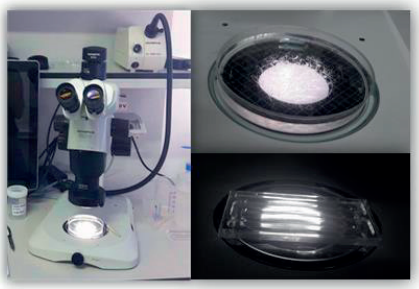

Figure 5. Analysis of mesozooplankton samples

The number of individuals and mean individual weights were used for estimating the density as $ind \cdot m^{-3}$, respectively the biomasses as $mg \cdot m^{-3}$ wet weight (Korshenko and Alexandrov, 2014). The study of the food array was performed by analyzing the gastro-intestinal content at sprat (Figure 6).

The length of each sprat specimen was measured, each fish was weighted, and after dissection the stomach was removed and stored in formalin solution until identification. The stomachs were cut longitudinally, and the contents of each stomach were transferred to a petri dish and identified under a binocular microscope (Figure 5). Food remains, which were not recognizable due to an advanced stage of digestion, were recorded as semi digested food. The qualitative analysis consisted of a complete identification of the organisms in the gut contents.

Figure 6. Sprat stomachal content analysis

A hierarchical cluster analysis was performed using the Bray-Curtis similarity in PRIMER. The data were square-root-transformed to handle zero-inflation and the few large values typical for density data sets, and standardized by range, which is one of the possible standardizations for the Bray-Curtis similarity coefficient.

RESULTS AND DISCUSSIONS

The mesozooplankton was represented by 17 species. Copepoda represented the bulk of the community, with seven species, followed by the meroplanktonic component with five species (Table 1).

Among the marine zooplankton, copepods are the most familiar and dominant constituent since they comprise around 55-95% of the total zooplankton abundance in the marine pelagic system (Angara, 2013)

Cladocera was represented by one species and other groups by three species (Table 1).

Table 1. List of identified mesozooplanktonic species

No.	Scientific Name Accepted	Scientific Name Author	Phylum	Class	Order
1	*Noctiluca scintillans*	Macartney Kofoid & Swezy, 1921	Myzozoa	Dinophyceae	Aphragmophora
COPEPODA					
2	*Acartia (Acartiura) clausi*	Giesbrecht, 1889	Arthropoda	Hexanauplia	Calanoida
3	*Pseudocalanus elongatus*	Boeck, 1865	Arthropoda	Hexanauplia	Calanoida
4	*Paracalanus parvus*	Claus, 1863	Arthropoda	Hexanauplia	Calanoida
5	*Centropages ponticus*	Karavaev, 1895	Arthropoda	Hexanauplia	Calanoida
6	*Calanus euxinus*	Hulsemann, 1991	Arthropoda	Hexanauplia	Calanoida
7	*Oithona similis*	Claus, 1866	Arthropoda	Hexanauplia	Cyclopoida
8	*Harpacticoida*	Sars M., 1903	Arthropoda	Hexanauplia	Harpacticoida
CLADOCERA					
9	*Pleopis polyphemoides*	Leuckart, 1859	Arthropoda	Branchiopoda	Onychopoda
MEROPLANKTON					
10	Bivalvia	Linnaeus, 1758	Mollusca	Bivalvia	
11	Gastropoda	Cuvier, 1795	Mollusca	Gastropoda	
12	Polychaeta	Grube, 1850	Annelida	Polychaeta	
13	Balanus	Costa, 1778	Arthropoda	Hexanauplia	Sessilia
14	Decapoda	Latreille, 1802	Arthropoda	Malacostraca	Deacpoda
OTHER GROUPS					
15	*Parasagitta setosa*	J. Müller, 1847	Chaetognatha	Sagittoidea	Aphragmophora
16	*Oikopleura (Vexillaria) dioica*	Fol, 1872	Chordata	Appendicularia	Copelata
17	*Mesopodopsis slabberi*	I. Van Beneden, 1861	Arthropoda	Malacostraca	Mysida

Regarding the mesozooplankton quantitative structure, the non fodder component recorded the highest density values in Mangalia, Midia and Gura Portitei 2, in the other sampling stations reaching lower densities (Figure 7).

Acartia clausi and *Pseudocalanus elongatus* had the highest density values in Periteasca station, being dominant species in all the analysed samples, the other copepods recording lower density values (Figure 7).

Bivalvia and Balanus which belong to the meroplanktonic component, were best represented from the quantitative point of view in Periteasca, Gura Portitei 1 and Chituc stations. From other groups, *Oikopleura dioica* was the species with the highest density values, the other two species recording low density values (Figure 6).

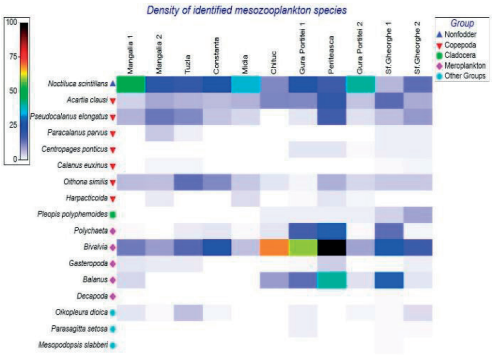

Figure 7. Shade plot showing the density (ind·m^{-3}) of mesozooplankton species

Analysing the similarities between the sampling stations, Chituc and Gura Portitei 1 have a similarity of over 90% (Figure 7). This is due to the fact that in these stations the mesozooplanktonic component was best represented from the quantitative point of view. High similarities (80%) were recorded between Sf. Gheorghe 2 and Constanta stations and Mangalia 2 and Tuzla, mainly driven by the species densities in the area (Figure 8). The more abundant a species is within a group or analysed area, its contribution to the intragroup similarities will be of great importance.

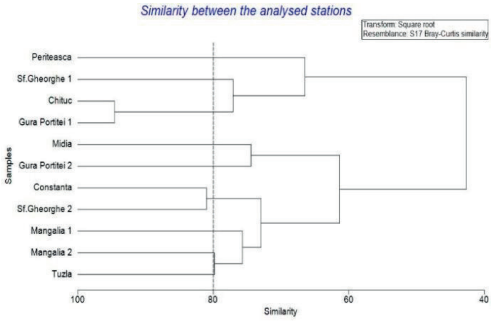

Figure 8. Bray-Curtis similarity matrix between the sampling stations

For the identification of sprat stomachal content, 100 fishes with length between 60-100 mm and a medium weight between 1.51-4,17 gr were analysed (Figure 9). Sprat, as a cold-water species attains much higher biomass during cold years and lower biomass in warm years (Shiganova and Öztürk, 2010).

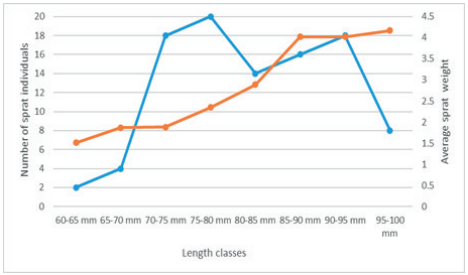

Figure 9. Distribution of average no. samples and medium weight of *Sprattus sprattus by* length classes

After analysing the sprat's stomachal content, species belonging to Copepoda, meroplankton and other groups were identified.

The major groups/species found in the sprat's stomach were represented by the following: Copepoda (a) Bivalvia (b) , Balanus nauplii (c), Balanus cypris (d), *Oikopleura dioica* (e) (Figure 10).

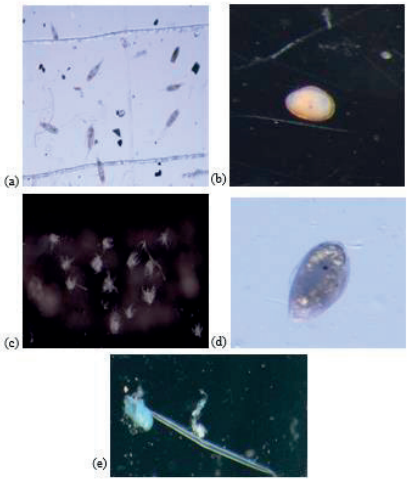

Figure 10. Major mesozooplankton groups/species found in the sprat's stomach

Individuals of *Sprattus sprattus* consumed mainly Copepoda species and food elements that belong to the meroplanktonic component. The highest consumption of copepods was in Periteasca, followed by Gura Portitei and Mangalia 1 and Midia (Figure 11).

From the meroplanktonic components, Bivalvia was highly consumed by sprat in Mangalia 1, Midia and Chituc. Balanus nauplii were preferred as food source only in three stations (Mangalia, Midia, Chituc) while the cypris stage was consumed only in Midia station (Figure 11).

Oikopleura dioica was found in the stomachal content at sprat from Periteasca station. Sprat individuals presented semidigested food in stations Gura Portitei, Sf.Gheorghe 1 and Sf.Gheorghe 2 (Figure 11).

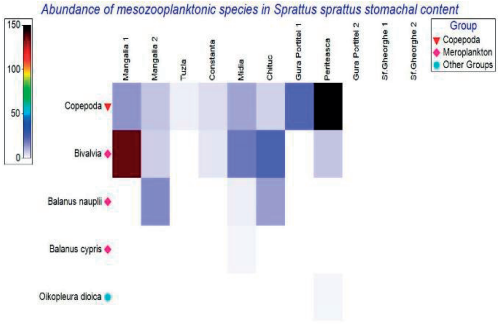

Figure 11. Shade plot showing the mesozooplanktonic species consumed by *Sprattus sprattus*

The Bray-Curtis matrix showed a very high similarity between Periteasca, Gura Portitei 1 and Tuzla stations since in this stations, sprat preferred Copepoda as a major source of food (Figure 12). Another high similarity was between Mangalia 2 and Chituc and Constanta and Midia. In these stations, sprat individuals consumed copepods and meroplanktonic components in similar quantities (Figure 12).

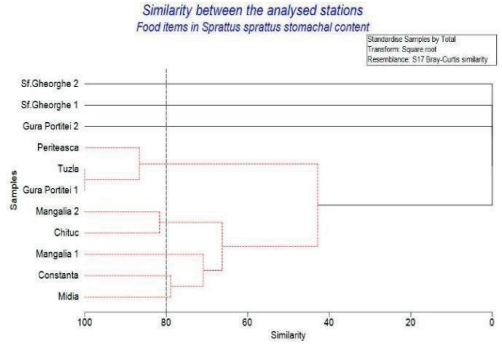

Figure 12. Similarity matrix between the stations based on the *Sprattus sprattus* stomachal content

Copepods are the most important mesozooplanktonic group constituting the primary food supply of fish larvae. Many organisms of commercial importance in many parts of the world depend mostly on copepods as a food source at the planktonic larvae stage (Yildiz and Feyzioğlu, 2014).

CONCLUSIONS

The mesozooplankton community was represented by 17 species. Copepoda represented the bulk of the community, with seven species followed by the meroplanktonic component with five species.

From the quantitative point of view, Copepoda and the meroplanktonic elements recorded the highest density values in all the analysed stations.

Stomach content analysis showed that Copepoda was preferred as food source, being followed by meroplankton.

Bivalvia was main source food for *Sprattus sprattus,* other organisms such as Balanus nauplii and Balanus cypris stages being consumed in smaller quantities.

Copepods were consumed in high amounts in Periteasca, Gura Portitei, Mangalia 1 and Midia.

Bivalvia was highly consumed in Mangalia 1, Midia and Chituc. Balanus nauplii were preferred as food source only in three stations (Mangalia, Midia,Chituc) while the cypris stage was consumed only in Midia station.

The Bray-Curtis matrixes showed that the analysed stations recorded high similarities driven mainly by the species abundance values, both for the mesozooplankton community and for the food items consumed by *Sprattus sprattus* individuals.

Taking into considerations the analysis we made, we conclude that the production of species involved in the trophic chain mesozooplankton organisms) represent a trophic base proper for the fish nutrition and lead to a good environment in which the sprat can develop in proper conditions appropriate for growth, reproduction and new generations sustainability.

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