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GENETICS AND BREEDING

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EVALUATION OF THE PRODUCTIVE AND REPRODUCTIVE QUALITIES OF BLACK MOTTLE COWS WITH THE USE OF FORAGE SORBENTS

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Abstract

Currently, the presence of toxins in the composition of all feed for cattle is recorded as a result of a violation of the technology of harvesting and storing feed. The most famous are aflatoxins, ochratoxins, zearalenone, T2 toxin, deoxynivalenol (DON), fumonisin, which, getting into the blood of an animal, quite negatively affect the body of cows, reducing their productivity and fertilizing ability. Of particular importance is the determination of toxins in feed when feeding highly productive livestock under conditions of intensive milk production technology. The purpose of the work is to establish the degree of influence of different dosages of feed sorbents on the productive and reproductive qualities of cows. The studies were carried out in the conditions of the breeding reproducer of the black-and-white breed LLC Barmino in the Nizhny Novgorod region. The objects of research were cows of the first lactation of the Black-and-White breed, but the subject of research was the milk productivity of animals and the efficiency of insemination of cows. It was found that milk productivity and higher rates of reproductive abilities were the best in cows of the third experienced groups that received the largest amount of feed sorbent - 2 kg per ton of concentrated feed.

Key words*: cows, black-and-white breed, toxins, sorbent, productivity.*

INTRODUCTION

Currently, the presence of toxins in the composition of all feed for cattle is recorded as a result of a violation of the technology of harvesting and storing feed - high humidity (more than 13%), the presence of oxygen, a sufficient amount of heat, a sharp temperature drop, damage to grain by insects and competition with other microorganisms for a nutrient substrate (Golovnya, 2017; Laptev et al., 2014; Klaenhammer et al., 2012; Zdorovinin et al., 2021).

The main known toxins found in feed include: aflatoxins, ochratoxins, zearalenone, T2 toxin, DON (deoxynivalenol), fumonisin.

Getting into the blood of an animal, toxins can affect the state of the body in a variety of ways, and symptoms often appear that veterinary specialists associate with other diseases (Malkov et al., 2016, 2018; Xue, 2020).

Two of the above toxins, such as zearalenone and DON, have a negative effect on the reproductive state of cows - they cause estrogen syndrome, provoke vaginitis, endometriosis, infertility, abortion, cause false hunts (Diaz D., 2006; Uyeno et al., 2015) (Table 1).

Table 1. The main toxins in feed and their effect on the body of cattle (Diaz D., 2006)

Toxins	Effect on the animal
Aflatoxins	Decreases appetite, causes lethargy, rough coat, affects the liver, poses a serious risk of contamination of milk
Ochratoxins	Affect the liver and kidneys, contribute to the removal of vitamins and minerals from the body, causing destruction of hooves, horns, teeth
Zearalenone	Causes estrogen syndrome, provoking vaginitis, infertility, abortion, false hunting, edema, genital and udder hypertrophy
T2 toxin	Causes a decrease in appetite, depression, a drop in productivity. The formation of necrosis on the skin and mucous membranes, worsens blood clotting
DON (deoxynivalenol)	Reduces appetite and, as a result, productivity, increases the number of somatic cells in milk, disrupts reproductive functions
Fumonisin	It affects the liver, has a nephrotoxic and carcinogenic effect, reduces the immunity of the animal.
Mycotoxins	They affect the health and productivity of animals, are found in meat, milk and offal.

Elimination or reduction of the concentration of toxins that enter the body of animals with food is possible with the help of various feed sorbents, which include both a mineral complex (coal, zeolite, silica), which is the primary filter for toxins, and live bacteria that are embedded on particles solid sorbent. Bacteria of the genus Bacillus are used as bacteria (Nekrasov et al., 2013; Filipyev, 2016). They have a significant advantage over other representatives of the microflora: they are able to increase the nonspecific resistance of the host organism, have antagonistic activity against a wide range of pathogenic and opportunistic microorganisms and high enzymatic activity, are resistant to lytic enzymes and thereby cause high viability throughout the gastrointestinal tract (Pozdnyakova et al., 2019, 2020).

Thus, determining the effect of feed sorbents on the productive and reproductive qualities of cows is a relevant and practically significant topic for scientific research.

MATERIALS AND METHODS

The studies were carried out in the conditions of a breeding reproducer of the black-and-white breed LLC Barmino, Lyskovsky district, s. Barmino, Nizhny Novgorod region.

The farm contains 1983 heads of purebred black-and-white cattle, of which 795 heads are dairy herds. The average productivity for 305 days of lactation is 8847 kg, the mass fraction of fat in milk is 4.06%, protein is 3.29%.

The method of keeping cows is mixed - some of the animals are kept loose in sections, milking takes place in the Tandem type milking parlor for 24 heads, and some are kept in stalls in a tethered way, milking is carried out in the milk pipeline.

The repair of the herd is carried out at the expense of own reproduction, the culling of cows is 21.5%.

The reasons for the withdrawal of cows from the herd are shown in Figure 1.

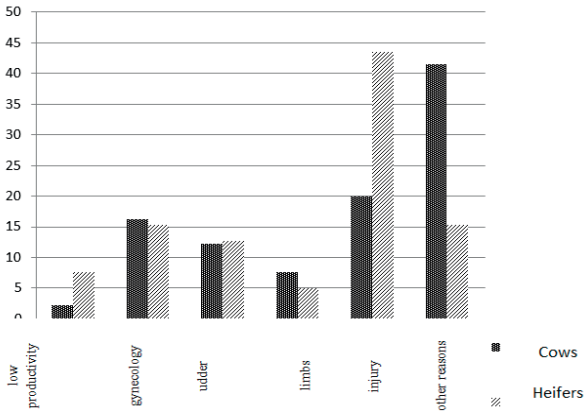

Figure 1. Reasons for culling cows from the herd

From Figure 1 it follows that the main reason for cow culling is technological problems 19.9% in adult cows and 43.5% in first-calf heifers, gynecological diseases and barrenness account for 16.3% and 15.3%, respectively.

An analysis of the reproductive qualities of the herd showed that the service period lasts 103 days, the dry period - 63 days, the number of inseminations per one fruitful - 1.9, and the yield of live calves per 100 cows - 79%.

These indicators can be considered satisfactory for a dairy farm with an average productivity of more than 8000 kg. However, the identification and elimination of the causes that keep reproduction at this level is practically necessary. To establish the effect of feed mycotoxins on the productive and reproductive qualities of cows, we conducted an examination of the concentrated part of the feed in accordance with GOST 34108-2017 - Feed, mixed feed, feed raw materials.

The presence of mycotoxins was determined by a direct solid-phase competitive enzyme immunoassay in the testing laboratory of the State Budgetary Institution of the Nizhny Novgorod Region "Regional Veterinary Laboratory" for the presence of mycotoxins (Table 2).

Table 2. Analysis of feed for the content of mycotoxins

No.	Indicator	Test result	Norm
1.	Aflatoxin B1, mg/kg	Less than 0.002	0.004
2.	Deoxynivalenol (DON), mg/kg	0.349	Not more than 1.0
3.	Zearalenone, mg/kg*	0.251	0.1
4.	Ochratoxin A, mg/kg*	0.021	0.005
5.	T-2 toxin, mg/kg	0.020	0.06

*excess of MPC

Thus, the conducted studies revealed the excess of the permissible norm of mycotoxins of zearalenone by 2.5 times and ochratoxin by 4.2 times.

Currently, the farm uses the Provitol feed additive to normalize the rumen digestion of cows, which accelerates the formation of rumen microflora and helps to increase productivity and improve the quality of milk (Yildirim et al., 2018, 2019, 2020). However, this feed additive, in our opinion, does not eliminate the negative impact on reproductive properties caused by feed toxicosis.

As a feed sorbent, we chose the Russian-made probiotic sorbent Vetosporin-aktiv, which is a multicomponent preparation. It is a suspension of live bacteria of natural strains of *Bacillus subtilis* 11B (with antifungal) and *Bacillus subtilis* 12B (with antifungal) activity.

The objectives of the research included studying the effect on the productivity and reproductive qualities of cows supplementing the main diet with the Provitol feed additive and the Vetosporin-Active feed additive.

Feed additive Provitol and drug Vetosporin-active were introduced in various dosages in the feed shop during the production of the concentrated part of the feed, and then fed as part of a mono-feed for 75 days - starting from the transfer of animals to the late dry group up to the 2nd month of lactation inclusive.

For the experiment, 5 groups of cows were selected by the method of pair-analogs, 12 heads each, in the physiological state of the late dry period (2 weeks before calving), of the same age and approximate live weight.

The first group is the control group, which did not receive a feed supplement to the main diet; the second group - experimental - received the Provitol feed additive at a dose of 1.5 kg per ton of feed (as recommended by the manufacturer); the third group - experimental - received the Provitol feed additive at a dose of 2.5 kg per ton; the fourth group - experimental – received the Vetosporin-active feed additive at a dose of 1.0 kg per ton of feed (as recommended by the manufacturer); the five group – experimental - received the Vetosporin-active feed additive at a dose of 2.0 kg per ton of feed.

RESULTS AND DISCUSSIONS

When assessing the reproductive qualities of experimental animals, we took into account the following features: ease of calving, the number of live calves at birth, the number of postpartum complications, the duration of the service period, the number of doses of semen used (Table 3).

Table 3. Indicators of the reproductive abilities of cows when using feed sorbents

Indicators	I counter	II Experienced 1.5 kg/t	III Experienced 2.5 kg/t	IV Experienced 1 kg/t	V Experienced 2 kg/t
Number of calves, head	12	12	12	12	12
Alive, head	10	7	12	12	11
%:	83.3	58.3	1	1	91.7
Stillborn, head	2	5	100	100	1
%	16.7	41.7	-	--	8.3
Ease of calving, score	1.67±0.15	1.58±0.16	1.88±0.9	1.17±0.12	1.08±0.09
There was no separation of the placenta, %	16.7	8.3	8.3	-	-
Registration of endometriosis, no of cases, %	25	25	16.7	8.3	0
Duration of the service period, days	103.5±1.1	100.5±1.1	98.2±0.8	90.7±1.2	83.3±0.8***
Doses of semen consumed	2.0	2.0	2.2	1.5	1.1

Note:* - p<0.05; ** - p<0.01; *** - p<0.001

The ease of calving was assessed according to the system adopted by the International Association of Animal Breeders, where points are distributed from 1 (calving with no problems) to 5 (extremely difficult calving, with fetal death).

In the first group, 1 case of 5-point calving was registered, which ended in the death of the fetus. In other groups, the ease of calving varied from 1 to 2 points. In the fifth experimental group, all calving was successful.

Childbirth in animals ends with the separation of the fetal membranes (after birth).

The retention of the placenta is considered to be the state of the cow when its separation did not occur - after 10-12 hours. In the 1st group, two cases of retention of the placenta (16.7%) were registered, and in the 2nd and 3rd one each.

Often, difficult births in cows and non-separation of the placenta lead to postpartum endometriosis, so in the 1st control group and in the 2nd experimental group, three cases each (25%) were recorded, in the third group - 2 cases, and in the fourth - only in one cow. In the fifth experimental group, all 12 cows were healthy.

The duration of the service period in the fifth group was significantly less compared to other groups ($p<0.001$). The number of doses of semen used in the fifth group was the lowest - 1.1 doses. Obviously, this is due to the well-tolerated calving and the absence of gynecological diseases in this group.

The degree of influence of various probiotics and their dosage on the milk production of experimental animals is presented in Table 4.

Table 4. Indicators of milk productivity of cows when using feed sorbents

Indicators	I counter	II Experienced 1.5 kg/t	III Experienced 2.5 kg/t	IV Experienced 1 kg/t	V Experienced 2 kg/t
Average daily milk yield for the milking period, kg	35.6±0.73	37.5±0.78	39.4±0.97**	38.8±0.67**	42.1±1.03***
Milk yield for the milking period (4 months), kg	4184.1±120.1	4314.3±98.9	4465±116.5	4556.9±62.1*	4754.4±141.6**
Milk yield for 305 days of lactation, kg	8405.7±243.8	8928.2±234.1	9127.5±191.9*	9174.2±157.2*	9220.3±197.5*
The content of the mass fraction of fat in milk,%	3.78±0.06	3.84±0.05	3.86±0.05	3.84±0.04	3.92±0.03
The content of the mass fraction of protein in milk,%	3.18±0.04	3.2±0.05	3.17±0.04	3.21±0.05	3.33±0.08
The number of somatic cells. thousand/cm^3	258.6±10.1	251.2±8.5	259.8±12.3	156.3±9.1***	125.6±3.8***

Note:* - $p<0.05$; ** - $p<0.01$; *** - $p<0.001$

The average daily milk yield for the milking period was the highest in the fifth experimental group and amounted to 42.1 kg, which is significantly higher than the milk yield in the control group by 6.5 kg ($p<0.001$). and in the second group by 4.6 kg ($p<0.01$). Animals from the 4th group that received the Vetosporin-active sorbent at a lower dose (1 kg/t of feed) also significantly exceeded ($p<0.01$) the average daily milk yield in cows of the control group by 3.2 kg. These data are also confirmed by the assessment of the total milk yield for the period of milking (from 1 to 4 months): on average, 11.9% more milk was received from cows of the 5th group than from cows of the control group ($p<0.01$) and 9.3% than in cows of the 2nd group ($p<0.05$), respectively.

Milk yield for 305 days of lactation was significantly higher ($p<0.05$) in animals receiving feed sorbents: in the 3rd, 4th and 5th groups, respectively, by 7.9%, 8.4 and 8.8%, than cows in the control group. However, no significant difference was found between the groups.

A reliable effect of feed sorbents from different companies and their dosages on the quality indicators of milk (mass fraction of fat and protein) has not been determined, however, a trend towards an increase in milk fat and protein content is noted in the milk of animals of the 5th experimental group.

For many years, the main indicator for evaluating milk was the mass fraction of fat in it, but at present, the purchase price of milk is

also determined by the content of somatic cells. When the body is poisoned with mycotoxins, a sharp increase in the number of somatic cells in milk is observed, which affects its quality (Olchowy et al., 2019).

The presence in feed of a hormone-like mycotoxin such as zearalenone (Table 2) causes mammary tissue hypertrophy with increased cell rejection. Thus, as a result of mycotoxicosis, the content of leukocytes in milk increases, the number of desquamated mucosal cells in the udder ducts increases, and the number of mammary gland cells increases, which leads to a significant increase in somatic cells in milk (Oikonomou et al., 2012; Spaniol et al., 2014).

When examining milk samples, it was found that the number of somatic cells in the 5th group was significantly ($p<0.001$) 2.1 times lower than in the control, 2 times than in the 2nd group, and 2.1 times than in group 3. There is also a significant difference between the milk samples of the 4th and 5th groups ($p<0.001$): a higher concentration of feed sorbent of 2 kg per ton of feed made it possible to reduce the number of somatic cells in the milk of the 5th group to 125.6 kg/cm^3.

CONCLUSIONS

The data obtained indicate that the animals (fourth and fifth experimental groups) that received the Vetosporin-Active feed sorbent in addition to the main diet had a higher assessment of productive and reproductive qualities, in comparison with analogues of other experimental groups. They noted the best ease of calving, the absence of problems with the separation of the placenta and endometriosis, the optimal duration of the service period and the minimum cost of doses of semen for fruitful insemination.

The cows of the fourth and fifth experimental groups used the nutrients of the feed more efficiently during the milking period, which had a positive effect on milk synthesis and higher productivity.

Of the quality indicators of milk, significant differences in favor of the fifth group were observed only in the number of somatic cells.

Thus, the research results allow us to recommend the use of Vetosporin-active feed sorbent at a dose of 2.0 kg per ton of feed as a feed additive in the main diet of highly productive cows to improve reproductive and productive qualities.

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BASIC PRINCIPLES OF SELECTION OF BULLS-PRODUCERS

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Abstract

Improving the methods of their evaluation is one of the most important links in breeding programs to increase productivity and fertility of livestock. The genetic improvement of the next generation and the population as a whole depends on the objectivity and accuracy of determining the tribal value of producers. We analyzed the basic principles of selection of bulls-producers, namely the use of producers of higher quality compared to the uterus; maximum use of the best manufacturers; replacement of the previous manufacturer by a manufacturer of even higher quality; regulation of kinship between the producer and the uterus with which he mates. As a result, it was found that when selecting producers for the herd, it is not rational to justify the choice with a high index of lifetime productivity.

Key words*: bulls-producers, dairy productivity, selection.*

INTRODUCTION

Widespread introduction of artificial insemination of cows imposes special requirements on breeding qualities of bulls-producers. Improving the methods of their evaluation is one of the most important links in breeding programs to increase productivity and fertility of livestock (Shishkina, 2020). The genetic improvement of the next generation and the population as a whole depends on the objectivity and accuracy of determining the tribal value of producers (Zdorovinin et al., 2021).

In this regard, we set a goal to study the peculiarities of the selection of bulls-producers according to international breeding bases and to develop a science-based system of selection of bulls-breeders (Shishkina, 2021). The basic principles of selection include the following: the use of producers of higher quality compared to the uterus; maximum use of the best manufacturers; replacement of the previous manufacturer by an manufacturer of even higher quality; regulation of kinship between the producer and the uterus with which he mates (Krykov, 2014; Nekrasov, 2014; Guseva, 2020).

MATERIALS AND METHODS

The research was conducted on the basis of LLC "Pachelmskoe khozyaystvo", part of the group of companies LLC "UK" Rusmolko", which is one of the largest producers of whole milk in the Penza region. The herd of LLC "Pachelmskoe khozyaystvo" is staffed with cattle from the Holstein breed. Data from primary zootechnical and breeding documenttation were used for analysis.

RESULTS AND DISCUSSIONS

In order to carry out a competent selection of bulls-producers, it is necessary to determine the quality of the heifer to which the producer is selected (productivity and body type) and to establish the genealogical affiliation of the herd cows.

The average milk yield per herd is 10,471 kg, and the average fat content and protein milk yield are 3.73 and 3.14%, respectively. The average yield of fat per cow is 390.6 kg, and the average yield of protein is 328.8 kg (Figures 1, 2), which significantly exceeds the standards set for Holstein breed in the Russian Federation (Table 1).

Figure 1. Cattle of LLC "Pachelmskoe khozyaystvo" (Penza region, Russia)

Figure 2. Cattle of LLC "Pachelmskoe khozyaystvo" (Penza region, Russia)

Table 1. Indicators of milk productivity of cows for 305 days of the last completed lactation

Groups of animals	Heads	Milk, kg	Milk fat		Milk protein	
			%	kg	%	kg
All livestock	3333	10471	3.73	390.6	3.14	328.8
And lactation	1684	9962	3.71	369.6	3.15	313.8
II lactation	1649	10991	3.75	412.2	3.14	345.1

The correct physique of animals testifies to their ability to show a high level of productivity and productive longevity. The result of the evaluation of herd cows is slightly above average; deep enough torso and long sacrum; udder tightly attached with long anterior and highly attached broad posterior lobes, and a deep furrow. Dairy forms are developed satisfactorily, muscularity is average. The position of the hind limbs and the angle of the hoof are normal. Disadvantages of the physique include insufficient strength of the physique, raised sacrum, narrow pelvis, closeness and some shortening of the front nipples.

Therefore, the cows of the herd of LLC "Pachelmskoe khozyaystvo" (Penza region, Russia) should be selected bulls-producers that enhance the positive expression of external features and are able to correct the shortcomings of the offspring (Figure 3).

Figure 3. Calves of LLC "Pachelmskoe khozyaystvo" (Penza region, Russia)

To do this, a comparison of the exterior profile of the cows of the herd and the daughters of the fixed bull is carried out. The cows of the herd are characterized on average by a rather tall stature and deep body, strong physique and pelvic width slightly above the middle, tightly attached and high-lying udder with the length of the forelegs and the width of the hindquarters to the middle to the rear. It should be noted that the position of the sacrum in animals of both countries of breeding is elevated, the position of the hind limbs deviates from the normal elephant side, the furrow of the udder is small,

In order to avoid undesirable related copulation, the method of line rotation is used in the selection of bulls. Which is that the cows of a certain lineage are selected bulls of other lines. The linear affiliation of the parent stock is given in Table 2.

Table 2. Genealogical structure of the parent stock by lineage

The line	Total uterine population, goal	Including, goal		
		Cows		heifers of all ages
		of all ages	of them first-timers	
R. Sovering	1526	1395	897	131
M. Cheftain	508	508	78	-
B. Ajdial	1789	1647	926	142
Total	3823	3550	1901	273

A study of the genealogical structure of the herd showed that the animals imported from the Netherlands and the United States come from 35 bulls from different breeding countries. Most of the cows come from bulls from the United States (52.3%) and Canada (26.1%), in addition, the share of animals from Dutch, French, German and Italian producers is 10.5; 6.3; 3.1 and 1.6%, respectively. It is obvious that the bulls of other countries were used in Canada and America for the purpose of "refreshing the blood." All bulls of the same lines, regardless of the country of origin, belonged to the same branches.

For example, let's base the selection of the manufacturer of one planned Vis Ajdiala line. Recently, since the introduction of the complex index of lifelong productivity, Russian milk producers have begun to focus on it when selecting bulls. The higher the index, the index of lifetime productivity, the greater the interest of the manufacturer.

However, it should be understood that the data of the international assessment could not be fully consistent with Russian conditions. And since the Russian Federation is not a member of Interbull and does not participate in the MASE rating system, it is not possible to make an adjustment in the assessment for local conditions.

In this regard, it is important to assess the priority areas in the improvement of the herd and to select bulls-producers according to individual indicators of the projected transmission capacity that meet the established priorities.

Improving the productivity and quality of cow's milk is, of course, a priority for the herd. But the question of the importance of choosing a bull according to the forecast of the transfer capacity of dairy productivity can be answered only by comparing the level of basic productivity of Holstein cows in the United States and LLC "Pachelmskoe khozyaystvo" (Table 3).

Table 3. Average dairy productivity of the Holstein population in the United States and LLC "Pachelmskoe khozyaystvo" (Penza region, Russia)

Indicator	USA	LLC "Pachelmskoe khozyaystvo"	Difference, %
Milk, kg	11813	10471	11.4
Yield of milk fat, kg	431	390	9.5
Yield of milk protein, kg	355	328	7.6

As mentioned above, the index of the projected transmittance of bulls on milk yield, milk fat and milk protein shows how many daughters of the producer exceed the genetic basis (the level of the US cow population in 2015). Table 3 shows that even the initial population of Holstein cattle in the United States exceeded Pachelmskoe khozyaystvo LLC by an average of 11.4% in all indicators of dairy productivity. Therefore, whatever the bull's transfer index, it will be an improver for the cows of this farm. However, it should be borne in mind that the yield of milk fat and protein depends not only on their percentage in the milk yield, but also on the amount of milk obtained. A cow with a high yield of protein and fat can be abundant, but liquid milk. At the same time as in LLC "Pachelmskoe khozyaystvo" it is necessary to increase the quality of milk. Therefore, when choosing a bull, it is necessary to pay attention to the quality of milk of its female ancestors. It is important that they are high enough.

The problem of modern dairy cattle breeding is the reduction of the term of economic use of cows. Therefore, the second priority is to increase the longevity of animals. Therefore, it is necessary to pay attention to the indices characterizing the health of the daughters of the bull-producer.

The third priority area of work with the herd is to improve the body type of herd cows. In this regard, you should pay attention to the assessment of the exterior of the daughters, the chosen bull.

For the sample, we selected two bulls from the Vis Ajdiala Alta Revolver and Alta Briar line. A comparison of the index score of these producers shows that Briar is inferior to Revolver in some priority features, but the differences are not significant and do not affect its improving ability in relation to the cows of the herd of LLC "Pachelmskoe khozyaystvo ". In addition, the Briar bull is descended from female ancestors with a high fat and protein content in milk: the fat content and protein milk content of the bull's mother are 4.2 and 3.4%, and the bull's mother's mother is 4.1 and 3.4%, respectively. For Revolver such data are not given in the catalog. It should also be noted that Briara's estimate was based on its use in 139 herds based on 721 daughters (MD/H-721/139), so the accuracy of its improving ability (MREL) is higher than 97%. The revolver (2014 year of birth) has not yet been

evaluated for lactating daughters. The forecast of its tribal value is given on the basis of research of its genome, therefore accuracy of this forecast is lower (75%). With all the above, the lifetime profit index, which sets the price of sperm, but not relevant for the Russian Federation in Briara below. Therefore, the purchase of Briara seeds for the economy is more profitable.

CONCLUSIONS

On the basis of research conducted on the basis of LLC "Pachelmskoe khozyaystvo" we can draw the following conclusion that when selecting producers for the herd is not rational to justify the choice of a high index of lifetime productivity. It is necessary to assess the parameters of the herd, to determine the priority areas for its improvement and to choose a producer according to the indicators of individual characteristics of its transmitting ability, corresponding to the priorities in the direction of selection of a particular farm.

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QUANTITATIVE AND SIMULTANEOUS GAS CHROMATOGRAPHIC DETERMINATION OF VARIOUS FORMS OF LONG-CHAIN FATTY ACIDS IN BIOLOGICAL MATERIAL

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Abstract

The literature describes non-quantitative gas chromatographic methods for determining the relative content of various forms of long chain fatty acids in the biological material under study. Our task was to improve the quantitative and simultaneous gas chromatographic method for determining the absolute content of various forms of long-chain fatty acids in the biological material under study (plant and animal tissues and liquids). To do this, the studied biological material, a simple and complex internal standard is treated with various extracting mixtures. Various forms of long chain fatty acids are isolated from extracted lipids and their methyl esters are chromatographed. The results of gas chromatographic studies are calibrated. The improved method for low error allows quantitatively and simultaneously in absolute units to determine the content of total long chain fatty acids, long chain fatty acids of common lipids, esterified, non-esterified and anionic long chain fatty acids in the studied biological material. Thus, using the improved gas-chromatographic method at a lower cost of reagents and time, quantitatively and simultaneously in the biological material under study in absolute units we determine the content of various forms of long chain fatty acids.

Key words*: accurate, biological material, definitions, gas chromatographic method, long chain fatty acids.*

INTRODUCTION

The literature describes gas chromatographic methods for determining the content of various forms of long-chain fatty acids in the biological material under study: total fatty acids (Brian & Gardner, 1991; Rivis & Danylic, 1995; Rivis et al., 1996a; Chen & Chuang, 2002), fatty acids of general lipids (Panagiotopoulou & Tsimidou, 2002), esterified (Ostermann et al., 2014; Liguon et al., 2015; Miwa, 2002; Weatherly et al., 2016), non-esterified (Miwa, 2002; Rivis & Danylic, 1997a) or anionic (Rivis et al., 1996b) fatty acids. Moreover, the existing methods are not quantitative (calibrated) (Ostermann et al., 2014; Liguon et al., 2015; Miwa, 2002) and give the results of studies in relative units (percentages) (Miwa, 2002; Rivis & Danylic, 1997b). It should be noted that in the literature there are limited data regarding quantitative and simultaneous gas chromatographic determination of the absolute content of different forms of individual long-chain fatty acids in biological material (Rivis et al., 1997a). Such gas chromatographic studies are necessary with the aim to obtain objective quantitative data and significant savings of reagents and time.

Based on the above, we have been set a task to improve the quantitative and simultaneous gas chromatographic method for determining the absolute content of total long-chain fatty acids, long-chain fatty acids of total lipids, esterified, non-esterified and anionic long-chain fatty acids in studied biological material (plant and animal tissues and liquids).

MATERIALS AND METHODS

For the analysis, 1.0-2.0 g or ml of plant tissues or liquids, 0.5-1.0 g or ml of tissues or liquids of the animal body, 0.08-1.00 g of microor-

ganisms were used. The amount of methyl ester of heptadecanoic acid (methylheptadecanoate), which is added to the sample as an internal standard, is (% of sample taken): to the liver of various species of animals, birds and fish - 0.39, to the mucous membrane of the wall of adult females - 0.145, to the mucous membrane of the small intestine of animals and birds - 0.066, to plasma or serum of animals, birds and fish - 0.027, to cow's milk - 0.645, to the rumen fluid of ruminants - 0.008, to insect tissues - 0.09, to ruminant rumen microorganisms - 0.116, to feces of livestock and poultry - 0.065 to natural fodder of ponds (zoobenthos, zooplankton, phytoplankton or higher aquatic vegetation) - 0.0095 to cereals and legumes or grains - 0.028.

Methylheptadecanoate is prepared as follows. The amount of heptadecanoate required for the tests introduced into a test tube (250 × 6 mm) with a ground. Then to the content of the test tube pour 8-10 drops of absolute methyl alcohol. Subsequently, 4-5 drops of acetyl chloride are poured into it. The latter is poured very carefully until a distinctive sound is heard from the test tubes. The tube is then quickly closed with a ground glass stopper and placed in a converted thermostat. In the latter, the upper, larger part of the test tube is cooled by flowing water from the water supply system for 1.5 hours and the lower, smaller part is in a water bath (65 C). After methylation is completed, the methanol from the test tube is evaporated in a vacuum cabinet at 45-50ºC. The obtained methylheptadecanoate is dissolved in a certain amount of hexane and used for research.

The amount of unesterified form of heptadecanoate, which is also added to the test sample as an internal standard, is (% of sample taken): 0.01 to the liver of different species of animals, birds and fish, 0.005 to the mucous membrane of the wall of mature females of animals, to the mucosa membrane of the small intestine of animals and poultry - 0.004, to plasma or serum of animals, poultry and fish - 0.003, to cow's milk - 0.005, to rumen fluid of ruminants - 0.004, to insect tissues - 0.01, to ruminant rumen microorganisms - 0.004, to feces of animals and birds - 0.005, to natural forage ponds (zoobenthos, zooplankton, phytoplankton or higher aquatic vegetation) - 0.0005, to cereals and legumes or cereals - 0.002.

The absolute content of total long-chain fatty acids, long-chain fatty acids of total lipids, esterified, non-esterified, and anionic long-chain fatty acids in the tissues of the honey bee (*Apis mellifera*) was determined quantitatively and simultaneously in three replicates. For this purpose, in the first 30 ml by volume, a vial of colored glass is placed 1,0 g of tissues of the abdomen and a simple internal standard (1.0 mg of heptadecanoate), and in the second - 1.0 g of tissues and a complex internal standard (mixture 0.95 mg methylheptadecanoate with 0.05 mg heptadecanoate). Next, 20 ml of chloroform-methanol-hydrochloric acid extraction mixture (200:100:1 by volume) poured into the contents of the first vial and 20 ml of chloroform-methanol extraction mixture (2:1 by volume) is added to the second vial. The vials are closed with plastic corks and placed on a shuttle apparatus, on which they will be shaken vigorously for 2 hours. After the extraction is completed, 7 ml of distilled water are poured into both vials. Subsequently, after delamination of the content of the bottles, the top water-methanol layers are selected and discarded by a water jet pump and a glass pipette with the spout is removed, and the lower (chloroform) pipette is transferred to a paper filter with a blue filter (180 × 13 mm). Moreover, the chloroform extract from the first bottle is filtered into one tube, and from the second into two tubes (in one of them only 1/10 of the total amount of chloroform extract is filtered). The chloroform of the above tubes is evaporated in a stream of dried nitrogen in a water bath or a vacuum cabinet (37-40ºC).

Then, 4 to 5 drops of 5% alcohol (methyl) sodium metal solution are added to the tube containing the first bottle extract and to the tube containing the smaller quantity of the extract from the second bottle. Test tubes are placed in a boiling water bath. After 30 seconds from the beginning of boiling 1.5 ml of distilled water are poured to the contained test tube`s content. Boiling continues until the smell of alcohol disappears (approximately 30 minutes). After boiling, the test tubes in a tripod are cooled in running water from the water supply system.

To convert the resulting saponification sodium salts of long-chain fatty acids to the free state, 5-7 drops of glacial acetic acid are added to the content of tubes. After that, free long-chain fatty acids are extracted with hexane (2 times in 1-2 ml). The hexane solution of free long-chain fatty acids is transferred automatically with the pipette into test tubes (250 × 6 mm) with a ground.

Hexane from the above tubes is evaporated in a stream of dried nitrogen in a water bath or a vacuum cabinet (37-40°C), and 6-8 drops of absolute methyl alcohol and 2-3 drops of acetyl chloride are poured into their content. The tubes are quickly closed with a ground glass stopper and placed in a converted ultra thermostat, in which their upper, larger, part is cooled by running water from the water main, and the lower, smaller part is in a water bath (65°C) for 1.5 hours.

After the methylation is completed, the methanol from the test tubes is evaporated in a vacuum cabinet at a temperature of 45-50°C. Then, the methyl esters of the long-chain fatty acids are dissolved in test tubes in a few drops of hexane. Afterward, using a microsyringe, 1 ml of hexane methyl esters of long-chain fatty acids are alternately selected from each tube and injected into the evaporator of the gas-liquid chromatographic apparatus. The chromatograms yield fatty acid peaks according to the total amount of long-chain fatty acids (esterified, non-esterified, and anionic forms of long-chain fatty acids) and long-chain fatty acids of general lipids (esterified and non-esterified long-chain acids). The difference in the content of long-chain fatty acids between the two test tubes is formed by the anionic forms of long-chain fatty acids.

The lipids in the second tube, which had a larger amount of extract from the second vial, were dissolved in 2 ml of hexane. Next, 5-6 drops of 5% alcohol (methyl) sodium metal solution are added to the content of this tube. The tubes will then be shaken vigorously for 2 minutes. After a few minutes, it is necessary to stratify the content of the tube, using a glass pipette with a stretched spout, select the upper hexane layer and transfer it to a glass tube (50 × 5 mm) with a conical bottom. Hexane from this tube is evaporated in a stream of dried nitrogen in a water bath or a vacuum cabinet (37-40°C) to several drops. Next, using a syringe, 1 ml of the hexane solution of pure methyl esters of long-chain fatty acids from this tube is introduced into the evaporator of a gas-liquid chromatographic apparatus. The chromatogram gives peaks of long-chain forms of fatty acids that have been esterified in phospholipids, esterified cholesterol (phytosterol), triacylglycerols, diacylglycerols and monoacylglycerols.

Next, add 2 ml of hexane to the content of the tube containing the larger extract from the second vial. After that, the test tube is shaken vigorously for 2 minutes. Subsequently, the upper hexane layer is removed and discarded after separation of the tube contents, with the aid of a water jet pump and a glass pipette with an extended nose. It is repeated another three times.

After that the contents of the above tubes is dissolved in 2 ml of hexane. Then we add 5-7 drops of glacial acetic acid to the test tube. Next, the test tube is shaken vigorously for 2 minutes. Subsequently, after separation of the content of the tube, using a glass pipette with a stretched spout, the upper hexane layer is removed and transferred to a tube (250 × 6 mm) with a ground. Hexane from this tube is evaporated in a stream of dried nitrogen in a water bath or a vacuum cabinet (37-40°C). Then, 8-10 drops of absolute methyl alcohol are poured into the content of the tube with the ground. After that, 4-5 drops of acetyl chloride are poured into it. The test tube is then quickly closed with a ground glass stopper and placed in a converted thermostat. In the latter, the upper, larger portion of the test tube is cooled with running water from the water pipe for 1.5 hours, and the lower, smaller part is in a water bath (65°C). After methylation is completed, the methanol from the test tube is evaporated in a vacuum cabinet at 45-50°C. The methyl esters of the obtained long-chain fatty acids are dissolved in a few drops of hexane. Next, using a syringe, 1 ml of the hexane solution of pure methyl esters of long-chain fatty acids is introduced into the evaporatorof the gas-liquid chromatographic apparatus. On the chromatogram peaks of unesterified forms of long-chain fatty acids are obtained.

We determined among the total long-chain fatty acids, long-chain fatty acids of general

lipids, esterified, non-esterified and anionic forms of long-chain fatty acids in the tissues of the honey bee belly, quantitatively in absolute units, the contents of saturated fat with pair (caprylic − 8:0, capric − 10:0, lauric − 12:0, myristic − 14:0, palmitic − 16:0, stearic − 18:0, arachic − 20:0) and an odd (pentadecanoic − 15:0) number of carbon atoms in the chain, monounsaturated fatty acids of families ω − 7 (palmitooleic − 16:1) and ω − 9 (oleic − 18:1, eicosaenoic − 20:1) and polyunsaturated fatty acids of families ω − 3 (linolenic − 18:3, eicosapentaenoic − 20:5, docosatriene − 22:3, docosapentaenoic − 22:5, docosahexaenoic − 22:6) and ω − 6 (linoleic − 18:2, eicosadiene − 20:2, eicosatriene − 20:3, eicosatetraene-arachidone − 20:4, docosadiene − 22:2, docosatetraene − 22:4).

A gas-liquid chromatographic apparatus "Chrom-5" (Laboratorni pristroye, Praha), which has a stainless steel column with a length of 3700 mm and an internal diameter of 3 mm, was used for the study of fatty acid methyl esters. The column was filled with Chromaton-N-AW, 60-80 mesh grain, silaneised HMDS (hexamethyldisilysane), coated with polydiethylene glycoladipinate (fixed liquid phase) in an amount of 10% ("LACHEMA", Praha). The consumption of carrier gas, chemically pure and dried nitrogen (mobile phase) through the column at an inlet pressure of $1{,}5x10^5$ PA was about 65 ml/min. The combustion of the flames was provided with hydrogen (25 ml/min) and air (380 ml/min). The isothermal mode of operation of the packed column with the polar liquid phase was kept at the level of 196ºC, and of the evaporator and the detector − 245ºC. The detector was flame ionized. The record of the results of the analysis is differential. The identification of peaks on the chromatogram was also performed by the method of calculation of "Carbon atoms", well as using chemically pure, standard, hexane solutions of long-chain fatty acid methyl esters.

To obtain quantitative data, the results of gas chromatographic studies are calibrated. Moreover, the calibration of the results of gas chromatographic studies is carried out by the method of internal normalization. The norm (unit) is accepted as peak parameters of a simple (heptadecanoate) or complex (a mixture of heptadecanoate with methylheptadecanoate) internal standard. To this norm, the parameters of the peaks of the investigated fatty acids are equated.

For calibration of the results of gas chromatographic studies in test tubes (250 × 6 mm) we prepared mixtures of chemically pure heptadecanoic and investigated fatty acids in mass ratios of 1:1, 1:2, 1:4, 1:8, 8:1, 4:1 and 2:1. Next such an amount of absolute methyl alcohol is poured into these tubes which covers the fatty acids present in them. Then, acetyl chloride is poured into the content of the tubes. Immediately thereafter, the tubes are closed with ground glass stoppers and placed in a converted thermostat. In the latter, the upper, larger part of the test tubes is cooled with running water from the water tubes for 1.5 hours, and the lower, smaller one is in a water bath (65ºC). After completing of the methylation, the methanol from the tubes is evaporated in a vacuum cabinet at the temperature of 45-50ºC. Further, the methyl esters of the long-chain fatty acids under study are dissolved in a small amount of hexane. Then, using a microsyringe, 1 ml of a hexane solution of pure methyl esters of long-chain fatty acids from each tube is introduced into the evaporator of the gas-liquid chromatographic apparatus. On the chromatograms, the peaks of the long-chain fatty acids under study are obtained and their parameters are measured. Then the peak parameters of the heptadecanoic acid (internal standard) are equated with the peak parameters of the fatty acids under study. The results of the comparisons are converted to a ratio of 1:1. As a result of such calibration, numerical values are obtained, which are the correction coefficients for each fatty acid under study.

The absolute content of individual long-chain fatty acids in the tested biological material is determined by the following formula:

$$X, \text{ g/kg or l} = [(P \times K \times C) / Pst] \times 1000 / P$$

where:

X - quantitative content of the investigated long-chain fatty acid in absolute units, g/kg or l;

P - peak parameters of the investigated long-chain fatty acid, mm^2 or mm;

K - correction factor for the investigated long-chain fatty acid;

C - the amount of internal standard added (heptadecanoate), mg;
P_{st} - internal standard (heptadecanoate) peak parameters, mm^2 or mm;
1000 - conversion factor in kg or l;
P - a sample of the tested biological material, g or ml.
Based on the above formula for each chromatogram, first of all, it was determined the exact peak parameters of the studied long-chain fatty acids, mm^2 or mm:

$$P_1 = P \times K$$

Next, we determined the conversion factor in g/kg or l:

$$K_1 = 1000/P$$

Then, we calculated the amount of internal standard (heptadecanoate), which should be in kg or l of the biological material under study:

$$C_2, \text{ mg} = K_1 \times C$$

Then, we determined the conversion factor of the exact peak parameters of the studied individual long-chain fatty acids in absolute units of g/kg or l:

$$K_2 = C_2/P_{st}$$

Finally, the absolute content of individual long-chain fatty acids is calculated in the studied biological material:

$$X, \text{ g/kg or l} = K_2 \times P_1$$

The obtained digital material was processed by the method of variational statistics (Lopach et al., 2011). We calculated the arithmetic mean (M), and error of the arithmetic mean (± m). For the calculations a special computer program was used, Microsoft Exel for Windows XP.

RESULTS AND DISCUSSIONS

The results of studies of the absolute content of total long-chain fatty acids, long-chain fatty acids of total lipids, esterified, non-esterified and anionic long-chain fatty acids in the tissues of the honey bee are shown in the Table 1. The data of the Table 1 indicate that the error of the advanced gas chromatographic method of quantitative and simultaneous determination of the content of different forms of long-chain fatty acids in the studied biological material does not exceed 1%.
It should be noted that the absolute results of the research obtained by the advanced method can easily be translated into relative quantitative indicators. They can also be easily converted to molar units.

Table 1. The content of various forms of long-chain fatty acids in the tissues of the abdomen of the honey bee, g • 10^{-3}/kg wet weight, M ± m, n = 3

Long-chain fatty acids and their code	Forms of long-chain fatty acids				
	Esterified, non-esterified and anionic fatty acids in the total amount of fatty acids	Esterified and non-esterified fatty acids in the composition of total lipids	The esterified fatty acids	Non-esterified fatty acids	Anionic fatty acids
Caprylic, 8:0	67.2±0.53	64.3±0.53	58.3±0.51	6.1±0.05	3.0±0.02
Capric, 10:0	21.7±0.19	20.6±0.16	18.3±0.12	2.2±0.01	1.2±0.01
Lauric, 12:0	23.9±0.18	22.6±0.18	20.4±0.16	2.3±0.02	1.2±0.01
Myristic, 14:0	31.3±0.22	29.9±0.21	27.0±0.20	4.1±0.03	1.7±0.01
Pentadecanoic, 15:0	67.2±0.59	64.5±0.56	54.7±0.49	9.5±0.07	2.9±0.02
Palmitic, 16:0	1353.5±10.22	1293.3±9.97	1176.1±9.80	114.3±0.95	62.2±0.49
Palmitooleic, 16:1	94.0±0.77	89.3±0.69	81.8±0.68	7.8±0.06	4.4±0.04
Stearic, 18:0	1004.2±8.33	954.8±8.90	877.5±8.17	80.5±0.67	46.2±0.39
Oleic, 18:1	4026.0±32.83	3852.4±31.58	3534.1±29.92	311.2±2.76	188.6±1.12
Linoleic, 18:2	3139.2±26.38	2949.7±24.03	2705.6±22.54	250.5±2.04	184.5±1.34
Linolenic, 18:3	4217.9±36.04	4028.2±34.52	3716.4±29.78	301.4±2.57	198.7±1.55
Arachic, 20:0	125.4±1.00	118.6±0.90	109.7±0.90	9.3±0.07	6.2±0.05
Eicosaenoic, 20:1	311.3±2.19	296.4±2.16	271.5±2.14	23.8±0.19	15.5±0.11
Eicosadiene, 20:2	357.8±3.05	340.2±3.10	315.0±2.84	26.7±0.21	16.8±0.12
Eicosatriene, 20:3	197.1±1.54	179.9±1.37	165.1±1.35	14.2±0.10	17.5±0.14
Eicosatetraene-arachidone, 20:4	3370.8±26.98	3198.0±26.07	3006.3±26.02	202.1±1.78	162.5±1.27
Eicosapentaenoic, 20:5	2502.3±19.78	2395.4±18.95	2238.1±18.88	150.6±1.17	111.9±0.96

Docosadiene, 22:2	335.4±2.98	319.6±2.87	298.7±2.27	22.1±0.16	16.4±0.12
Docosatriene, 22:3	336.8±2.77	320.2±2.80	298.0±2.42	21.1±0.15	15.8±0.13
Docosatetraene, 22:4	387.3±3.21	369.4±3.12	348.8±3.05	21.2±0.16	18.8±0.15
Docosapentaenoic, 22:5	588.9±5.17	558.7±5.18	527.5±4.90	33.4±0.28	28.8±0.21
Docosahexaenoic, 22:6	668.4±6.22	641.3±5.96	601.6±5.17	37.1±0.30	30.5±0.24

CONCLUSIONS

The improved gas chromatographic method at lower costs of reagents and time allows quantitatively and simultaneously to determine the content of common long-chain fatty acids, long-chain fatty acids of common aliphatic alkaline chains acids in the studied biological material (vegetable and animal tissues and liquids) in absolute units. The error of the advanced gas chromatographic method of quantitative and simultaneous determination of different forms of long-chain fatty acids in the biological material under study does not exceed 1%.

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HISTORY GENETIC ANALYSIS OF POLWARTH SHEEP BREED

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Abstract

The research was carried out on a population of Polwarth sheep, intensely inbred belonging to the Research and Development Institute for Sheep and Goats Breeding, Palas - Constanta. Morpho-productive indices, reproduction indices, current number compared to the one acquired in 1995, inbreeding depression, intergenerational depression, inbreeding population, body weight differentiation and quantity of wool in the year of acquisition compared to 2020 were determined. From the presented data it results that the reproduction of the sheep was not affected instead in rams the body weight and the amount of wool decreased. Surprisingly, instead of shrinking and disappearing over time, the herd has increased and continues to grow, contrary to what is known to date from the effect of intense inbreeding on a breed of sheep.

Key words*: effective size, inbreeding, inbreeding depression, relationship in itself.*

INTRODUCTION

The Polwarth sheep breed is originated from Australia, around 1800 when the Lincoln breed crossed with the Merino breed. This combination was made in order to increase the weight of the Merino breed so that they could be raised more in grazing conditions.

The creation of the Polwarth breed was a success, the breed being able to live in regions with a more humid climate that favoured and maintained a vegetation on pastures for a longer time, being also imported to other parts of the world. It was imported into New Zealand in 1932 and has adapted very well to higher and colder temperature conditions in areas of hills and plains, making it an ideal choice for sheep farmers in this country.

The Polwarth sheep breed was imported from Australia and brought to Romania in November 1976. A number of 5125 heads were brought and distributed to 4 state agricultural enterprises (Oradea, Borcea, Liebling and Vetrișoaia).

In 1995, the Palas Institute purchased 50 female sheep and 10 male sheep from the Baitalu farm belonging to the Borcea I.A.S., Călărași County.

This population was reproductively isolated for a period of 25 years, during which time it has been monitored in terms of productive and reproductive performance.

The present study aimed to estimate the level of inbreeding of the breed and its effect on the morpho-productive parameters achieved.

The study looked at the evolution of the body weight of sheep and rams and young male and female sheep, wool production and reproductive rates.

Determining the level of inbreeding in any closed population is inevitable in determining its future growth. Inbreeding depression, expressed as a change in performance per inbreeding unit, is associated with decreased performance and depends on the rate of inbreeding. The process of inbreeding increases homozygosity for all present generations, leading to a decrease in their value for certain characters.

Research on the effect of inbreeding on productive and reproductive parameters in sheep has been done by Lax and Brown (1967),

Galal et al. (1981), Lamberson et al. (1982), Lamberson & Thomas (1984), Wiener et al. (1992), Ercanbrach & Knight (1991) and Van Wyk et al. (1993).

MATERIALS AND METHODS

The research was conducted on the current number of sheep (91 heads) consisting of 61 females and 30 males, reproductive isolate since 1995.

For this study, 4 random line pedigrees from 6 generations of sheep and rams (80 pedigrees) were used, according to the method presented by Draganescu & Sandu (1986).

Sheep and wool coats were weighed individually, establishing morpho-productive and reproductive indices compared to those in Australia.

Inbreeding depression, kinship, total inbreeding, inbreeding per generation, actual size (Ne), and other parameters were calculated

The generation interval was calculated in years, taking into account the date of birth of the ram, male offspring and female offspring and the date of birth of the ewe and male and female offspring's (Drăgănescu et al., 1986).

For the calculation of the effective size of the population (Ne) the formula mentioned by Draganescu (1986) was used.

$$Ne = \frac{4Nm \times Nf}{Nm + Nf}$$

where: Nm - the number of rams active in reproduction.

Nf - the number of ewes.

All data on morpho-productive indices were processed and interpreted statistically. The Fisher test was used to analyze the significance of the differences. (Snedecor, 1965)

RESULTS AND DISCUSSIONS

Table 1 shows the current Polwarth sheep herd.

It is observed that out of a total of 91 heads, 30 heads are rams and young males and 61 heads are sheep and young female.

Table 1. Current herd of Polwarth sheep (2020)

No. crt.	Category	Number of heads	Observations
1.	Rams	13	-
2.	Young Males	17	Young reproductive males and youth from this year
3.	Sheep	34	-
4.	Young Females	18	Youth from this year
		9	Young reproductive sheep
5.	Total effective	91	

Table 2. Polwarth sheep dynamics (adults + youth) from 1995 to 2020

No. crt.	Specification	Herd (heads)		Dynamics of the herd	
		Total in 1995	Total in 2020	± heads in 2020	± % in 2020
1.	Polwarth breed	60	91	+ 31	+ 52,0

Table 2 shows the dynamics of the workforce in the period 1995-2020.

It turns out that the total number of heads increased by 52% compared to 1995 (the year of acquisition).

Table 3. Polwarth body weight and wool quantity in 1995

No. crt.	Category	Body weight (kg/head)	Quantity of wool (kg/head)
		$x \pm s_x$	$x \pm s_x$
1.	Rams	73.43 ± 2.2023	7.37 ± 0.3153
2.	Sheep	42.14 ± 0.7723	4.52 ± 0.1223

Table 3 shows the body weight and the amount of physical wool in Polwarth sheep in the year of purchase.

It turns out that the rams had an average body weight -73.43 kg/head and the average amount of wool -7.37 kg/head and the sheep weighed 42.14 kg/head and 4.52 kg/head the average amount of physical wool.

Table 4. Body weight and quantity of wool in the current flock of sheep (Polwarth 2020)

No. crt.	Category	Body weight (kg/head)	Quantity of wool (kg/head)
		$x \pm s_x$	$x \pm s_x$
1.	Rams	61.36 ± 4.1429	5.70 ± 0.4493
2.	Sheep	48.84 ± 1.0171	3.82 ± 0.1344

Table 4 shows the body weight and the amount of wool in the current herd.
It is observed that the rams weighed 61.36 kg/ head and 5.70 kg wool and the sheep 48.84 kg/ head and 3.82 kg/head physical wool.

Table 5. Body weight and amount of wool in Polwarth sheep in Australia

No. crt.	Category	Body weight (kg / head)	Quantity of wool (kg / head)
1.	Rams	80 - 90	8-12
2.	Sheep	50	4-6

Table 5 shows that in Australia Polwarth rams have a body weight in the range of 80-90 kg/head and have a quantity of wool in the range of 8-12 kg/head.
Sheep have an average body weight of 50 kg/ head and 4-6 kg / head of physical wool.

Table 6. Body weight differentiation in Polwarth sheep, 1996 compared to 2020

No. crt.	Category	Body weight (kg / head)		± The difference between 2020 and 1996	
		1996	2020	kg	%
1.	Rams	73.43	61.86	- 11.57	- 18.70
2.	Sheep	42.14	48.84	+ 6.7	+ 13.72

Table 6 shows that compared to 1996, in 2020 the rams were lighter by about 17% and the sheep were heavier by about 14%.

Table 7. Differentiation of the quantity of wool for Polwarth sheep, 1996 compared to 2020

No. crt.	Category	Quantity of wool (kg / head)		± The difference between 2020 and 1996	
		1996	2020	kg	%
1.	Rams	7.37	5.70	- 1.67	- 29.30
2.	Sheep	4.52	3.82	- 0.70	- 18.32
3.	The significance of the differences				
4.	Rams	$p < 0,001$			
4.	Sheep	$p < 0,001$			

Table 7 shows that the amount of wool in rams decreased by about 29% and in sheep it decreased by about 18% in 2020 compared to 1996.

Table 8. Reproductive indices in Polwarth sheep

No. crt.	Category	Fecundity (%)**	Prolificacy (%)***	Weaned lambs (%)*
1.	Sheep	92.59	120.00	86.67
2.	Young reproductive sheep	88.89	112.50	88.89
3.	Total	91.67	118.20	87.18

*Average weaning age = 80 days; **Fertility in Australia is 93-95%; ***Sheep prolificacy in Australia = 110.0 - 120.0%

Table 9. Average weaning weight in Polwarth breed

No. crt.	Category	Average weaning weight kg/head		
		Males	Females	Total
1.	Sheep	22.16	21.64	21.90
2.	Young reproductive sheep	22.47	23.74	23.26
3.	Total	22.21	21.78	22.22

It is observed that the sheep had a fecundity of 91.67% and a prolificacy of 118.2%, values similar to those in Australia (Table 8).
At weaning at the age of 80 days, the male lambs had an average weight of 22.21 kg/head and the lambs weighed 21.78 kg/head, which indicates good growth increases (235-240 g/ head and day) and a high milk production of sheep (Table 9).

Table 10. Inbreeding depression

No. crt.	Decreased performance by a 10% increase in Fx	Body weight kg/head		The quantity of wool kg/head	
		Rams	Sheep	Rams	Sheep
1.	+ 1.4	- 1.16	+ 0.67	- 0.167	- 0.070

From the Table 10 it results that for a 10% increase of Fx, in rams the body weight decreases by approx.1.16 kg/head, in sheep the body weight increased by 0.67 kg/head.
The amount of wool decreases in rams by 167 g/ head and in sheep it decreases by 70 g/head.

Table 11. The evolution of the generation gap in the Polwarth breed

Specification	Father - son	Father - daughter	Mother - son	Mother - daughter	Total
Interval in years	3.30	5.12	3.12	4.58	4.85

Table 11 shows that the average interval was 4.85 years with variations between 3.30-5.12 years, the father-son interval, father-daughter and 3.12-5.58 years, mother-son interval, mother-daughter.

Table 12. Inbreeding of Polwarth breed

No. crt.	Specification	Medium values
1.	Relationship in itself R (%)	23.70
2.	Total inbreeding F (%)	67.50
3.	Non-current inbreeding (%)	64.40
4.	Current inbreeding (incest) (%)	3.13
5.	Average inbreeding per generation ΔF (%)	14.00
6.	Possible inbreeding (%)	13.44
7.	Inbreeding of strain (%)	50.96
8.	Medium number of generations	4.85
9.	Effective size of population (N_e)	3.59
10.	Effective number of males (N_m)	0.9
11.	Best number of males	12.0

Table 12 shows that the number of animals in 2020 had a total inbreeding of 67.50%, the increase in inbreeding per generation being 14%.

Incest (current inbreeding) reached 3.13%. The inbreeding by ΔF generations was 14%, and the line inbreeding was 50.96%.

CONCLUSIONS

From the presented data it results that the reproduction of the sheep was not affected instead in rams the body weight and the amount of wool decreased by approx. 19% and 29.3% respectively and in sheep the body weight increased by approx. 14% and the amount of wool decreased by about 18%.

Surprisingly, instead of shrinking and disappearing over time, the herd has increased by 52% and continues to grow, contrary to what is known to date from the effect of intense inbreeding on a breed of sheep.

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PEROXIDE PROCESSES AND BIOSYNTHESIS OF CHOLESTEROL DERIVATIVES IN RABBIT TISSUES AT ACUTE L-ARGININE-INDUCED PANCREATITIS AND ITS CORRECTION

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Abstract

The positive corrective effect of fed flaxseed oil on the condition of the pancreas in acute L-arginine-induced pancreatitis was shown, the development of which was assessed by the number of necrotized acinar epitheliocytes in the head and tail of the pancreas and the activity of lipase and α-amylase in blood plasma. Feeding sunflower oil does not show a similar corrective effect. The normalizing effect of fed flaxseed oil on the state of the antioxidant defense system in rabbits in acute L-arginine-induced pancreatitis, on the content of thiobarbituric acid-positive products and the activity of superoxide dismutase, catalase and glutathione peroxidases in blood. Feeding sunflower oil leads to a deterioration of the oxidative-prooxidant balance. The ability of fed flaxseed oil to prevent disorders of content of non-esterified and esterified cholesterol in blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis has been established. Feeding sunflower oil under the above conditions impairs the lipid composition of rabbit tissues. The positive effect of fed flaxseed oil on the ratio of anti-inflammatory polyunsaturated fatty acids of the ω-3 family to proinflammatory polyunsaturated fatty acids of the ω-6 family in the fatty acid spectrum of esterified cholesterol in blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis. The result of the fed sunflower oil action on the studied indicators is quite the opposite. In acute L-arginine-induced pancreatitis, feeding flaxseed oil stimulates the conversion of cholesterol to bile acids, 25-OH-vitamin D_3, testosterone, aldosterone and cortisol in rabbits. The stimulating ability of fed sunflower oil in this regard is less pronounced.

Key words*: acute arginine pancreatitis, correction, derivatives of cholesterol, fatty acids, lipids, rabbits.*

INTRODUCTION

Factors protecting the pancreas of humans and animals from their own digestion include: 1) synthesis of proteolytic and lipolytic enzymes in an inactive state, their isolation from the cytosol of cells in zymogenic granules during maturation (Eydoux, 2006); 2) stable connection of enzymes and inhibitors, which keeps them inactive; 3) the specificity of the action of active lipases only in relation to triacylglycerols in the emulsified state, which are not present in acinar cells (Human & Jain, 2001); 4) protection of acinar cells from the reflux of pancreatic juice, the possibility of its exit into the interstitial space and lymphatic capillaries (Chen et al., 1999); 5) the presence in the blood nonspecific factors of proteolytic enzymes inactivation – α_2-macroglobulin and α_1-antitrypsin (Morgado et al., 2005). The effects of pro-inflammatory cytokines such as IL-1, IL-6, IL-8, TNF-α in humans and animals are inhibited by such "anticytokines" as IL-1 receptor antagonists or soluble receptors (soluble TNF-receptor-55, soluble TNF-receptor-75) (Bozza et al., 2011; Tverdokhlib et al., 2011). Together with some anti-inflammatory cytokines (IL-4, IL-10), these endogenous anticytokines form the basis for a fragile balance between pro-inflammatory and anti-inflammatory mediators (Ansell & Hawthorne, 2000; Puppo et al., 2001). It should be noted that proinflammatory eicosanoids (certain groups of prostaglandins) in humans and animals are synthesized from

polyunsaturated fatty acids of the family ω-6 (linoleic, eicosatrienic, eicosatetraenoic-arachidonic, docosatetraenoic) (Rivis et al., 1997; Jorristma et al., 2001). Therefore, the above mentioned polyunsaturated fatty acids are called pro-inflammatory. Polyunsaturated fatty acids of the family ω-3 (linolenic, eicosapentaenoic, docosatrienic, docosapentaenoic, docosahexaenoic) in humans and animals are precursors of anti-inflammatory eicosanoids (Ang et al., 2006; Ang et al., 2009). In addition, polyunsaturated fatty acids of the family ω-6 (linoleic, eicosatrienic, eicosatetraenoic-arachidonic, docosatetraenoic) stimulate the synthesis of proinflammatory cytokines, and polyunsaturated fatty acids of the family ω-3 (linolenic, eicosapentaenoic, docosacotrienic, docosapentaenoic, docosahexaenoic) stimulate the synthesis of anti-inflammatory ones (Curley, 1996). The pathogenesis of acute pancreatitis in humans and animals is based on damage to the pancreas by its own enzymes and the development of systemic inflammatory response syndrome (Joshi et al., 2005; Wang & Chan, 2015). Acute pancreatitis in humans and animals develops on the background of gallstone disease, chronic alcohol poisoning (Makhija and Kingsnorth, 2002), traumatic and burn injuries (Chypre et al., 2012), surgery in the bio-pancreato-duodenal area (Schmidt et al., 1992), the use of various drugs and poisons (Zanotti et al., 2015), infectious and parasitic diseases (Rasilainen et al., 2002), tumor obstructions and atherosclerotic lesions of the vascular system (Rollins et al., 2006). In order to study the pathogenetic and metabolic aspects of acute pancreatitis, various methods of its experimental reproduction using small laboratory animals have been proposed. These include direct administration of calcium chloride solution to pancreatic tissue (Drogomyretska et al., 2010), intraperitoneal administration of L-arginine solution (Posokhova & Bukovska, 2002; D**e Roos** et al., 2009), injection of ethyl alcohol into the common bile duct (Drogomyretska, 2010), temporary ligation of the bile and pancreatic ducts (Jayaraman et al., 2011) and so on. Currently, there are two versions of the mechanism of acute pancreatitis with intraperitoneal administration of L-arginine. One version indicates that due to its strong activation of oxide-nitrate synthase and the formation of nitrogen oxide, which, in excessive formation, with superoxide anion-radical produces peroxynitrite, which damages the lipids of cell membranes (Vlasov et al., 2011; Akon & Min, 2013). In another version, L-arginine promotes the overproduction by the pancreas of enzymes that digest the gland itself (Yin, 2013). Regardless of the etiology, acute pancreatitis in humans and animals manifests itself in two forms: edematous and destructive (Shchipunov, 1996). In order to study certain aspects of acute pancreatitis use small laboratory animals with edematous form (Pereyaslov et al., 2000). In laboratory animals with edematous form of acute pancreatitis, the activity of lipolytic blood enzymes increases (Sweiry & Mann, 1996). At the same time in their blood the level of reactive forms of Oxygen, primary and secondary products of lipid peroxidation, sharply increases (Datsenko et al., 2000; Guicciardi, 2005). The above is observed on the background of a decrease in the concentration of fat-soluble vitamins, which are involved in the non-enzymatic chain of antioxidant protection (Donaldson, 1979). At the same time in the blood of laboratory animals with edematous form of acute pancreatitis decreases the content of trace elements (Zinc, Manganese, Selenium), which activate the enzymatic link of antioxidant protection, primarily sureroxide dismutase and glutathione peroxidase (Donaldson, 1979; Neoptolemus & Bhuani, 2006). On this background, the activity of lipase and the content of non-esterified cholesterol in their blood increases (Makhija & Kingsnorth, 2002). Changes in the activity of individual enzymes and the content of lipids in the blood of laboratory animals with edematous form of acute pancreatitis are accompanied by changes in the concentration of lipids and fatty acids in the tissues of the whole organism (Pereyaslov, 2001). However, in the absence of sound research methods, changes in the content of lipids and fatty acids in the tissues of laboratory animals with edematous form of the acute pancreatitis are insufficiently studied. They are fragmentary in nature.

The purpose of the research was to investigate the effect of flaxseed oil to prevent pathological changes in the pancreas, disorders of oxidative-prooxidant balance and composition of lipids

and fatty acids and stimulate the conversion of cholesterol into appropriate derivatives in the tissues of rabbits with acute L-arginine-induced pancreatitis.

MATERIALS AND METHODS

The experiments were conducted in the vivarium of Danylo Halytsky Lviv National Medical University on male rabbits of the Gray Giant breed weighing 3.8-4.0 kg. Animals were divided into four groups (5 rabbits each): I - control (K); II - animals with experimental L-arginine-induced acute pancreatitis (P); III - animals with experimental L-arginine-induced acute pancreatitis, which was fed flaxseed oil (P+flaxseed oil); IV - animals with experimental L-arginine-induced acute pancreatitis, which was fed sunflower oil (P+sunflower oil).

Rabbits of all groups received standard granulated feed in the amount of 225 g/head/day for one month and without restrictions drinking water. However, during this period, rabbits of group P+flaxseed oil received daily compound feed with flaxseed oil (manufacturer "Elit-Pharm", Dnipro city, Ukraine), while rabbits of group P+sunflower oil received daily compound feed with sunflower oil (manufacturer "MACHNO", Dnipro city, Ukraine) at the rate of 1 ml/kg of body weight. In addition, 5 days before the end of the experiment, rabbits of group K were administered once intraperitoneally 2 ml/kg of body weight of saline solution Sodium chloride, and rabbits of groups P, P+flaxseed oil and P+sunflower oil (in the same amount of saline solution) - L-arginine at a dose of 4 g/kg of body weight. At the end of the experiment, the experimental rabbits after sampling blood from the ear vein under ether anesthesia were killed by decapitation. Blood, pancreas, liver and skeletal muscle samples were used as test material.

All animal interventions and slaughter were carried out in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Strasbourg, 1985) and the decision of the First National Congress on Bioethics (Kyiv, 2001).

Histological examinations of the pancreas were performed following Tverdokhlib et al. (2011). The number of necrotized acinar epitheliocytes in the head and tail of the pancreas was assessed. Lipase activity (KE 3.1.1.3) and α-amylase (KE 3.2.1.1) were determined in blood plasma. Lipase activity was determined by the chemical method described by Vlizlo et al. (2012) and α-amylase activity – using a standard set of reagents ("α-Amylase", "Filisit-Diagnostic", Ukraine).

According to the methods described by Vlizlo et al. (2012), the content of lipid peroxidation products, namely diene conjugates, lipid hydroperoxides and thiobarbituric acid-positive products, was determined in the blood plasma, liver and skeletal muscles of rabbits. In addition, in erythrocytes, liver and skeletal muscle of rabbits we determined the activity of the main enzymes of antioxidant protection – superoxide dismutase (KE 1.15.1.1), catalase (KE 1.11.1.6) and glutathione peroxidase (KE 1.11.1.9). The protein concentration in the test material was determined by Lowry.

The concentration of non-esterified and esterified cholesterol was determined in the blood plasma, liver and skeletal muscles according to the methods of Rivis et al. (2017). The fatty acid composition of the latter was determined by extraction of total lipids, their chromatography in a thin layer of silica gel, development of plates in Iodine vapor, isolation of the esterified cholesterol fraction and preparation from it fatty acid methyl esters by transesterification. It was determined total content of saturated fatty acids with pair (caprylic - 8:0, capric - 10:0, lauric - 12:0, myristic - 14:0, palmitic - 16:0, stearic - 18:0, arachinoic - 20:0) and the odd (pentadecanic - 15:0) number of Carbon atoms in the chain. It was determined also monounsaturated fatty acids of the families ω-7 (palmitoleic - 16:1) and ω-9 (oleic - 18:1 and eicosaenoic-20:1) and polyunsaturated fatty acids of the families ω-3 (linolenic - 18:3, eicosapentaenoic - 20:5, docosatrienic - 22:3, docosapentaenoic - 22:5 and docosahexaenoic - 22:6) and ω-6 (linoleic - 18:2, eicosatrienic - 20:3, eicosatetraenoic-arachidonic - 20:4, docosadienic - 22:2 and docosatetraenoic - 22:4). The ratio of polyunsaturated fatty acids of the ω-3 family to polyunsaturated fatty acids of the ω-6 family was determined also.

Obtained by the above method, methyl esters of fatty acids were introduced into the evaporator

of the gas-liquid chromatographic apparatus[41]. For studies of methyl esters of fatty acids we used gas-liquid chromatographic apparatus "Chrom-5" (Laboratorni pristroye, Praha) with stainless steel column 3700 mm in length and internal diameter of 3 mm. The column was filled with Chromaton-N-AW, 60-80 mesh, silanized HMDS (hexamethyldisilizan), coated with polydiethylene glycol adipinate (fixed liquid phase) in the amount of 10%. The consumption of carrier gas, chemically pure and dried Nitrogen (mobile phase) through the column at an inlet pressure of 1.5 x 10^5 Pa was about 65 ml/min. Burning flame was provided with hydrogen (25 ml/min) and air (380 ml/min). The isothermal mode of operation of the filled column with a polar liquid phase was kept at the 196^0C, evaporator and detector - 245^0C. Detector was of flame-ionizing type as the most sensitive. Recording of analysis results – differential. The column efficiency determined by McNair and Bonelli for the conventional mean peak on the chromatogram, palmitic acid methyl ester, was 1917 ± 110 theoretical plates. Peak identification on the chromatogram was performed by the method of "Carbon numbers" calculation as well as using chemically pure, standard, hexane solutions of fatty acid methyl esters.

The concentration of bile acids in the serum of animals was determined by fluorometric method after their separation by chromatography on paper according to Gromashevskaya et al. (1971). The content of vitamins (25-OH-vitamin D_3), androgens (testosterone) and corticosteroles (aldosterone and cortisol) in blood plasma was determined by enzyme-linked immunosorbent (solid phase) method (Vlizlo et al., 2012). Moreover, the content of 25-OH-vitamin D_3 was determined using the test system "Immunodiagnostic", and hormones content - with "DRG" reagents (Germany).

Obtained digital material was processed by the method of variation statistics using Student's criterion (Lopach et al., 2001). The arithmetic mean values (M), the arithmetic mean error (± m) and the probability of differences between the investigated arithmetic mean values (P) were calculated. Changes were considered probable at $P<0.05$. A special computer program Microsoft Exel for Windows XP was used for the calculations.

RESULTS AND DISCUSSIONS

An increase in the number of necrotized acinar epitheliocytes in the head and tail of the pancreas of rabbits with acute L-arginine-induced pancreatitis was found (Table 1). These data indicate the development of an inflammatory process in the pancreas and significant damage to its cells. This may be due to the fact that L-arginine is the main substrate of the enzyme NO-synthase and therefore increases the synthesis of Nitrogen oxide (Ang et al., 2009). The latter, in case of excessive formation, together with the superoxide anion radical, produces peroxynitrite (Posokhova & Bukovska, 2002), which in free radical oxidation reactions is able to oxidize and damage the lipid bilayer of cell membranes (Yaremchuk & Posokhova, 2011).

Feeding flaxseed oil, which contains in its composition according to our data 65.1% of anti-inflammatory linolenic acid, is able to correct the condition of the pancreas in rabbits with acute L-arginine-induced pancreatitis. In particular, the number of necrotized acinar epitheliocytes in the head and tail of the pancreas of rabbits is normalized in acute L-arginine-induced pancreatitis corrected by feeding flaxseed oil. The number of necrotized acinar epitheliocytes in the head and tail of the pancreas of rabbits increases sharply in acute L-arginine-induced pancreatitis and feeding sunflower oil, which also contains, according to our data, 61.8% of proinflammatory linoleic acid.

The activity of lipase and α-amylase in the blood plasma of rabbits increases significantly in acute L-arginine-induced pancreatitis. This is a consequence of inflammatory processes in the acinar cells of the pancreas, which activate exocrine cells that secrete a large number of hydrolytic enzymes into the blood. The latter with excessive activity are able to "digest" the tissues of the pancreas.

The activity of lipase and α-amylase in the blood plasma of rabbits in acute L-arginine-induced pancreatitis, corrected by fed flaxseed oil, is normalized. Fed sunflower oil, on the contrary, intensifies the lipase and α-amylase activity of rabbit blood plasma in acute L-arginine-induced pancreatitis.

It was found that in the blood plasma, liver and skeletal muscles of rabbits with acute L-arginine-induced pancreatitis, compared with the control, the concentration of primary and secondary products of lipid peroxidation – diene conjugate, lipid hydroperoxides and thiobarbituric acid-positive products increases significantly (Table 2). This is due to the fact that inflammatory processes in the pancreas cause oxidative stress of a systemic nature (Chuklin et al., 2011).

Feeding flaxseed oil normalizes and sunflower oil increases the concentration of primary and secondary products of lipid peroxidation in blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis. The activity of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase in rabbit red blood cells, liver and skeletal muscle in acute L-arginine-induced pancreatitis is greatly increased due to changes in the content of free radicals. In this biological material, catalase activity is significantly reduced. The results of our research are consistent with the literature (Biradar & Veeresh, 2013).

Feeding flaxseed oil normalizes and sunflower oil increases the concentration of primary and secondary products of lipid peroxidation in blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis. The activity of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase in rabbit red blood cells, liver and skeletal muscle in acute L-arginine-induced pancreatitis is greatly increased due to change in the content of free radicals. In this biological material catalase activity is significantly reduced. The results of our research are consistent with the literature (Biradar & Veeresh, 2013).

Fed flaxseed oil normalizes, and sunflower oil - increases the activity of superoxide dismutase, glutathione peroxidase and reduces catalase activity in erythrocytes, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis. Changes in superoxide dismutase, catalase, and glutathione peroxidase activity in rabbit red blood cells in acute L-arginine-induced pancreatitis and feeding of flaxseed and sunflower oils may be related to changes in the functional state of erythrocyte membranes. Apparently, the change in their activity was also influenced by the increased release of myeloid cells from the red bone marrow, which led to a change in the population composition of erythrocytes by age.

Acute L-arginine-induced pancreatitis showed an increase in esterified cholesterol in the blood plasma, liver and skeletal muscles of rabbits (Table 3). In addition, the concentration of non-esterified cholesterol increases in the blood plasma and liver of rabbits under conditions of pathology, which may be due to the inhibition of its conversion into the corresponding derivatives - bile acids, 25-OH-vitamin D_3, testosterone and corticosterols (Tsyupko, 2008).

Feeding flaxseed oil to rabbits with acute L-arginine-induced pancreatitis normalizes non-esterified and esterified cholesterol levels in the blood plasma, liver and skeletal muscle of rabbits. This reduces the level of non-esterified cholesterol in the liver and skeletal muscles of rabbits, which may be associated with its more intensive conversion into the corresponding derivatives (Neoptolemus & Bhuani, 2006).

Sunflower oil, fed to sick animals, aggravates the pathological condition and unbalances the content of non-esterified and esterified cholesterol in blood plasma, liver and skeletal muscle. In particular, the increase of non-esterified and esterified cholesterol in the blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis and feeding sunflower oil may be associated with a decrease of their esterification and conversion into appropriate derivatives.

The above data may indicate a positive effect of feeding flaxseed oil, because the use of its fatty acids normalizes the processes of lipid metabolism (Neoptolemus & Bhuani, 2006; De Roos et al., 2009; Drogomyretska et al., 2010).

It was found that in the fatty acid composition of esterified cholesterol of blood plasma, liver and skeletal muscle of rabbits with acute L-arginine-induced pancreatitis increases the relative content of saturated fatty acids with even and odd number of Carbon atoms in the chain and monounsaturated fatty acids of ω-7 and ω-9 families, but decreases - polyunsaturated fatty acids of the families ω-6 and, especially, ω-3 (Tables 4, 5 and 6). However, in esterified cholesterol of blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced

pancreatitis decreases the inclusion of more long-chained and more unsaturated derivatives of linoleic and linolenic acids.

In the fatty acid spectrum of blood plasma esterified cholesterol, liver and skeletal muscle of rabbits with acute L-arginine-induced pancreatitis corrected by fed flaxseed oil, the relative level of monounsaturated fatty acids of the ω-9 family decreases, but increases – polyunsaturated fatty acids of the ω-3 family (Tables 4, 5 and 6). The above leads to an increase in the ratio of polyunsaturated fatty acids of the family ω-3 to polyunsaturated fatty acids of the family ω-6. Along with that in the esterified cholesterol of blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis, corrected with fed flaxseed oil, increases the inclusion of more long-chained and more unsaturated derivatives of linolenic and linoleic acids.

In the fatty acid composition of esterified cholesterol in blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis and feeding sunflower oil, compared with the control, the relative content of saturated fatty acids with even amount of Carbon atoms in chain and polyunsaturated fatty acids of the ω-6 family, but decreases content of monounsaturated fatty acids of the ω-9 family and, especially, polyunsaturated fatty acids of the ω-3 family (Tables 4, 5 and 6). The above leads to a sharp decrease in the ratio of polyunsaturated fatty acids of the ω-3 family to polyunsaturated fatty acids of the ω-6 family.

Polyunsaturated fatty acids of the ω-3 family, compared with polyunsaturated fatty acids of the ω-6 family, have a more pronounced targeted effect on humans and animals through prostaglandins, thromboxanes and leukotrienes (Flaming & Kelly, 2004; Tsyupko, 2008). Polyunsaturated fatty acids of the ω-3 family in humans and animals stimulate the synthesis of such direct anti-inflammatory factors as cytokines IL-4, IL-10 (Trukhan, 2000). In addition, synthesized from them more long-chained and more unsaturated fatty acids are precursors of prostaglandins E_2, $F_{1\alpha}$, which are also anti-inflammatory factors, but already direct (Datsenko et al. 2000; De Roos et al., 2000; Wang & Chan, 2015).

At the same time, polyunsaturated fatty acids of the ω-6 family in humans and animals stimulate the synthesis of such direct proinflammatory factors as cytokines IL-1, IL-6, IL-8, TNF-α.

The ratio of anti-inflammatory polyunsaturated fatty acids of the ω-3 family to polyunsaturated fatty acids of the ω-6 family in the fatty acid spectrum of esterified cholesterol in blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis is significantly reduced. In acute L-arginine-induced pancreatitis, corrected by feeding flaxseed oil, this ratio increases significantly, and with feeding sunflower oil to sick animals - it decreases significantly. That is, fed sunflower oil significantly exacerbates the disease of animals.

Esterified cholesterol, rich in polyunsaturated fatty acids, especially of the ω-3 family, in human and animal tissues is a precursor of bile acids, corticosteroles, 25-OH-vitamin D_3, estrogens and androgens (Smolyar, 2003). Polyunsaturated fatty acids of the ω-3 family, compared to polyunsaturated fatty acids of the ω-6 family, convert cholesterol into a less crystalline compound (Flaming & Kelly, 2004). Such cholesterol in metabolic processes penetrates better through cell membranes and is much less deposited in the walls of blood vessels. In general it can be stated that in relation to humans and animals, cholesterol esterified with polyunsaturated fatty acids of the family ω-3, has less atherogenic properties (Drogomyretska et al., 2010).

It has been recorded that in rabbits with high plasma, liver and skeletal muscle content of esterified cholesterol, rich in saturated and monounsaturated fatty acids, but poor in polyunsaturated, the concentration of taurocholic, glycocholic, glycodeoxycholic, cholic and deoxycholic acids in blood serum and 25-OH vitamin D_3 in blood plasma decreases (Table 7).

Table 1. The number of necrotized acinar epitheliocytes in the head and tail of the pancreas and lipase/α-amylase activity of rabbit blood plasma in acute L-arginine-induced pancreatitis + feeding flaxseed and sunflower oils (M ± m, n=5)

Material and indicators	Groups of rabbits			
	К	Р	P+flaxseed oil	P+sunflower oil
	The number of necrotized acinar epitheliocytes in the pancreas,%			
Head	5.2±0.2	24.1±1.1*	5.1±0.1	26.4±1.1*
Tail	1.6±0.1	14.5±1.3*	1.8±0.2	16.0±1.3*
	Activity of lipase (units/l) and α-amylase (Megaunits/l) in blood plasma			
Lipase	5.9±0.3	13.5±0.4*	6.0±0.4	15.7±0.5*
α-Amylase	73.8±1.6	120.5±2.9*	71.8±1.8	131.4±2.7*

Note: hereinafter * – the difference is probable in comparison with group K.

Table 2.The content of lipid peroxidation products and the activity of the main enzymes of antioxidant protection in the blood, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis and its correction (M ± m, n=5)

Researched indicators and units of measurement	Rabbits' groups			
	К	Р	P+flax-seed oil	P+sunflo-wer oil
	Blood plasma			
Diene conjugates. μmol/l	4.3±0.1	8.4±0.2*	4.5±0.1	9.3±0.2*
Lipid hydroperoxides. units E480/ml	1.3±0.1	4.9±0.2*	1.5±0.1	5.5±0.2*
Thiobarbituric acid-positive products. nmol/ml	3.5±0.1	5.8±0.2*	3.7±0.1	6.5±0.2*
	Liver			
Diene conjugates. μmol/l	88.2±1.6	135.0±4.2*	90.3±1.2	140.7±3.1*
Lipid hydroperoxides. units E480/ml	1.3±0.1	3.5±0.1*	1.4±0.1	4.1±0.1*
Thiobarbituric acid-positive products. nmol/ml	4.9±0.3	9.8±0.4*	5.2±0.3	10.6±0.5*
	Skeletal muscles			
Diene conjugates. μmol/l	86.4±1.5	131.3±2.0*	88.3±1.5	141.0±2.9*
Lipid hydroperoxides. units E480/ml	1.5±0.1	3.7±0.2*	1.6±0.1	4.2±0.2*
Thiobarbituric acid-positive products. nmol/ml	3.5±0.1	7.0±0.3*	3.8±0.1	7.8±0.3*
	Erythrocytes			
Superoxide dismutase. conventional units/mg protein	1.2±0.1	3.3±0.1*	1.2±0.1	3.4±0.1*
Glutathione peroxidase. mmol GSH/min mg protein	39.6±0.1	42.7±0.4*	39.7±0.1	43.4±0.2*
Catalase. mmol H_2O_2/min mg protein	4.3±0.1	3.7±0.1*	4.2±0.1	3.6±0.1*
	Liver			
Superoxide dismutase. conventional units/mg protein	22.3±0.3	29.6±0.3*	22.9±0.2	31.7±0.4*
Glutathione peroxidase. mmol GSH/min mg protein	3.3±0.1	4.6±0.1*	3.4±0.1	4.8±0.1***
Catalase. mmol H_2O_2/min mg protein	7.4±0.3	4.6±0.2*	7.0±0.3	4.2±0.2*
	Skeletal muscles			
Superoxide dismutase. conventional units/mg protein	19.5±0.4	23.9±0.4*	20.1±0.4	25.3±0.5*
Glutathione peroxidase. mmol GSH/min mg protein	5.8±0.1	8.6±0.1*	6.0±0.1	9.0±0.1*
Catalase. mmol H_2O_2/min mg protein	1.6±0.1	0.9±0.1*	1.5±0.1	0.8±0.1*

Table 3. The content of non-esterified and esterified cholesterol in blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis by feeding flaxseed and sunflower oils (M ± m, n=5)

Cholesterol classes	Groups of rabbits			
	К	Р	P+flaxseed oil	P+sunflower oil
	Blood plasma. g/l			
Unesterified cholesterol	0.20±0.01	0.25±0.01*	0.18±0.01	0.27±0.01*
Esterified cholesterol	0.83±0.06	1.01±0.01*	0.79±0.06	1.05±0.01*
	Liver. g/kg of raw weight			
Unesterified cholesterol	2.73±0.03	3.06±0.09*	2.60±0.01*	3.20±0.06*
Esterified cholesterol	5.95±0.17	6.46±0.03*	5.64±0.15	7.30±0.26*
	Skeletal muscle. g/kg raw weight			
Unesterified cholesterol	0.88±0.03	0.94±0.03	0.74±0.01*	1.03±0.04*
Esterified cholesterol	2.79±0.05	3.04±0.04*	2.68±0.05	3.13±0.06*

Table 4. Fatty acid spectrum of esterified cholesterol in blood plasma of rabbits in acute L-arginine-induced pancreatitis + flaxseed/sunflower oils feeding, % (M ± m, n=5)

Fatty acids and their code	Rabbits' groups			
	К	Р	Р+flaxseed oil	Р+sunflower oil
Caprylic, 8:0	0.16±0.01	0.21±0.01*	0.17±0.01	0.23±0.01*
Caprynic, 10:0	0.22±0.01	0.28±0.01*	0.24±0.01	0.30±0.01*
Lauric, 12:0	0.30±0.01	0.31±0.01	0.32±0.01	0.35±0.01*
Myristic, 14:0	0.49±0.01	0.60±0.02*	0.52±0.01	0.62±0.02*
Pentadecanic, 15:0	0.30±0.01	0.36±0.01*	0.32±0.01	0.30±0.01
Palmitic, 16:0	7.45±0.12	8.28±0.16*	7.62±0.11	8.52±0.19*
Palmitoleic, 16:1	0.96±0.02	1.07±0.03*	1.00±0.03	1.08±0.06
Stearinic, 18:0	10.54±0.30	11.73±0.06*	9.99±0.39	13.44±1.08*
Oleic, 18:1	36.65±0.63	38.74±0.79*	34.26±0.85*	32.31±0.24*
Linoleic, 18:2	12.37±0.38	11.16±0.10*	12.03±0.40	13.21±0.15*
Linolenic, 18:3	5.44±0.10	4.94±0.06*	5.93±0.06*	4.80±0.12*
Arachinic, 20:0	0.35±0.01	0.44±0.01*	0.37±0.01	0.50±0.02*
Eicosaenoic, 20:1	0.21±0.01	0.23±0.01	0.19±0.01	0.24±0.01*
Eicosadienoic, 20:2	0.30±0.01	0.25±0.01*	0.33±0.01	0.33±0.01*
Eicosatrienoic, 20:3	1.74±0.04	1.52±0.03*	1.80±0.04	1.90±0.02*
Eicosatetraenoic (arachidonic), 20:4	5.47±0.13	5.00±0.04*	5.60±0.13	5.79±0.05*
Eicosapentaenoic, 20:5	1.53±0.09	1.22±0.03*	1.94±0.05*	1.33±0.02*
Docosadienoic, 22:2	0.98±0.02	0.86±0.02*	1.02±0.02	1.08±0.02*
Docosatrienoic, 22:3	1.15±0.05	0.93±0.02*	1.36±0.03*	0.86±0.02*
Docosatetraenoic, 22:4	2.85±0.07	2.47±0.04*	3.00±0.06	3.09±0.08*
Docosapentaenoic, 22:5	4.71±0.11	4.23±0.05*	5.54±0.16*	4.28±0.08*
Docosahexaenoic, 22:6	5.82±0.14	5.18±0.07*	6.44±0.06*	5.43±0.15*
Total content of fatty acids	100.00	100.00	100.00	100.00
Including saturated	19.81	22.20	19.56	24.27
monounsaturated	37.82	40.64	35.46	33.63
polyunsaturated	42.37	37.76	44.98	42.10
ω-3/ω-6	0.79	0.78	0.89	0.66

Table 5. Fatty acid composition of esterified cholesterol of the rabbits' liver in acute L-arginine-induced pancreatitis + feeding of flaxseed/sunflower oils, % (M ± m, n=5)

Fatty acids and their code	Rabbits' groups			
	К	Р	Р+flaxseed oil	Р+sunflower oil
Caprylic, 8:0	0.16±0.01	0.22±0.01*	0.18±0.01	0.24±0.01*
Caprynic, 10:0	0.20±0.01	0.25±0.01*	0.22±0.01*	0.27±0.01*
Lauric, 12:0	0.29±0.01	0.34±0.01*	0.32±0.01*	0.36±0.01*
Myristic, 14:0	0.52±0.02	0.62±0.02*	0.55±0.02	0.65±0.02*
Pentadecanic, 15:0	0.31±0.01	0.40±0.02*	0.34±0.01	0.40±0.01*
Palmitic, 16:0	8.46±0.22	9.69±0.21*	8.79±0.22	9.78±0.21*
Palmitoleic, 16:1	0.95±0.02	1.00±0.03	0.99±0.02	1.02±0.05
Stearinic, 18:0	8.82±0.22	9.78±0.11*	8.55±0.23	10.44±0.30*
Oleic, 18:1	29.08±0.62	31.85±0.53*	25.09±0.83*	28.40±0.51
Linoleic, 18:2	14.51±0.30	13.14±0.10*	15.05±0.37	15.17±0.18*
Linolenic, 18:3	6.48±0.14	5.87±0.07*	7.14±0.09*	5.03±0.11*
Arachinic, 20:0	0.34±0.01	0.40±0.01*	0.28±0.01*	0.45±0.02*
Eicosaenoic, 20:1	0.19±0.01	0.21±0.01*	0.20±0.01	0.23±0.01*
Eicosadienoic, 20:2	0.30±0.01	0.24±0.01*	0.32±0.01	0.33±0.01*
Eicosatrienoic, 20:3	1.95±0.05	1.64±0.04*	2.04±0.05	2.14±0.06*
Eicosatetraenoic (arachidonic), 20:4	7.07±0.10	6.59±0.06*	7.34±0.10	7.38±0.10*
Eicosapentaenoic, 20:5	1.85±0.06	1.48±0.05*	2.20±0.06*	1.20±0.07*
Docosadienoic, 22:2	0.95±0.02	0.82±0.02*	0.97±0.02	1.02±0.03*
Docosatrienoic, 22:3	1.30±0.06	0.99±0.03*	1.58±0.04*	1.14±0.05*
Docosatetraenoic, 22:4	3.21±0.07	2.85±0.04*	3.43±0.07*	3.43±0.07*
Docosapentaenoic, 22:5	6.13±0.17	5.42±0.07*	6.84±0.06*	5.08±0.13*
Docosahexaenoic, 22:6	6.93±0.14	6.19±0.08*	7.57±0.08*	5.82±0.13*
Total content of fatty acids	100.00	100.00	100.00	100.00
Including saturated	19.10	21.70	19.23	22.60
monounsaturated	30.23	33.07	26.29	29.65
polyunsaturated	50.67	45.23	54.48	47.75
ω-3/ω-6	0.81	0.79	0.87	0.62

Table 6. Fatty acid composition of skeletal muscles' esterified cholesterol of rabbits in acute L-arginine-induced pancreatitis + feeding flaxseed/sunflower oils, % (M ± m, n=5)

Fatty acids and their code	Rabbits' groups			
	К	Р	Р+flaxseed oil	Р+sunflower oil
Caprylic, 8:0	0.12±0.01	0.17±0.01*	0.13±0.01	0.18±0.01*
Caprynic, 10:0	0.18±0.01	0.24±0.01*	0.20±0.01	0.26±0.01*
Lauric, 12:0	0.29±0.01	0.36±0.01*	0.31±0.01	0.38±0.01*
Myristic, 14:0	0.51±0.02	0.62±0.02*	0.54±0.02	0.65±0.02*
Pentadecanic, 15:0	0.31±0.01	0.37±0.01*	0.33±0.01	0.37±0.01*
Palmitic, 16:0	10.53±0.43	12.03±0.08*	10.86±0.47	12.82±0.36*
Palmitoleic, 16:1	1.05±0.05	1.37±0.05*	1.14±0.06	1.41±0.07*
Stearinic, 18:0	12.51±0.51	15.01±0.45*	11.91±0.42	15.79±0.51*
Oleic, 18:1	37.58±0.58	37.12±0.62	34.77±0.73*	33.34±0.62*
Linoleic, 18:2	9.01±0.15	8.32±0.09*	9.33±0.15	9.37±0.10*
Linolenic, 18:3	4.86±0.09	4.29±0.08*	5.52±0.08*	3.92±0.07*
Arachinic, 20:0	0.29±0.01	0.37±0.02*	0.27±0.01	0.40±0.02*
Eicosaenoic, 20:1	0.20±0.01	0.21±0.01	0.18±0.01	0.19±0.01
Eicosadienoic, 20:2	0.36±0.01	0.28±0.01*	0.38±0.01	0.39±0.01*
Eicosatrienoic, 20:3	1.72±0.03	1.46±0.05*	1.81±0.04	1.93±0.02*
Eicosatetraenoic (arachidonic), 20:4	4.82±0.10	4.19±0.09*	4.97±0.10	5.09±0.06*
Eicosapentaenoic, 20:5	1.22±0.03	1.01±0.04*	1.46±0.04*	0.80±0.03*
Docosadienoic, 22:2	1.08±0.04	0.90±0.02*	1.15±0.05	1.25±0.05*
Docosatrienoic, 22:3	1.13±0.03	0.95±0.02*	1.31±0.03*	0.88±0.02*
Docosatetraenoic, 22:4	2.63±0.06	2.27±0.05*	2.73±0.07	2.90±0.05*
Docosapentaenoic, 22:5	4.35±0.09	3.83±0.07*	4.87±0.06*	3.42±0.09*
Docosahexaenoic, 22:6	5.25±0.11	4.62±0.09*	5.85±0.08*	4.24±0.05*
Total content of fatty acids	100.00	100.00	100.00	100.00
Including saturated	24.74	29.17	24.54	30.87
monounsaturated	38.83	38.70	36.10	34.94
polyunsaturated	36.43	32.13	39.36	34.19
ω-3/ω-6	0.86	0.84	0.93	0.63

Table 7. The content of bile acids, 25-OH vitamin D_3, testosterone, aldosterone and cortisol in the blood of rabbits depending on the concentration and fatty acid composition of esterified cholesterol (M ± m, n=5)

The studied indicator	Rabbits' groups			
	К	Р	Р+flaxseed oil	Р+sunflower oil
Esterified cholesterol and polyunsaturated fatty acids of the families ω-3 and ω-6				
Esterified cholesterol, g/l	0.83±0.06	1.01±0.01*	0.79±0.06	1.05±0.01*
Fatty acids of ω-3 and ω-6 families, %	42.4±1.1	37.8±0.8*	45.2±1.2	40.66±1.2
including fatty acids of ω-3 family, %	18.6±0.5	16.5±0.5*	21.2±0.5*	16.78±0.4*
Cholesterol derivatives				
Taurocholic, g • 10^{-3}/l	0.42±0.03	0.33±0.01*	0.54±0.01*	0.29±0.01*
Glycocholic, g • 10^{-3}/l	0.57±0.02	0.45±0.02*	0.67±0.01*	0.40±0.01*
Glycodeoxycholic, g • 10^{-3}/l	0.23±0.01	0.16±0.01*	0.31±0.01*	0.14±0.01*
Cholic, g • 10^{-3}/l	0.20±0.01	0.14±0.01*	0.27±0.01*	0.12±0.01*
Deoxycholic, g • 10^{-3}/l	0.74±0.03	0.59±0.01*	0.89±0.02*	0.54±0.02*
25-OH vitamin D_3, g • 10^{-6}/l	3.88±0.18	3.21±0.04*	4.62±0.05*	3.05±0.07*
Testosterone, g • 10^{-6}/l	2.75±0.11	2.86±0.10	3.19±0.04*	2.52±0.05*
Aldosterone, g • 10^{-9}/l	988.3±35.7	1040.3±25.7	1172.5±21.0*	907.4±24.4*
Cortisol, g • 10^{-6}/l	40.4±1.9	43.6±2.0	51.7±2.1*	36.5±0.8*

The above table also shows that the concentration of taurocholic, glycocholic, glycodeoxycholic, cholic and deoxycholic acids in the serum, 25-OH vitamin D_3, testosterone, aldosterone and cortisol in the blood plasma of rabbits increases with normal content of rich in polyunsaturated fatty acids of the families ω-6 and, especially, ω-3 esterified cholesterol in blood plasma.

Predominant esterification of blood plasma, liver and skeletal muscle of rabbits with polyunsaturated fatty acids in acute L-arginine-induced pancreatitis, corrected by fed flaxseed oil, may indicate a decrease in its crystallinity and improvement of interstitial transport (Drogomyretska et al., 2010). In the liver, skin, adrenal glands and gonads it is converted into appropriate derivatives: bile acids, vitamin D_3, estrogens, androgens and corticosteroles (Chen et al., 1999).

CONCLUSIONS

The positive corrective effect of fed flaxseed oil on the condition of the pancreas in acute L-arginine-induced pancreatitis was shown, the development of which was assessed by the number of necrotized acinar epitheliocytes in the head and tail of the pancreas and the activity of lipase and α-amylase in blood plasma. Feeding sunflower oil does not show a similar corrective effect.

The normalizing effect of fed flaxseed oil on the state of the antioxidant defense system in rabbits in acute L-arginine-induced pancreatitis, on the content of thiobarbituric acid-positive products and the activity of superoxide dismutase, catalase and glutathione peroxidases in blood. Feeding sunflower oil leads to a deterioration of the oxidative-prooxidant balance.

The ability of fed flaxseed oil to prevent disorders of content of non-esterified and esterified cholesterol in blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis has been established. Feeding sunflower oil under the above conditions impairs the lipid composition of rabbit tissues.

The positive effect of fed flaxseed oil on the ratio of anti-inflammatory polyunsaturated fatty acids of the ω-3 family to proinflammatory polyunsaturated fatty acids of the ω-6 family in the fatty acid spectrum of esterified cholesterol in blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis. The result of the fed sunflower oil action on the studied indicators is quite the opposite.

In acute L-arginine-induced pancreatitis, feeding flaxseed oil stimulates the conversion of cholesterol to bile acids, 25-OH-vitamin D_3, testosterone, aldosterone and cortisol in rabbits. The stimulating ability of fed sunflower oil in this regard is less pronounced.

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BIOLOGICAL ASSESSMENT OF THE CONSTITUTION OF THE POLISSIAN BEEF CATTLE IN THE CONDITIONS OF THE PRECARPATHIAN REGION

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Abstract

The aim of the study was to investigate the growth of body weight, linear development, physiological and biochemical parameters of the blood of the first cows of Polissian beef breed of different constitution before and after weaning calves in the Precarpathians. To achieve this goal provides for the following tasks: to study the growth of live weight of cows of different types of constitution before and after weaning calves; to study the linear development of first-born cows of different types of constitution before weaning; to study the physiological and biochemical parameters of the blood of first-born cows of different types of constitution before and after weaning calves. In terms of body weight growth, linear development of physiological parameters of the blood of the first-born cows of the experimental group, both before weaning and after weaning calves significantly outperformed control peers, on average by 10-12%. In the Carpathian region, the breeding of Polissya meat breed should use animals with high physiological selection index, compared with analogues with low physiological selection index, it will accelerate the selection process in livestock populations and increase the production of biologically valuable beef in the region.

Key words*: body structure indices, cattle breeding, constitution, measurements of the bodies, Polissian beef cows.*

INTRODUCTION

The Polissian beef breed has been created in the Polissya area, at the main breeding farm "Zapovit" ("Testament"). There are a significant number of subsidiary farms in the Polissya region. The breed has been created by complex reproductive breeding of Chernihiv ("ChB -1"), Dnieper ("PM -1") and Znamyansk types ("ZT").
In Polissya beaf breed (Boyko, 2006; Kobylins'ka, 2012; Kosyr, 2016; Logosha, 2012; Melnyk, 2010; Speka, 2010; Speka et al., 2011; Azizov, 2011), lines Iris 559, Tonaka 662, Cascadera 530 have been created, final work is underway to approve lines Omar 814, Paketa 93, Liner 65. All line ancestors underwent a two-stage evaluation of their own productivity and the quality of their offspring and obtained the following results:
- Iris 559: for its own productivity - A-565-1030-8.6-54.0- elite record; by the quality of descendants - B-14530-1070-8.5-52.3-elite-record - 102.2;
- Stuntman 530: for its own performance - A-520-995-8.9-52.0 - elite record; by the quality of descendants - B-7-515-1008-8.5-52.3-elite-record - 101.3;
- Tonak 662: for its own productivity - A-520-935-8.9-52.0 - elite record - 102.8.
Control slaughter of the descendants of these breeders showed that in 15 months they had a slaughter yield of almost 64%, carcass yield - almost 61%, in 18 months - respectively 65-66%, 62-63%, which meet the breed standard.

The best bull' calves of the Iris 559 line at the age of 15 months had an average live weight of 495 kg, average daily gains (from 8 to 15 months) - 1196 g on average; in the line of Cascade 530 figures were 506 kg and 1121 g, respectively; Tonaka - 462 kg and 981 g.

The ancestors of the created lines - Omar 814, Package 93, Liner 85 also passed a two-stage evaluation:

- Omar 814: for its own productivity - A-545-1037-8.8-52.0-elite-record; by the quality of descendants - B-9-522-1025-8.6-52.0-elite-record - 101.6;
- Package 93: for its own performance - A-520-1015-8.6-52.0-elite-record; by the quality of the descendants of B-9-502-985-8.7-52.0 - elite record;
- Liner 65: on own productivity - A-525-1020-8.5-52.0 - elite-record; by the quality of the descendants of B-15-495-995-8.6-52.0 - elite record.

Control slaughter of Omar 814 offspring showed that they had a slaughter yield of 64% at 15 months, carcass yield of 61%, at 18 months - 66 and 62%, respectively, which corresponds to the parameters of the Polissian beef breed standard.

In terms of body structure, Polissian beef breeds are close to the Aberdeen Angus of American selection: long, broad-bodied, with a large head and short neck, deep chest, well-developed back of the torso, have relatively low limbs, light color.

Animals of Polissian beef breed are characterized by the following indicators: live weight of newborn calves - 28-34 kg, bull' calves at weaning at 8 months -260-303 kg, heifers - 240-280 kg, bull' calves at 18 months - 540-604 kg, heifers - 410-450 kg, physiologically mature bulls 1055-1150 kg, adult cows - 560 - 600 kg, first-born cows - 460-525 kg, cows of the second calf - 500-625 kg, the third calf - 550-645 kg.

Indicators of meat productivity of Polissian beef breed are as follows: growth rate of bulls on cultivation - 1064-1250 g, carcass weight of bulls at 18 months - 330-370 kg, carcass yield - 63-64%, slaughter yield - 65%, content bones in the carcass - 14-15%, meat quality - 4-5 points, fertility, ease of calving - 4.5-5.0 points, feed consumption per kilogram of live weight gain - 6.0-7.5 feed units, The yield of calves per 100 cows - 85-93 heads (Boyko, 2006).

Simultaneously with the derivation of the lines, selection work was carried out to establish families. The lines are based on the families in which the mothers of the future successors of the lines are received. Through families, some lines enrich others with heredity.

Today, the Polissian beef breed has more than 3,000 breeding stock, more than 5 lines and 18 families have been approved. Animals of this breed are bred mainly in farms of Zhytomyr, Rivne and Lviv regions. Breeding work with Polissian beef breed at this stage is carried out in the direction of accelerated genetic improvement of the population from high fertility, intensive live weight gain, good meat forms, meat quality and adaptation of animals to climatic and environmental conditions Polissya and the Precarpathian region.

The development of beef cattle breeding is important for increasing beef production in Ukraine, including the Carpathian region. A number of meat breeds are bred in the Precarpathian region: Limousine, Simmental, Polissya, Volyn, Aberdeen-Angus. All these breeds are very well adapted to the conditions of the Precarpathian region. The study of biological and zootechnical laws of these breeds is of great scientific, economic and practical value in the Precarpathian region

The assessment of beeg breeds in the Precarpathian region according to the type of constitution on the basis of physiological selection index is carried out for the first time. This process makes it possible to speed up the selection process when breeding beef breeds by an average of 4-5 years. The use in the breeding process of animals with a high physiological and breeding index, compared with low physiological and breeding index, makes it possible to increase beef production by 8-10% (Fedak, 2011). This development is also of practical importance for improving the quality of beef cattle in the Precarpathian region.

The aim of the study was to investigate the growth of body weight, linear development, physiological and biochemical parameters of the blood of the first cows of Polissian beef breed of different constitution before and after weaning calves in the Precarpathians.

To achieve this goal provides for the following tasks:

- to study the growth of live weight of cows of different types of constitution before and after weaning calves;
- to study the linear development of first-born cows of different types of constitution before weaning;
- to study the physiological and biochemical parameters of the blood of first-born cows of different types of constitution before and after weaning calves.

MATERIALS AND METHODS

The research was conducted at the "Bilak" farm in the Sambir district of the Lviv region on the first-born cows of the Polissian beef breed after the first calving before weaning and after weaning the calves. There were 10 cows in the experimental and control groups.

Materials of growth of live weight of cows, the basic measurements of sexes of a body, indices of a structure of a body and the basic biochemical indicators of blood of cows for 2-3 and 7-8 months of lactation of various type of the constitution are given. The type of constitution was performed according to the physiological-selection index developed by us. The control group included cows with a low physiological selection index (103.66 units), in the experimental group with a high physiological selection index (140.37 units) (Fedak, 2008).

Biochemical parameters of the blood of experimental first-born cows before and after weaning calves of different types of constitution were performed according to the generally accepted methods in biological practice of Vlizlo (2012).

Statistical processing of research materials was performed in Microsoft Office Excel and Microsoft Office Word.

RESULTS AND DISCUSSIONS

Live weight of cows in the experimental group (Tabel 1) was higher than in the control group by 5.6%. Thus, animals with a high physiological selection index of the type of constitution (experimental group) had higher growth energy than analogues with a low physiological selection index of the type of constitution (control group).

In terms of height at the withers (Table 2), the cows of the experimental group outperformed the control analogues by 1.3%, and in terms of height in the buttocks - -2.6%, respectively. Thus, in terms of altitude measurements, the cows of the experimental group significantly outperformed the control analogues.

In terms of breast depth, cows of the experimental group outperformed the control analogues by 10.4%. In terms of breast width, the animals of the experimental group also outperformed the control peers by 4.4%. Thus, in terms of volumetric measurements, the animals of the experimental group have rather large advantage over control analogues.

According to the oblique length of the body, the animals of the experimental group outperformed the control analogues by 2.8%.

The chest girth (behind the shoulder blades) was higher in animals of the experimental group by 1.8% compared with control counterparts.

The width in the hip joints and in the clubs was higher in animals of the experimental group by 3.5 and 3.2%.

By their lower back development, the cows of the experimental group outperformed the control analogues (vertical and horizontal half-girth of the buttocks) by 1.6 and 5.75.

The animals of the experimental group outperformed the control analogues by 2.1%.

The main body structure indices of Polissian beef breed corresponded to the breed standard (Table 3).

According to the indices of distension, pelvic, thorax, beating, overgrowth and bone of the animals of the experimental group slightly outperformed control counterparts.

According to the long-legged and thoracic indices, there is some advantage of control animals over experimental analogues.

In general, both experimental and control animals developed harmoniously, the front and back of the torso were proportional.

An important object of study of the interior of livestock is the morphological and biochemical composition of blood (Table 4), because in fact this substance plays an important role in the body and due to a number of different internal and external factors can change significantly and can predict at different stages of ontogenesis their future performance.

This report presents the biochemical parameters of the blood of the first cows of Polissian beef breed, which is bred in the Precarpathians.

The content of erythrocytes in the blood of cows of the experimental group outperformed the control peers by 6.4%, and the content of hemoglobin, respectively - 6.4%.

In terms of protein metabolism in the serum of animals of the experimental group also outperformed the control analogues.

Thus, the protein content in the serum of experimental animals outweighed the control animals by 5.3%. The activity of reamination enzymes in blood serum was also higher in cows of the experimental group compared with control analogues.

According to the content of total, residual and protein sulfhydryl groups of cows of the experimental group prevailed control by 7.4%, 1.1% and 5.8%.

The content of total, reduced and oxidized glutathione in the blood of the experimental groups was higher than in the control by 4.8%, 3.8% and 8.3%.

Redox processes in the blood of the experimental group were also higher compared to control counterparts, as evidenced by the activity of catalase in the blood. Thus, the cows of the experimental group outperformed the analogues of the control group in catalase activity by 8.1%.

Thus, in terms of body weight growth, linear development and physiological and biochemical parameters of the blood of animals of the experimental group significantly outperformed control counterparts. This indicates that in experimental animals the metabolic processes were more intense than in control analogues, as evidenced by the live weight of animals.

Table 5 presents the live weight of cows after weaning calves. It should be noted that the live weight of the experimental animals was 5.8% higher than that of control counterparts. Animals of both the control and experimental groups significantly exceeded the breed standard by 8.16 and 14.47%, respectively.

The main measurements of bodies characterize the linear development of animals. In our studies, as can be seen from the Table 6 in altitude measurements of cows of the experimental group prevailed control analogues by 2.91-3.18%. In terms of chest width and depth, individuals of the experimental group had an advantage over control analogues. It should be noted that the oblique length of the body of cows of the experimental group after weaning outperformed the control peers by 2.90%. In terms of volumetric measurements of the articles, there is also an advantage of the cows of the experimental group over the control peers.

Higher skeletal development was found in cows of the experimental group compared with control counterparts.

Thus, the linear development of the cows of the experimental group obviously outperformed analogues of the control group. A similar pattern was observed for live weight of cows.

Indices of body structure of experimental animals are shown in the Table 7.

The main indices of body structure in animals of the control and experimental groups were within the physiological norm and corresponded to the breed standard according to these indicators

The interior performance of experimental animals characterizes the biochemical components of the blood (Table 8).

According to the morphological parameters of the blood there is a probable advantage of the animals of the experimental group over the control analogues and this difference is 9.0-11.0%.

In terms of protein metabolism in the blood of experimental cows, there is also a significant advantage of animals of the experimental group over control peers.

Redox processes in the blood of cows were more intense in animals of the experimental group, compared with control counterparts and this difference was 10.0-12.0%.

Table 1. Live weight of first-born cows of Polissian beef breed before weaning calves, kg

Indicators	Groups (M ± m)		± to control
	Control (n = 10)	Experimental (n = 10)	
Live weight of cows at 2-3 months of lactation	505.11 ± 1.10	529.11 ± 1.55*	+ 24.0
Breed standard by live weight, I class	450	450	-
± to standard	+55.11	+79.11	-

Table 2. The main measurements of the bodies of the Polissian beef breed before weaning calves, cm

Measurements	Groups (M ± m)		± to control
	control (n = 10)	experimental (n = 10)	
Cows at 2-3 months of lactation			
Height at withers	125.8±0.11	129.5±0.13**	+ 3.7
Height in the lower back	132.8±0.14	136.4±0.17****	+ 3.6
Chest depth	63.4±0.20	66.3±0.21****	+ 2.7
Oblique body length	144.3±0.15	148.4±0.16****	+ 4.1
Chest width	45.0±0.13	46.9±0.14	+1.9
Width in the hip joints	49.4±0.18	51.0±0.19*	+ 1.6
Width in clubs	46.9±0.20	50.1±0.22***	+ 3.2
Chest girth behind the shoulder blades	184.5±0.40	188.9±0.45****	+4.4
Half girth of the back - vertical	162.0 ±0.99	174.8±1.05*	+12.8
Half girth of the back - horizontal	109.5±0.66	115.8±0.77*	+6.3
Wrist girth	18.7±0.11	19.3±0.14	+0.6

Table 3. Indexes of body structure of Polissian beef breed before weaning calves, %

Indexes	Groups (M ± m)		± to control
	control (n = 10)	experimental (n = 10)	
Cows at 2 - 3 months of lactation			
Leg length	49.7	50.6	+0.9
Lengthiness	113.3	114.5	+ 1.2
Pelvic-thoracic	92.8	95.1	+ 2.3
Pectoral	68.2	70.2	+ 2.0
Muscles structure	127.3	127.4	+ 0.1
Overgrowth	104.1	105.2	+ 1.1
Bones' structure	14.5	14.7	+ 0.2

Table 4. Biochemical parameters of Polissian beef breed before calving

Blood indicators	Groups (M ± m)		± to control
	control (n = 10)	experimental (n = 10)	
The number of erythrocytes in the blood, 10-12 liters	6.43 ± 0.13	6.85 ± 0.09**	+ 0.42
The content of hemoglobin in the blood, g/l	109.0 ± 1.5	118.0 ± 1.7**	+ 9.0
The content of total protein in blood serum, g/l	75.2 ± 0.8	86.9 ± 3.5**	+ 11.7
ACT activity in serum, units/l^{-3}	34.18 ± 0.44	40.82 ± 0.29***	+6.64
ALT activity in blood serum, units / l^{-3}	23.17 ± 0.50	28.11 ± 1.24***	+ 4.94
The content of total sulfhydryl groups in the blood, g^{-3} / l cysteine	611.7 ± 10.1	657.3 ± 8.8**	+ 45.6
The content of residual sulfhydryl groups in the blood, g^{-3} / l cysteine	143.4 ± 4.0	160.8 ± 4.0***	+ 17.4
The content of protein sulfhydryl groups in the blood, g^{-3} / l cysteine	6015.0 ± 290.0	6375.0 ± 110.0	+ 360.0
The content of total glutathione in the blood, g^{-3} / l	457.3 ± 4.2	480.9 ± 2.8**	+ 23.6
The content of reduced glutathione in the blood, g^{-3} / l	379.2 ± 2.6	393.7 ± 3.6**	+ 14.2
The content of oxidized glutathione in the blood, g^{-3} / l	82.4 ± 3.2	94.8 ± 1.9	+ 12.4
Catalase activity in the blood, g^{-3} H_2O_2 / liter	9.22 ± 0.13	9.78 ± 0.05*	+ 0.56

Table 5. Live weight of the first-born cows of the Polissian beef breed after weaning calves, kg

Indicators	Groups (M ± m)		± to control
	control (n = 10)	experimental (n = 10)	
Live weight of cows at 7-8 months of lactation	482.68 ± 2.41	513.11 ± 2.78***	+30.43
Breed standard by live weight, I class	450	450	-
± to standard	+32.68	+63.11	-

Table 6. The main measurements of the Polissian beef breed cows' bodies after weaning calves, cm

Measurements (cows at 7-8 months of lactation)	Groups (M ± m)		± to control
	control (n = 10)	experimental (n = 10)	
Height at withers	122.3±0.12	126.2±0.16*	+3.9
Height in the lower back	129.3±0.15	134.1±0.17**	+4.8
Chest depth	61.2±0.19	64.4±0.11*	+3.2
Oblique body length	141.2±0.16	145.5±0.17*	+4.3
Chest width	41.7 ±0.12	45.3±0.14*	+3.6
Width in the hip joints	46.3±0.19	49.4±0.14*	+3.1
Width in clubs	44.1±0.18	49.3±0.20*	+5.2
Chest girth behind the shoulder blades	180.9±0.30	186.7±0.50*	+5.8
Half girth of the back - vertical	157.9±0.80	168.0±0.99**	+10.1
Half girth of the back - horizontal	107.3±0.55	111.0±0.78*	+3.7
Wrist girth	18.0±0.09	18.9±0.13*	+0.9

Table 7. Body structure indices of Polissian beef cows after calving, %

Indexes	Groups (M ± m)		± to control
	control (n = 10)	experimental (n = 10)	
cows at 7 - 8 months of lactation			
Leg length	48.97	49.80	+0.83
Lengthiness	115.03	115.35	+0.32
Pelvic-thoracic	92.28	94.58	+2.30
Pectoral	68.12	70.40	+2.28
Muscles structure	128.13	128.32	+0.19
Overgrowth	104.51	106.15	+1.64
Bones' structure	14.72	15.01	+0.29

Table 8. Biochemical parameters of blood of Polissian beef cows after calving

Blood indicators	Groups (M ± m)		± to control
	control (n = 10)	experimental (n = 10)	
The number of erythrocytes in the blood, 10-12 liters	6.10±0.12	6.65±0.10*	+0.55
The content of hemoglobin in the blood, g/l	109.0±0.99	114.2±1.2**	+5.2
The content of total protein in blood serum, g/l	68.9±0.90	82.6±3.4*	+13.7
ACT activity in serum, units / l^{-3}	31.01±0.30	37.05±0.30*	+6.04
ALT activity in blood serum, units / l^{-3}	21.11±0.40	26.90±1.11*	+5.79
The content of total sulfhydryl groups in the blood, g^{-3} / l cysteine	592.4±9.8	629.5±7.7*	+37.1
The content of residual sulfhydryl groups in the blood, g^{-3} / l cysteine	143.5±3.5	152.1±3.5*	+8.6
The content of protein sulfhydryl groups in the blood, g^{-3} / l cysteine	5885.0±190.0	6179.0±100.0*	+294.0
The content of total glutathione in the blood, g^{-3} / l	428.3±3.8	448.7±2.8*	+20.4
The content of reduced glutathione in the blood, g^{-3} / l	348.3±2.4	369.7±3.5*	+21.2
The content of oxidized glutathione in the blood, g^{-3} / l	73.3±2.9	89.3±1.7**	+16.0
Catalase activity in the blood, g^{-3} H_2O_2 / liter	8.66±0.14	9.16±0.04*	+0.50

Thus, according to the main biochemical parameters of blood, the animals of the experimental group significantly outperformed the control analogues, which also affected the growth of live weight and linear development of individuals.

CONCLUSIONS

In terms of body weight growth, linear development of physiological parameters of the blood of the first-born cows of the experimental group, both before weaning and after weaning calves significantly outperformed control peers, on average by 10-12%.

In the Carpathian region, the breeding of Polissya meat breed should use animals with high physiological selection index, compared with analogues with low physiological selection index, it will accelerate the selection process in livestock populations and increase the production of biologically valuable beef in the region.

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GENETIC PARAMETERS ESTIMATES FOR GROWTH TRAITS OF GOATS FROM THE ARGAN GROVE OF AGADIR IN MOROCCO

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Abstract

The main objectives of this study were to estimate the heritability and genetic advance for growth traits in the Argan grove of Agadir in Morocco goat. Traits included, birth weight (BW), 10 days weight (W10), 30 days weight (W30) and at 70 days weight (W70), average daily gain from birth to 30 days (ADG1) and average daily gain from 30 days to 70 days (ADG2). The measurements concern 81 parent-offspring pair distributed in two generations G1 (maternal parent) and G2 (offspring). Genetic parameters are estimated using the parent-offspring regression method after adjusting data for significant non genetic factors. In G2, the birth weights (BW), 10 days (W10), 30 days (W30) and at 70 days (W70) are respectively 1.75 ± 0.23 kg, 2.42 ± 0.24 kg, 3.57 ± 0.27 kg and 5.54 ± 0.46 kg. The average daily gains (ADG1: 0-30 d) and (ADG2: 30-70 d) are 62.38 ± 5.38 g and 50.34 ± 10.62 g respectively. The heritability and genetic advance estimates of different growth traits under study were found moderate in magnitude. Genetic correlations between body weight traits ranged from 0.18 to 0.77, whereas phenotypic correlations ranged from 0.13 to 0.52. these estimates suggest that there is no genetic contradiction between these traits and their assigned genes which were responsible for phenotypic expression. The exploitation of this variability could be used for the selection of better performing goats kids adapted to local breeding conditions.

Key words*: arganeraie (argan forest), genetics parameters, goats, growth trais.*

INTRODUCTION

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. 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 & Tazi, 1996). Several authors have pointed out the important role of this adaptation of these local breeds to the difficult farming conditions and for the production in harch environments (Hoffmann, 2013; Alexandre & Mandonnet, 2005).

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 & 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 & El Madidi, 2020).

The main objective of this study is the analysis were to estimate the heritability and genetic advance for growth traits in the Argan grove of Agadir in Morocco goat.

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. Goat farming is extensive, and its diet is based on silvopastoral resources (forest resources), either through direct grazing or lopping, all year round, except for the duration of the grazing period. of "Agdal" for the argan tree, between July and September. During this period the animals exploit the areas occupied by *Juniperus phoenicea* [Arar] and other fodder

shrubs. During the winter, the animals graze on the neighboring paths. The argan tree is a predominant pastoral resource, the goats take in both pulp and leaves, on the ground, or during aerial grazing. The history of the goat in the argan tree is part of a long process of continuous presence in the family activities of the local populations of South-West Morocco and the goat is the only species able to take advantage of the agropastoral resources of the argan tree (Lacombe & Casabianca, 2015).

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.

Birth date, birth weight, gemellarity and sex of kid were recorded within 24 h of the new birth.

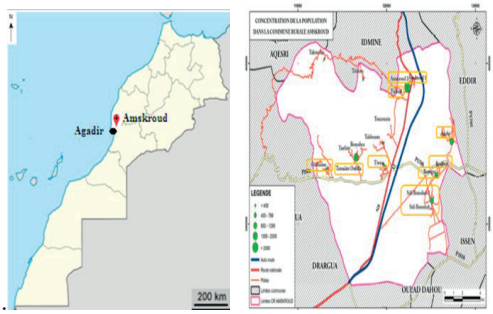

Figure 1. Map showing location of the study area (left) and location of villages sampled (right)

Kids were weighed using Brecknell 235 10S Hanging Scale having 50 kg capacity and 200 g division within 24 h after birth. The data used in present study were collected between 2018 and 2019 from a total of 81 kids (33 males and 48 females), the progeny 81 dams. The birth weights (BW), 10 days (W10), 30 days (W30) and at 70 days (W70) were recorded. Based on the weight records of individual kids, the average daily gains ADG1 (from birth to 30 days), ADG2 (from 30 to 70 days) were calculated assuming linear growth rate between the appropriate weights under the existing management conditions by recruited enumerators.

Genetic parameters are estimated using the parent-offspring regression method after adjusting data for significant non genetic factors. The major non-genetic factors included for the adjustment are age of dam, birth season, sex and type of birth.

The heritability for any measurable characteristic and production performance indicate the ability of both the parents to transmit superior productivity to their offspring. Heritability in the narrow sense is the ratio of the additive genetic variance (σ_A^2) to total phenotypic variance (σ_P^2), which is given by: $h^2 = \sigma_A^2 / \sigma_P^2$. Heritability can be calculated in a number of ways. One method uses phenotypic measures for some trait in parents and their offspring. The slope of the line relating the performance of offspring to that of their parents or midparent average gives a measure of heritability in the narrow sense

The covariance parents offspring**:**

$Cov(P,O) = 1/2\ \sigma_A^2$ and the regression coefficient of the value of the offspring (y) as a function of the value of the parent (x) is written:

$$b_{PO} = \frac{(1/2)\sigma_A^2}{\sigma_P^2} = \frac{\sigma_A^2}{2\sigma_p^2}$$

The genetic advance was calculated by:

GA = i h^2 σ_P were I = 1.76 at 10 % of selection intensity (standardized selection differential), h^2 is heritability in the narrow sense and σ_P is the phenotypic standard deviation.

The relative genetic advance (GA as % of the mean): RGA= ($\sqrt{GA}$) / m) ×100, where: *GA* = genetic advance and m = mean of the population.

Phenotypic correlations (r_p) were estimated based on variances and covariances estimates calculated from half sib family variances, parent-offspring covariances. To estimate the phenotypic correlation between two characters we compute what might be called the cross-covariance obtained from the product of the value of trait X in parents and value of trait Y in offspring or vice versa.

$$\mathbf{r_P} = \frac{Cov_{P(XY)}}{\sqrt{V_{P(X)}V_{P(Y)}}}$$

where:
$COV_{P(XY)}$ is the phenotypic covariance of X and Y traits and $V_{P(X)}$ and $V_{P(Y)}$ are the phenotypic variance value of the traits X and Y.

Genetic correlations (r_G) were estimated by regressing average offspring values of trait X on average parent values of trait Y, and vice versa, in accordance with the methods described in (Lynch & Walsh, 1998). Prior to these analyses, all traits were corrected for significant effects of age, sex, year and ringer (see above).
The calculation of r_G involves dividing the covariances between different traits X and Y (Cov_{XY}) in parents and off spring with the square-root product of the covariances between the same traits (Cov_{XX} and Cov_{YY}, respectively). Since there are two possible products of Cov_{XY} there are also two estimates of r_G (r_{G1} and r_{G2}). The data used for estimating r_{G1} and r_{G2} were balanced in the sense that there were no missing values for trait X and Y in neither parents nor offspring. Thus, the calculation of r_{G1} and r_{G2} for trait X and Y are based on the same individual samples. To estimate the standard error of r_G, we applied the procedures described in (Robertson, 1959; Falconer & Mackay, 1996).
The cross covariance is half the genetic covariance of the two characters that is ½ $COV_{A(XY)}$. In addition, the covariance of offspring and parents for each of the trait are also needed separately for the estimation of genetic correlation and the genetic correlation is given by

$$\mathbf{r_G} = \frac{(1/2)Cov_{A(XY)}}{\sqrt{(1/2)V_{A(X)}(1/2)V_{A(Y)}}} = \frac{Cov_{A(XY)}}{\sqrt{V_{A(X)}V_{A(Y)}}}$$

where:
$COV_{A(XY)}$ is the genetic covariance of the additive deviations between X and Y traits and $V_{A(X)}$ and $V_{A(Y)}$ are the additive genetic variance value of the traits X and Y.

All statistical analyzes were performed using SAS version 9.3 software (SAS, 2010).

RESULTS AND DISCUSSIONS

In G1 (maternal parent), the weight at birth, weights at 10 days, weights at 30 days and weights at 70 days varied between 1.17 to 2.68, 1.40 to 3.76, 2.30 to 5.26 and 3 to 9.12 kg, respectively. The average daily gains, ADG 1 (0-30) and ADG 2 (30-70) varied between 10.3 to 101 and 10.5 to 110.8 g respectively (Table 1).

Table 1. Descriptive statistics for the measured characters (growth traits) in G1

Parameters	n	Mean	Min	Max	SD	CV (%)
BW (Kg)	81	1.91	1.17	2.68	0.35	18.20
W10 (Kg)	76	2.47	1.40	3.76	0.53	21.32
W30(Kg)	72	3.71	2.30	5.26	0.73	19.82
W70(Kg)	69	5.60	3.00	9.12	1.38	24.64
ADG1 (g)	72	62.28	18.3	101.0	8.95	30.42
ADG2 (g)	69	47.79	10.5	110.8	14.6	51.5

ADG: Average Daily Gains, BW: birth weight, 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).

For weights at typical age, the coefficient of variation (CV) estimates ranged from 18.20% to 24.64 % whereas for the average daily gain, we observed that the coefficient of variation are higher and are equal to 30.42 and 51.50 % for ADG 1 (0-30) and ADG 2 (30-70) respectively. In G2 (offspring), the average values recorded for birth weights, weights at 10 days, weights at 30 days and weights at 70 days was 1.75 ± 0.23 kg, 2.42 ± 0.24, 3.57 ± 0.27 and 5.54 ± 0.46 kg, respectively. The average daily gains, ADG 1 (0-30) and ADG 2 (30-70) was 62.38 ± 5.34 and 50.8 ± 10.8 g respectively (Table 2).

Table 2. Descriptive statistics for the measured characters (growth traits) in G2

Parameters	Mean	Min	Max	SD	CV (%)
BW (Kg)	1.75	1.19	2.25	0.23	12.95
W10 (Kg)	2.42	1.98	2.91	0.24	10.11
W30(Kg)	3.57	2.94	4.12	0.27	7.55
W70(Kg)	5.54	4.13	6.75	0.46	8.30
ADG1 (g)	62.38	44.5	79.31	5.34	8.56
ADG2 (g)	50.34	16.1	78.21	10.6	21.09

For weights, the coefficient of variation (CV) estimates ranged from 7.55% to 12.95% whereas for the average daily gain, the coefficient of variation is equal to 8.56 and 21.1% for ADG 1 (0-30) and ADG 2 (30-70) respectively.

Heritability in the narrow sense refers to the part of the genetic variance in the phenotypic variance of a trait and it is a statistical datum allowing an estimation by genetic factors in the expression of the character (Nyquist, 1991; Falconer & Mackay, 1996). The estimation of heritability is a first step in establishing a genetic evaluation for a given trait and the knowledge of this parameter is particularly important for the objective of selection. The h^2 estimates ranged from 0.20 to 0.34 for weights and ranged from 0.20 to 0.28 for The average daily gains. The estimated values for the genetic gain for the different weights ranged from 190 to 490 g while the values recorded for the daily gain are equal to 6.61 g for ADG 1 and 12.12 g for ADG 2 (Table 3).

Table 3. Heritability and genetic advance estimates for the growth traits

	b_{op}	h^2	GA (g)	RGA %
BW	0.1549	0.31	190	124.85
W10	0.1718	0.34	320	106.26
W30	0.1714	0.34	440	90.54
W70	0.1001	0.20	490	50.50
ADG 1	0.0981	0.20	6.67	13.63
ADG 2	0.1396	0.28	12.12	14.16

b_{PO}: regression slope, h^2: heritability in the narrow sense, GA: genetic advance and RGA : relative genetic advance.

Table 4. Estimates of phenotypic (P) and genetic correlation (G) among the growth traits analyzed

	W10	**W30**	**W70**	**ADG 1**	**ADG 2**
WB	P 0.398 G 0.745	P 0.320 G 0.507	P 0.227 G 0.394	P 0.521 G 0.775	P 0.187 G 0.367
W10	- -	P 0.392 G 0.444	P 0.129 G 0.179	P 0.394 G 0.557	P 0.226 G 0.431
W30		- -	P 0.212 G 0.274	P 0.341 G 0.411	P 0.421 G 0.677
W70			- -	P 0.192 G 0.365	P 0.514 G 0.767
ADG 1				- -	P 0.412 G 0.524

P: phenotypic correlation, G: genetic correlation

These results indicated that the genetic component played a relatively important role in the expression of these traits and its significant genetic progress could be obtained by selection based on phenotypic performance. In this study, low birth weights were recorded for kids. Similar results have been observed in other local breeds (Zahraddeen, 2008; Montaldo et al., 2010; Mabrouk et al., 2010). This low weight is considered as an indicator of adaptation to harsh environments (Najari et al., 2007).

The values of the phenotypic and genetic correlations are presented in Table 4. The estimates of genetic and phenotypic correlations were moderate to high and showed no genetic antagonisms among the growth traits analyzed. The estimated phenotypic correlations were positive and were between 0.129 (W10-W70) and 0.521 (WB-ADG1). Genetic correlations varied from 0.179 and 0.775. The highest genetic correlation was recorded between BW and ADG1 and the lowest was found between W10 and W70.

The positive genetic correlations between the traits studied suggest that the genetic factors that influence these traits play an important role in the correlation between traits and vary in the same directions and suggest that there is no genetic contradiction between these traits and their assigned genes which were responsible for phenotypic expression, therefore selection for one of these traits will bring a positive response to selection for others. Similar to our estimates, moderate to high and positive genetic correlations have been reported by several authors in various breeds of goats (Al-Shorepy et al., 2002; Baneh et al., 2012; Rashidi et al., 2008).

Goat meat production is an important source of income for farmers in this region. This production is based exclusively on local breeds because of their adaptation to climatic conditions unfavorable to intensive farming. The improvement of the production requires an evaluation of the variability of the characters related to the growth of the animals and the selection of the best performing individual kids. Moderate heritability values were observed for growth traits analysed. Similar results have been reported by several authors (Rout et al., 2018; Anggraeni et al., 2020; Mohammed et al., 2018). These moderate values of genetic parameters for the growth traits gives hope for the possibility to improving the traits by selecting the best performing individuals in selection programs.

CONCLUSIONS

Genetics parameters for growth traits were moderate to high and genetics correlations between measured traits are positive. Consequently the genetic selection for local goats are possible to improve meat production. 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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FATTENING AND MEAT QUALITIES OF YOUNG PIGS OF DIFFERENT INTRABREED DIFFERENTIATIONS BY ORIGIN AND BREEDING VALUE

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Abstract

The paper presents the results of the study on fattening and meat qualities of young pigs of different intrabreed differentiation by origin and breeding value. The research was conducted on farms and processing enterprises of Dnipropetrovsk region, as well as in the Laboratory of Animal Husbandry of the State Institution "Institute of Grain Crops of NAAS of Ukraine". It was established that Large White young pigs from the controlled population at the age of achievement of live weight in 100 kg, at the fatback thickness at the level of 6-7 thoracic vertebrae and at the length of the chilled carcass exceed the minimum requirements of the elite class by 13.85% in average. Significant differences between animals of English and Hungarian origin were established in the average daily gain of live weight (2.28%) and the age of achievement of live weight in 100 kg (3.58%). Young pigs from group I (intrabreed differentiation by selection index "I") and from group II (intrabreed differentiation by Sazer-Fredin index) exceeded peers from opposite groups in the average daily gain in live weight by 7.48-2.75%; in the age of achievement of live weight in 100 kg by 3.87-3.41%. The difference between the groups in the fatback thickness at the level of 6-7 thoracic vertebrae, in the length of the chilled carcass and in the length of the bacon side of the chilled carcass varied from 0.51 to 12.16%.
The maximum increase in additional products was obtained from the sale of one head of Large White young pigs of English origin (+1.86%), animals from group I of intrabreed differentiation by selection index "I" (+3.39%) and animals from group II of intrabreed differentiation by index Sazer-Fredin (+2.11%). Based on the obtained data, it was established that the criterion for selection of highly productive animals is the value of the selection index "I" +0.181 - +3.205, Sazer-Fredin index -1.017 - -0.102.

Key words*: breed, correlation, economic efficiency, fattening and meat qualities, index, variability, young pigs.*

INTRODUCTION

It was established that the increase in pig productivity is due to such important factors as optimization of feeding and keeping of animals of different gender and age, effective use of modern gene pool and implementation of innovative technological solutions (Bankovska & Voloshchuk, 2015; Anikhovska, 2007; Voloshchuk et al., 2013; Likhach, 2015; Likhach & Likhach, 2020; Nechmilov, 2019; Khramkova, 2021; Likhach et al., 2018; Povod et al., 2021).

An important issue is to improve breeding work in the pig husbandry. It is based on a system for assessing the breeding value of breeding boars, sows and their offspring, as well as the selection of high-yielding livestock for further breeding. To assess the animals of these production groups the main provisions of the Instruction on pig determination (Instruction on pig determination…, 2003) are used, namely: "Scale for assessing young animals in live weight and body length", "Scale for assessing boars and sows in live weight and body length", "Scale for assessing young animals in

the age of 100 kg", "Scale for assessment of young animals in fat thickness", "Scale for assessing boars and sows in fattening and meat qualities of offspring" etc.

However, studies of native scientists show that the use of breeding boars and sows of foreign selection has improved the fattening and meat qualities of young pigs (Onishchenko, 2019; Krasnoshchok, 2020; Susol, 2014; Rybalko & Floka, 2014; Sheiko et al., 2013; Gryshyna & Krasnoshchok, 2018), and the consequence of this is not an objective assessment of animals according to this normative document. Therefore, it is important to find effective methods of assessing the breeding value of fattening and meat qualities of young pigs in the conditions of purebred breeding.

The aim of the work is to study the fattening and meat qualities of young pigs of different intrabreed differentiation by origin and breeding value; based on the obtained data, to determine the criteria for selection of highly productive animals taking into account these factors and calculate the economic efficiency of research results.

MATERIALS AND METHODS

The research was conducted on farms and processing enterprises of Dnipropetrovsk region and the Laboratory of Animal Husbandry of the State Institution "Institute of Grain Crops of the National Academy of Agrarian Sciences of Ukraine".

Large White young pigs, obtained from sows of native selection and breeding boars of Azuro (Hungarian selection), C 61203 Tafftus and C 57801 Kotilo (English selection), were the object of the study.

Evaluation of young pigs in fattening and meat qualities was carried out taking into account the following indicators: average daily gain in live weight during control fattening, kg; age of achievement of live weight in 100 kg, days; fatback thickness at the level of 6-7 thoracic vertebrae, mm; length of chilled carcasses, cm (Berezovskiy & Khatko, 2005).

The average daily gain in live weight (1) of young pigs and the age of achievement of live weight in 100 kg (2) were calculated by the following formulas:

$$X = \frac{T_2 - T_1}{A_2 - A_1} \times 1000 \quad (1)$$

where: X - average daily gain, g; T_1 - weight of animals at the beginning of the accounting period, kg; T_2 - weight of animals at the end of the accounting period, kg; A_1 - age of animals at the beginning of the accounting period, days; A_2 - age of animals at the end of the accounting period, days; 1000 - conversion factor in grams;

$$X = B + \frac{100 - m}{A} \quad (2)$$

where: X - age of achievement of live weight in 100 kg, days; B - the actual age of the animals on the day of the last weighing, days; m - the actual weight of animals on the day of the last weighing, kg; A - average daily gain of animals during the accounting period, kg (Berezovsky & Khatko, 2005).

Comprehensive evaluation of young pigs in fattening and meat qualities was performed according to the following mathematical models of selection indices:

$$E = \frac{1}{(G_i \times \Delta_i)} - \frac{1}{(G_ø \times \Delta_ø)} \quad (3)$$

where: I - selection index, points; $G_п$ - quadratic average deviation of the index "average daily gain of live weight, g"; $G_ш$ - quadratic average deviation of the index "fatback thickness at the level of 6-7 thoracic vertebrae, mm"; $\Delta_п$ та $\Delta_ш$ - deviation of an individual trait from the average population value (*Getia, 2009);*

$$I = \frac{1}{\sigma_g} \times \Delta G_1 - \frac{1}{\sigma_f} \times \Delta F_1 \quad (4)$$

where: I - Sazer-Fredin index, ΔG_1 – growth rate in deviations from the mean value; ΔF_1 - fatback thickness in deviations from the mean value; σ_g – phenotypic standard deviation of growth rate; σ_f – phenotypic standard deviation of the fat thickness (Kozlovsky et al., 1982). Biometric processing of the obtained data (Kovalenko et al., 2010) and calculation of economic efficiency of research results (Method of definition of economic…, 1983) were carried out according to the generally accepted methods.

RESULTS AND DISCUSSIONS

The research results show that young pigs in the controlled population are characterized by high rates of fattening and meat qualities. Thus, the average daily gain in live weight during the period of control fattening is 780.4 ± 5.91 kg (Cv = 4.91%); the age of achievement of live weight in 100 kg is 171.8 ± 1.44 days (Cv = 5.10%); fatback thickness the at the level of 6-7 thoracic vertebrae is 22.3 ± 0.41 mm (Cv = 11.36%); the length of the chilled carcass - 96.8 ± 1.62 cm (Cv = 4.10%); the length of the bacon side of the chilled carcass is 82.6 ± 5.03 cm (Cv = 14.93%). The selection index "И" ranges from -3.561 to +3.205, the Sazer-Fredin index from -1.017 to +4.738 points, respectively. The results of studies of fattening and meat qualities of Large White young pigs of English and Hungarian origin are shown in Table 1.

Table 1. Fattening and meat qualities of Large White young pigs of English and Hungarian origin

Indexes, units of measurement	Biometric indexes	Group	
		I	II
Average daily gain in live weight during the period of control fattening, g	*n*	*10*	*32*
	$X \pm Sx$	801.2±8.96	775.9±6.26
	$\sigma \pm X_\sigma$	38.89±8.700	37.59±4.698
	$Cv \pm Sc_v$, %	4.85±1.085	4.84±0.605
Age of achievement of live weight in 100 kg, days	$X \pm Sx$	173.5±1.37	178.3±0.83
	$\sigma \pm X_\sigma$	4.12±0.921	5.02±0.627
	$Cv \pm Sc_v$, %	2.37±0.530	2.81±0.351
Fatback thickness at the level of 6-7 thoracic vertebrae, mm	$X \pm Sx$	19.7±0.97	20.9±0.31
	$\sigma \pm X_\sigma$	2.91±0.651	1.91±0.238
	$Cv \pm Sc_v$, %	14.77±3.304	9.13±1.141
Length of chilled carcass, cm	*n*	*3*	21
	$X \pm Sx$	95.7±0.75	96.7±0.34
	$\sigma \pm X_\sigma$	1.50±0.641	1.67±0.257
	$Cv \pm Sc_v$, %	1.56±0.639	1.72±0.265
Length of bacon side of chilled half-carcass, cm	$X \pm Sx$	84.0±1.22	85.7±0.64
	$\sigma \pm X_\sigma$	2.44±1.000	3.08±0.475
	$Cv \pm Sc_v$, %	2.90±1.188	3.59±0.554

It was found that young pigs from group I exceeded peers from group II in terms of average daily live weight gain during the period of control fattening by 25.3 g (td = 2.32; P<0.05); age of achievement of live weight in 100 kg by 4.8 days (td = 3.01; P <0.01); fatback thickness at the level of 6-7 thoracic vertebrae by 1.2 mm (td = 1.18; P> 0.05). The difference between the groups in the length of the chilled carcass is 1.0 cm (td = 1.21; P>0.05), in the length of the bacon side of the chilled carcass is 1.7 cm (td = 1.24; P>0.05).

Analysis of the results of control fattening of young pigs of different intrabreed differentiation according to the selection index "И" showed that animals from group I exceeded peers from group II in average daily live weight gain by 60.5 g (td = 8.56; P <0.001); in age of achievement of live weight in 100 kg by 7.0 days (td = 5.26; P<0.001) (Table 2).

Table 2. Fattening and meat qualities of Large White young pigs of different intrabreed differentiation according to the selection index "*И*"

Indexes	Biometric indexes	Gradations of selection index «*И*»	
		+0.181 - +3.205	-3.561- -0.249
		group	
		I	II
Average daily gain in live weight during the period of control fattening, g	*n*	*23*	*19*
	X±*Sx*	807.8±5.55	747.3±4.38
	σ±$X_σ$	26.65±3.930	19.10±3.100
	Cv±Sc_v, %	3.29±0.485	2.55±0.413
Age of achievement of live weight in 100 kg, days	*X*±*Sx*	173.8±0.89	180.8±1.00
	σ±$X_σ$	4.31±0.635	4.40±0.714
	Cv±Sc_v, %	2.47±0.364	2.43±0.394
Fatback thickness at the level of 6-7 thoracic vertebrae, mm	*X*±*Sx*	19.5±0.41	22.2±0.32
	σ±$X_σ$	1.99±0.293	1.40±0.227
	Cv±Sc_v, %	10.20±1.504	6.36±1.032
Length of chilled carcass, cm	*n*	*16*	*10*
	X±*Sx*	96.8±0.38	96.2±0.61
	σ±$X_σ$	1.54±0.272	1.83±0.409
	Cv±Sc_v, %	1.59±0.281	1.90±0.425
Length of bacon side of chilled half-carcass, cm	*X*±*Sx*	85.6±0.61	84.8±0.86
	σ±$X_σ$	2.47±0.437	2.74±0.612
	Cv±Sc_v, %	2.88±0.509	3.23±0.722

According to fatback thickness the at the level of 6-7 thoracic vertebrae, the difference between the groups is 2.7 mm (td = 5.29; P <0.001); in the length of the chilled carcass is 0.6 cm (td = 0.84; P>0.05); in the length of the bacon side of the chilled carcass is 0.8 cm (td = 0.76; P>0.05).

Intrabreed differentiation of young pigs according to the Sazer-Fredin index shows that animals from group II exceeded peers from group I in average daily live weight gain by 22.0 g (td = 1.64; P>0.05); in age of 100 kg live weight by 6,1 days (td = 4.17; P <0.001) (Table 3).

It was found that young pigs, which Sazer-Fredin index ranges from +0.136 to + 4,738 points, are characterized by the smaller fatback thickness at the level of 6-7 thoracic vertebrae (by 1.8 mm; td = 3.52; P<0.01), the bigger length of the chilled carcass (by 0.5 cm; td = 0.71; P>0.05) and the bigger length of the bacon side of the chilled carcass (0.7 cm; td = 0.67; P>0.05).

Table 3. Fattening and meat qualities of Large White young pigs of different intrabreed differentiation according to the Sazer-Fredin index

Indexes	Biometric indexes	Graduations of the Sazer-Fredin index	
		+0.136 - +4.738	-1.017- -0.102
		group	
		I	II
Average daily gain in live weight during the period of control fattening, g	*n*	*32*	*10*
	X±*Sx*	775.2±6.70	797.2±11.57
	σ±$X_σ$	37.91±4.738	36.60±8.187
	Cv±Sc_v, %	4.89±0.611	4.59±0.560
Age of achievement of live weight in 100 kg, days	*X*±*Sx*	178.9±0.84	172.8±1.20
	σ±$X_σ$	4.77±0.596	3.80±0.850
	Cv±Sc_v, %	2.67±0.333	2.19±0.489
Fatback thickness at the level of 6-7 thoracic vertebrae, mm	*X*±*Sx*	20.4±0.41	22.2±0.32
	σ±$X_σ$	2.35±0.293	1.40±0.313
	Cv±Sc_v, %	11.52±1.440	6.30±1.409
Length of chilled carcass, cm	*n*	*20*	*6*
	X±*Sx*	96.7±0.37	96.2±0.61
	σ±$X_σ$	1.65±0.261	1.83±0.528
	Cv±Sc_v, %	1.71±0.270	1.90±0.549
Length of bacon side of chilled half-carcass, cm	*X*±*Sx*	85.5±0.58	84.8±0.86
	σ±$X_σ$	2.60±0.411	2.74±0.791
	Cv±Sc_v, %	3.04±0.481	3.23±0.933

The calculation of the economic efficiency of the research results shows that the maximum increase in additional products was obtained from Large White young pigs of English origin (+1.86%), I group of intrabreed differentiation by selection index "*И*" (+3.39%) and II group of intrabreed differentiation according to the Saser-Fredin index (+2.11%) (Table 4).

Table 4. Economic efficiency of research results

Group	Average daily gain in live weight during the period of control fattening, kg	Increase in additional products, %	The cost of additional products hryvnia / head USD / head
Total sample	780.4±5.91	-	-
intrabreed differentiation by origin			
II	777.0±6.59	-0.43	-21.30 / -0.67
I	795.2±12.95	+1.86	+89.87 / +2.80
intrabreed differentiation according to the selection index «И»			
II	747.3±4.38	-4.24	-213.01 / -6.65
I	807.8±5.55	+3.39	+163.72 / +5.11
intrabreed differentiation according to the Sazer-Fredin index			
II	797.2±11.57	+2.11	+101.31 / +3.16
I	775.2±6.70	-0.67	-33.30 / -1.04

***Note:** * - the selling price of young pigs at the time of research is 47.50 hryvnias or 1.48 US dollars per 1 kg of live weight.*

Its cost, received from the sale of 1 head, is +89.87, +163.72 and 101.31 hryvnias or +2.80, +5.11 and +3.16 US dollars, respectively.

CONCLUSIONS

1. It was found that Large White young pigs from the controlled population at the age of achievement of live weight in 100 kg, in fatback thickness at the level of 6-7 thoracic vertebrae and in the length of the chilled carcass exceed the minimum requirements of the elite class by 13.85% on average.
2. Significant differences between animals of English and Hungarian origin were established in the average daily gain of live weight (2.28%) and in the age of achievement of live weight in 100 kg (3.58%).
3. Young pigs from group I of intrabreed differentiation by selection index "И" and from group II of intrabreed differentiation by Sazer-Fredin index exceeded peers from opposite groups in average daily live weight gain by 7.48-2.75%, in the age of achievement of live weight in 100 kg by 3.87-3.41%.
4. The difference between the groups in fatback thickness at the level of 6-7 thoracic vertebrae, in the length of the chilled carcass and in the length of the bacon side of the chilled carcass varied from 0.51 to 12.16%.
5. The maximum increase in additional products was obtained from the sale of one head of Large White young pigs of English origin (+1.86%), animals from group I of intrabreed differentiation by selection index "И" (+3.39%) and animals from group II of intrabreed differentiation by index Sazer-Fredin (+ 2.11%). Based on the obtained data, it was established that the criterion for selection of highly productive animals is the value of the selection index "I" +0.181 - +3.205, Sazer-Fredin index -1.017 - -0.102.

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ADAPTATION LEVEL, MANAGEMENT VALUE AND PRODUCTIVITY OF LARGE WHITE SOWS OF HUNGARIAN ORIGIN IN THE STEPPE ZONE OF UKRAINE

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Abstract

The paper presents the results of a study of long-term adaptation, management value and productivity of Large White sows of Hungarian origin in the conditions of steppe zone of Ukraine. It was found that Large White sows of Hungarian origin are characterized by high rates of long-term adaptation, management value and reproductive qualities. Thus, their age of life is 44.1 ± 1.97 months (Cv = 35.27%); the duration of breeding use is 32.8 ± 1.95 months (Cv = 46.91%); the index "level of adaptation" varies in the range from 5.48 to 27.20 points. Taking into account the intra-breed differentiation according to the index "level of adaptation", a significant difference between the groups of sows of class M + and M- was found according to the following indicators: "farrowing received", "live piglets in total, heads", "multifetation, heads", "nest weight during the weaning at the age of 28 days, kg". The indicator "livability of piglets before weaning at the age of 28-32 days, %" in the experimental groups of sows ranges from 90.1 to 94.4%. Sows from the category "high management value" exceeded peers from the category "low management value" in terms of "farrowing received", "live piglets in total, heads", "multifetation, heads", "nest weight during the weaning at the age of 28-32 days, kg" by 46.67% on average. The indicator "livability of piglets before weaning at the age of 28-32 days, %" in different management value groups of sows ranges from 91.3 to 100.0%. The maximum increase in additional products was obtained from sows which index "level of adaptation" ranges from 5.48 to 8.20 points (+4.99%), as well as from animals of the category "high management value" (+3.41 %), respectively. These indicators are proposed to be used as criteria for selection of highly productive animals in the controlled population.

Key words*: adaptation, breed, correlation, economic efficiency, management value, reproductive qualities, sow, variability.*

INTRODUCTION

An objective assessment of the level of pig adaptation to environmental conditions, management value and productivity indicators is the relevant issue in working with the population of animals of a particular breed (Shulga et al., 2011; Topikha & Konovalov, 2009; Khalak et al., 2021; Khalak, 2009, 2020; Khokhlov et al., 2011; Kislinskaia et al., 2012).
Thus, according to Dudka (2020), it was established that sows of the Ukrainian Spotted Steppe breed in the process of ontogenesis adapt better to breeding conditions and to the environment. This is manifested in an increase in age of life and breeding use of sows from the main herd and a reduction in the adaptation index. The age of life of sows of the Ukrainian White Steppe breed is 35.7 months, the duration of breeding use is 21.9 months, the adaptation index is 37.7 points. The author notes that the high level of adaptability of genotypes from the studied breeds to breeding conditions is confirmed by the results of

assessment of their management value, which is 22.3-35.3 heads per one sow for all viable piglets, and it meets the requirements of the "average" level at scale for assessing the management value of sows.

Important population indicators are flexibility and stability of pigs, which depend on the genotype (Dudka, 2020). The author identified four highly flexible (bi = 3.020-4.601) lines with a positive genotypic effect (0.11-0.44 heads) in the Ukrainian Spotted Steppe breed and two (bi = 1.545-1.683 and 0.12-0.21 heads) in the Ukrainian White Steppe breed. It is proved that lines with a combination of high flexibility and low stability should be used in further breeding work to increase the genetic potential of animals, and low flexibility and high stability to consolidate the breeding traits of breeds.

It is established that in the process of adaptation in the herd replacements of Large White breed of Hungarian origin in the Black Sea region there is a decrease in growth, development and productivity for three consecutive generations. The most significant decrease in the above mentioned indicators is specific to the second generation of descendants of imported animals (Kislinska, 2012). The process of adaptation has negatively affected the level of reproductive qualities of the sows being tested. The lowest rates were obtained in the first generation of animals compared to the generation of acclimatizers. But with each following generation, an increase in these indicators was found, the maximum manifestation of which was found in the third generation of animals.

The works of other native and foreign scientists are confirmation of the relevance of the chosen research area (Khalak, 2020; Vashchenko et al., 2015; Kovalenko, 2011; Berezovskyi, 2014; Vashchenko, 2011; Tolokontsev, 2010; Kislinskaia et al., 2012).

The aim of the work is to investigate the indicators of long-term adaptation, management value and productivity of sows of Large White breed of Hungarian origin in the steppe zone of Ukraine, and based on the obtained data, to determine the criteria for selection of highly productive animals according to the index "level of adaptation" and calculate the economic efficiency of research results.

MATERIALS AND METHODS

The experimental part of the work was carried out on farms in Dnipropetrovsk region and in the laboratory of the State Institution "Institute of Grain Crops of NAAS of Ukraine". The work was performed according to the research program of NAAS of Ukraine № 31 "Genetic improvement of farm animals, their reproduction and conservation of biodiversity (Genetics, conservation and reproduction of biological resources in animal husbandry)", task "To determine the adaptive features and nature of inheritance of polygenic-hereditary traits of pigs of different genotypes and to develop an integrated system for creating a highly productive population".

Large White sows of Hungarian origin are the object of the study.

Evaluation of sows on the indicators of long-term adaptation, management value of reproductive qualities was carried out taking into account the following quantitative characteristics: "age of life of sows, months"; "duration of breeding use of sows, months"; "farrowing received"; "piglets in total, heads"; "live piglets in total, heads"; "multifetation, heads"; "nest weight during the weaning at the age of 28 days, kg"; "livability of piglets before weaning, %". Comprehensive assessment of sows on the indicators of reproductive qualities was carried out according to the index of M.D. Berezovskyi (quoted Vashchenko, 2019):

$$I = B + (2 \times W) + (35 \times G) \quad (1)$$

where: I - M.D. Berezovskyi index, points; B - the number of piglets at birth, heads; W - the number of piglets at the time of weaning, heads; G - average daily gain of live weight of piglets before weaning, kg.

The index "level of adaptation" was calculated according to the following method:

$$ILA = \frac{AL^2}{farrowings \times DBU\ (months)}, \quad (2)$$

where: ILA - index "level of adaptation", points; AL - age of life of sows (from birth to the last weaning of piglets), months; DBU - duration of breeding use (from the beginning of the first gestation to the last weaning of piglets), months (Smirnov, 2003).

The management value of sows was determined by the Koriazhnov scale (1983) (Table 1).

Table 1. The scale of management value of sows

The level of management value	Management value per farrowing sow		Management value per inseminated sow	
	E_1 (piglets in total)	E_1 (including viable)	E_2 (piglets in total)	E_2 (including viable)
Low	Up to 25	Up to 20	Up to 25	Up to 15
Medium	26-40	21-30	21-44	16-34
High	more 50	more 40	more 45	more 35

The cost of additional products was calculated by the following formula:

$$Å = P \times \frac{A \times M}{100} \times L \times N, \quad (3)$$

where: A - cost of additional products, UAH; P - purchase price per unit of output, according to existing current prices in Ukraine; A - average productivity of animals; M - the average raise of the main product (%), which is expressed as a percentage per 1 head when applying a new and improved breeding achievement compared to the productivity of animals of basic use; L - constant coefficient of reduction of the result, which is associated with additional costs for profitable products (0.75); N - the number of livestock of new or improved breeding achievement, heads ("Methods of determining the economic…", 1983).

Biometric processing of the obtained material was performed according to the methods of Kovalenko et al. (2010), using the software module "Data Analysis" in Microsoft Excel

RESULTS AND DISCUSSIONS

The research results show that Large White sows of Hungarian origin in the steppe zone of Ukraine are characterized by high rates of long-term adaptation (Table 2). Thus, their age of life is 50.1, the duration of breeding use is 41.9 months. The "adaptation level" index is 10.56 points.

Table 2. Indicators of long-term adaptation of Large White sows from controlled population, n = 153

Indexes, units of measurement	Biometric indicators		
	$\overline{X} \pm S\overline{x}$	$G \pm S_G$	$Cv \pm S_{Cv}$, %
Age of life, months	50.1±1.45	18.03±1.030	35.98±2.057
Duration of breeding use, months	41.9±1.39	17.28±0.987	41.24±2.357
Index "level of adaptation", points	10.56±0.279	3.46±0.197	32.76±1.873

Taking into account the intra-breed differentiation according to the index "level of adaptation" (deviation from the average value of the index is 0.67 × σ), it was found that sows from group III according to the indicators of "farrowing" exceeded peers from group I by 5.8 farrowings (td = 16.57; P<0.001); "live piglets in total, heads" by 70.9 heads (td = 16.26; P<0.001); "multifetation, heads" by 1.0 head (td = 4.16; P (0.001); "nest weight at the time of weaning at the age of 28-32 days, kg" by 5.2 kg (td = 2.98; P<0.01); Berezovskyi index by 3.9 points (td = 2.60; P<0.01) (Table 3).

Table 3. Reproductive qualities of Large White sows of Hungarian origin of different intra-breed differentiation according to the index "level of adaptation"

Indexes, units of measurement	Biometric indicators	Gradation of the index "level of adaptation", points		
		12.97-27.20	*8.30-12.80*	*5.48-8.20*
		distribution class		
		M^+	M^0	M^-
		group		
		I	II	III
Farrowing received	*n*	*31*	*77*	*45*
	$\overline{X} \pm S\overline{x}$	4.0±0.20	6.3±0.28	9.8±0.29
	$G \pm S_G$	1.12±0.142	2.46±0.198	1.94±0.204
	$C_v \pm S_{Cv}$, %	28.00±3.557	39.04±3.148	19.79±2.087

Live piglets in total, heads	$\overline{X} \pm S\overline{x}$	42.6±2.37	71.0±3.35	113.5±3.67
	$G \pm S_G$	13.24±1.682	29.41±2.371	24.56±2.590
	$C_v \pm S_{Cv}$, %	31.10±3.951	41.43±3.341	21.64±2.282
Multifetation, heads	$\overline{X} \pm S\overline{x}$	10.6±0.19	11.2±0.11	11.6±0.16
	$G \pm S_G$	1.10±0.139	0.98±0.079	1.09±0.114
	$C_v \pm S_{Cv}$, %	10.37±1.317	8.75±0.705	9.39±0.990
Average live weight of piglets at birth, kg	$\overline{X} \pm S\overline{x}$	1.39±0.025	1.37±0.111	1.31±0.014
	$G \pm S_G$	0.14±0.017	0.98±0.079	0.08±00.008
	$C_v \pm S_{Cv}$, %	10.08±1.280	9.47±0.763	6.45±0.680
Nest weight during the weaning at the age of 28 days, kg	$\overline{X} \pm S\overline{x}$	74.7±1.44	75.9±0.91	79.9±0.98
	$G \pm S_G$	8.07±1.025	8.02±0.646	6.61±0.697
	$C_v \pm S_{Cv}$, %	10.80±1.372	10.56±0.851	8.27±0.092
Berezovskyi index, points	$\overline{X} \pm S\overline{x}$	37.98±0.595	38.31±0.253	39.93±1.40
	$G \pm S_G$	3.31±0.420	2.22±0.179	9.43±0.994
	$Cv \pm S_{Cv}$,%	8.73±1.109	5.80±0.467	23.62±2.491
Livability of piglets at the age of 28-32 days, %	$\overline{X} \pm S\overline{x}$	94.4±1.89	91.2±0.65	90.1±0.86

The coefficient of variability of traits that characterize the level of long-term adaptation and reproductive qualities of sows from experimental groups ranges from 6.45 (class of distribution of sows according to the index "level of adaptation" is M-, indicator is "live weight of piglets at birth, kg") to 41.43% (class of distribution of sows according to the index "level of adaptation" is M0, indicator is "live piglets in total, heads").

The research results of long-term adaptation and reproductive qualities of sows of different management value are shown in Table 4.

Table 4. Reproductive qualities of sows of large white breed of Hungarian origin of different management value

Indexes, units of measurement	Biometric indicators	management value		
		high	medium	low
		group		
		I	II	III
Farrowing received	*n*	*126*	*25*	*2*
	$\overline{X} \pm S\overline{x}$	7.8±0.25	3.4±0.09	2.0
	$G \pm S_G$	2.87±0.180	0.50±0.068	-
	$Cv \pm S_{Cv}$,%	36.79±2.318	14.70±2.00	-
Live piglets in total, heads	$\overline{X} \pm S\overline{x}$	87.3±2.91	33.5±0.98	18.0
	$G \pm S_G$	32.70±2.060	5.12±0.697	-
	$Cv \pm S_{Cv}$,%	37.45±2.359	15.28±2.081	-
Multifetation, heads	$\overline{X} \pm S\overline{x}$	11.2±0.08	9.8±0.15	9.0
	$G \pm S_G$	1.00±0.063	0.80±0.108	-
	$Cv \pm S_{Cv}$,%	8.92±0.562	8.16±1.111	-
Average live weight of piglets at birth, kg	$\overline{X} \pm S\overline{x}$	1.32±0.08	1.41±0.18	1.45
	$G \pm S_G$	0.09±0.005	0.09±0.001	-
	$Cv \pm S_{Cv}$,%	6.81±0.429	6.96±0.948	-
Nest weight during the weaning at the age of 28 days, kg	$\overline{X} \pm S\overline{x}$	78.7±1.01	74.9±1.32	68.2
	$G \pm S_G$	11.40±0.718	6.85±0.933	-
	$Cv \pm S_{Cv}$,%	14.48±0.912	9.14±1.245	-

Berezovskyi index, points	$\overline{X} \pm S\overline{x}$	39.04±0.538	37.19±0.334	34.14
	$G \pm S_G$	6.03±0.379	1.74±0.237	-
	$Cv \pm S_{Cv}$,%	15.44±0.972	4.67±0.636	-
Livability of piglets at the age of 28-32 days, %	$\overline{X} \pm S\overline{x}$	91.3±0.64	94.2±1.11	100.0

Studies have shown that the number of sows from the category "high management value" in the controlled population is 82.3%, from the category of "low management value" is 1.31%.
The difference between the animals from these groups in terms of "farrowing" is equal to 5.8 farrowings, in "live piglets total, heads" is 69.3 heads, in "multiplicity, heads" is 2.2 heads, in "nest weight at the time of weaning at the age of 28-32 days" is 10.5 kg, in Berezovskyi index is 4.90 points.
It was established that sows from the category "high management value" exceeded peers from the category "medium management value" in terms of "farrowing" by 4.4 farrowings (td = 16.60, P<0.001); "live piglets in total, heads" by 53.8 heads (td = 17.52, P<0.001); "multiplicity, heads" by 1.4 heads (td = 8.23, P<0,001); "nest weight at the time of weaning at the age of 28-32 days" by 3.8 kg (td = 2.28, P<0.05), Berezovskyi index by 1.85 points (td = 2.93, P<0.01)
The maximum rates of survival of piglets before weaning at the age of 28-32 days were found in group of sows where the index "level of adaptation" ranges from 12.97 to 27.20 points (94.4%), and in the category of animals of "low management value" it is 100.0%.
The results of the calculation of the pairwise correlation coefficient between the index "level of adaptation" and indicators of reproductive qualities of sows are shown in Table 5.
It was established that the coefficient of pair correlation between the traits that characterize the level of adaptation of sows and indicators of their reproductive qualities ranges from -0.670 (tr = 15.03) to +0.257 (tr = 3.40).

Table 5. Pairwise correlation coefficients between the index "level of adaptation", age of life and breeding use and sows' reproductive performance

Indicator (attribute)		Biometric indicators	
x	*y*	r±Sr	tr
Index "level of adaptation", points	1	–0.420±0.0667***	6.30
	2	–0.588±0.0529***	11.11
	3	–0.670±0.0446***	15.03
	4	–0.667±0.0449***	14.85
	5	–0.222±0.0769**	2.89
	6	+0.257±0.0755***	3.40
	7	+0.043±0.0808	0.53
	8	+0.142±0.0793	1.79

Note: *1 – age of life, months; 2 – duration of breeding use of sows, months; 3 – farrowing received; 4 – live piglets in total, heads; 5 – multifetation, heads; 6 – live weight of piglets at birth, kg; 7 – nest weight during the weaning at the age of 28-32 days, kg; 8 – livability of piglets at the age of 28-32 days, %; *** - P<0.001*

Significant correlation coefficients were established by the following pairs of features: index "level of adaptation" × age of life (r = -0.420, tr = 6.30), index "level of adaptation" × duration of breeding use (r = -0.588, tr = 11.11), index "level of adaptation" × farrowing received (r = -0.670, tr = 15.03), index "level of adaptation" × live piglets in total (r = 0.667, tr = 14.85), index "level of adaptation" × multifetation (r = -0.222, tr = 2.89), index "level of adaptation" × live weight of piglets at birth (r = +0.257, tr = 3.40).
The calculation of the economic efficiency of the research results shows that the maximum increase in additional products was obtained from sows which index "level of adaptation" ranges from 5.48 to 8.20 points (+4.99%), as well as from animals of "high management value" - (+3.41%), respectively (Table 6).

Table 6. Economic efficiency of research results

Group	n	nest weight during the weaning at the age of 28-32 days, kg	Increase in additional products, %	The cost of additional products UAH/heads/farrowing * US dollar/head/farrowing
intra-breed differentiation according to the index "level of adaptation"				
Total sample	153	76.1±0.86	-	-
I	31	74.7±1.44	-1.83	-49.61 / -1.50
II	77	75.9±0.91	-0.26	-7.04 / - 0.21
III	45	79.9±0.98	+4.99	+135.28 / +4.11
intra-breed differentiation by management value				
III	2	68.2	-10.38	-281.40 / -8.55
II	25	74.9±1.32	-1.57	-42.56 / -1.29
I	126	78.7±1.01	+3.41	+92.44 / +2.81

***Note:** * - the price of selling young pigs to processing enterprises of the region at the time of the experimental part of the study was 47.5 UAH /kg, or $ 1.44*

The cost of additional products received from one sow of these groups is +135.28 and +92.44 hryvnias or +4.11 and +2.81 US dollars.

CONCLUSIONS

1. It is established that Large White sows of Hungarian origin are characterized by high rates of long-term adaptation, management value and reproductive qualities. Thus, their age of life is 44.1 ± 1.97 months (Cv=35.27%), the duration of breeding use is 32.8 ± 1.95 months (Cv=46.91%), the index "level of adaptation" varies in the range from 5.48 to 27.20 points. According to the indicators of reproductive qualities ("multifetation, heads"; "weight of the nest at the time of weaning, kg"), 82.28% of sows belong to class I and elite class, 14.51% to class II, 3.21% to non-class animals.
2. Taking into account intra-breed differentiation according to the index "level of adaptation", a significant difference between groups of sows of class M + and M- was found in the following indicators: "farrowing received", "live piglets total, heads", " multifetation, heads" and "nest weight at the time of weaning at the age of 28-32 days, kg". The indicator "livability of piglets before weaning at the age of 28-32 days, %" in the experimental groups of sows ranges from 90.1 to 94.4%.
3. Sows from the category "high management value" exceeded peers from the category "low management value" in terms of "farrowing received", "live piglets in total, heads", "multifetation, heads", "nest weight at the time of weaning aged 28-32 days, kg" by 46.67% on average. The indicator "livability of piglets before weaning at the age of 28-32 days, %" in groups of sows of different management value ranges from 91.3 to 100.0%.
4. Coefficient of pair correlation between traits that characterize the level of adaptation of sows and indicators of their reproductive qualities ranges from -0.670 (tr = 15.03) to +0.257 (tr = 3.40).
5. The maximum increase in additional products was obtained from sows which index "level of adaptation" ranges from 5.48 to 8.20 points (+4.99%), as well as from animals of the category "high management value" (+3.41%), respectively. These indicators are proposed to be used as criteria for selection of highly productive animals in the controlled population.

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THE BENEFICIAL EFFECT OF *BACILLUS* SPP. AS PROBIOTICS IN POULTRY NUTRITION - A REVIEW

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Abstract

Over the last few decades, the use of probiotics as source of feed additives in animal nutrition has increased considerably. As you know, sub-therapeutic levels of antibiotics were used as growth promoters (AGP) in the animal field, with extensive use in poultry industries, but due to their multivarious side effects, it was necessary to find some alternatives in order to satisfy the consumer's demands. Probiotics are considered one of the options as a significant alternative to antibiotics for improving health, growth, and poultry production. In our day, among the extensive number of probiotic products in use are bacterial spore formers, mostly of the genus Bacillus. The current review presents the benefits of probiotic utilization based on Bacillus spp. in poultry feed highlighting their potential to form spores that can withstand harsh environmental stress and transition during poultry gastrointestinal tract. Furthermore, Bacillus spores involve more than 80% survivability during the probiotic in vitro tests, remaining stable in a fairly high concentration. Based on the information found from published articles, this review summarizes stronger information about the properties of Bacillus spp. obtained from in vitro and in vivo screening, which can provide researchers with a better understanding of the use of this species in poultry nutrition.

Key words*: Bacillus spp., poultry, probiotics.*

INTRODUCTION

The use of antibiotics as growth promoters (AGP) in animal diets has been of concern and has even been banned in many countries globally, due to the appearance of resistant bacteria to antibiotics, which was associated with human and animal illnesses (Cartman et al., 2004; Bajagai et al., 2017; Jiang et al., 2021). Excessive antibiotic utilization involves the appearance and transfer of gene resistance between bacteria, the disequilibrium of normal microflora, and the decline of beneficial intestinal bacteria (Sinol et al., 2012). Moreover, Cervantes (2015) affirmed that the elimination of antibiotics involves considerable consequences with negative effects on performance production, infections of the gut, and the possibility of a high mortality rate in the poultry industry.

Since 2006, many European Union (EU) countries prohibited using all commonly feed antibiotics added as growth promoters (EC Regulation No. 1831/2003). The EU has included this issue as the main point of ***"the farm to fork concept"*** and, shortly, the European Commission will act to decrease the total antimicrobials sales for livestock animals (European Commission, 2020). Therefore, probiotics are increasingly popular as considerable safe alternatives to replace and reduce antibiotics (Meng et al., 2010; Nguyen et al., 2018; Luise et al., 2022), as a viable solution to save the animal livestock sector (Hmani et al., 2017; Park et al., 2015), especially for young animals like broilers and piglets (Aar et al., 2016; Idriceanu et al., 2020).

According to the Food and Agriculture Organization (FAO, 2002) and World Health Organization (WHO, 2006) guidelines, probiotic bacteria are an important solution and have been proved the most favourited alternative to antibiotics as AGP and inhibitor of pathogens in the animal industry (Zhang & Kim, 2014).

Probiotics are defined as a preparation containing viable or inactivated known bacteria (Ramlucken et al., 2020a) and generally are recognized as safe (GRAS). Also, the use of probiotics or direct in-fed microbial (DFM)

which, when administered in adequate amounts, confer "a health benefit to the host" (Fuller, 1989; Schrezenmeir & De Vrese, 2001) and seems to be one of the most promising strategies (Barba-Vidal et al., 2019).

Probiotics occur an important place due to their beneficial impact on body weight host, growth performance, improving the health profile (Abd El-Hack et al., 2020; Zhang & Kim, 2014), gut immunity by regulating the metabolism, and bacteria compositions from this area (Luise et al., 2022).

Generally, a probiotic strain is recommended to be isolated from the same source for which it was created. Based on the probiotic perspective, it is proclaimed that the candidate probiotic should be isolated from the source of the target population, which helps them to grow well inside the selected host (Elshaghabee et al., 2017).

Before a probiotic product can be included in poultry feed, it is essential to assay its stability (viability and growth) under simulation of the gastrointestinal tract (GIT) harsh conditions. As a level of inclusion, a probiotic must retain less than 1 x 10^6 CFU g^{-1} (Millette et al., 2013).

Probiotics improve digestion and nutrients absorption by inhibiting potentially pathogenic bacteria, regulating intestinal affection (Ding et al., 2021), and modulating the gut microbiota, which plays a critical role in sustaining beneficial health status (Patil et al., 2015). Also, probiotics addition as feed additives or supplements can re-establish the ecologic stability of gut microbiota by inhibiting pathogens and promoting the growth of representative bacteria (Bermúdez-Humarán et al., 2019; Del Toro-Barbosa et al., 2020).

An ideal probiotic is necessary to have the capacity to adhere to the intestinal mucosa, grow rapidly, and maintain its viability (Luise et al., 2022). Manufacturing is an additional trait of probiotics including transport and storage conditions, applied usually in the processes for obtaining animal feed, to keep as much possible the vital properties of these products (Banjagai et al., 2016), especially after feed pelleting, storage, and manipulation (Cutting, 2011).

The most commonly used probiotics are Gram-positive bacteria from the genus *Bacillus, Lactobacillus, Lactococcus, Streptococcus, Bifidobacterium, Enterococcus,* and non-bacteria (yeast or fungal) including *Aspergillus oryzae, Candida pintolopesii, Saccharomyces boulardii,* and *Saccharomyces cerevisiae,* which are widely used to prevent poultry diseases, pathogens multiplication and improve the growth performance (Mountzouris et al., 2007; Gaggia et al., 2010; Elshaghabee et al., 2017; Kerry et al., 2018; Dumitru et al., 2020; Yoha et al., 2021).

Currently, through the large number of probiotic products, bacterial spore formers are in use today (Hong et al., 2005) and have been most extensively studied. Species from the *Bacillus* genus present a distinct advantage over other probiotics due to the capacity of sporulation (Kim et al., 2019), germination, and proliferation within the GIT of animals (Dumitru et al., 2019; Ciurescu et al., 2020; Dumitru et al., 2021).

As Gram-positive or Gram-variable rods, catalase producing and efficient probiotic product, *Bacillus* spp. is necessary to survive during environmental stress, preparation conditions and application processes, tolerance to low pH (Lee et al., 2017; Penaloza-Vazquez et al., 2017), bile salts concentrations, and other severe conditions for the keep of their viability and properties within GIT (Barbosa et al., 2005; Shivaramaiah et al., 2011; Jiang et al., 2021; Dumitru et al., 2020). Morphologically, *Bacillus* species have rod-shaped cells with squared or rounded ends between 0.5 x 1.2 to 2.5 x 10 µm, occurring singly or in chains, and chains stability determines the colony form, which may differ from strain to strain (Logan & De Vos, 2009).

In comparison with other probiotic bacteria *Bacillus* spp. have notable advantages due to the capacity that are endospore-forming aerobic or facultative anaerobic bacteria. Sosa et al (2016) affirmed that *Bacillus* spp. under stressful environmental conditions can produce spores that remain in a dormant state for long periods (more than 2 years).

This trait makes them thermostable for storage and processing (i.e., extrusion and pelleting), with resistance to extreme temperatures up to 113°C for 8 min (Grant et al., 2018). This property makes it easier to control and enhances its probability of surviving during the animal feed production process. Further,

Cartman et al. (2008) affirmed that *Bacillus* spp. can utilize nitrate or nitrite to facilitate anaerobic respiration, which enables them to survive in anoxic conditions.

Additionally, *Bacillus* spores were confirmed to survive at low pH in the stomach, bile salts, harsh conditions in the GIT environment of the host (Barbosa et al., 2005; Chaiyawan et al., 2010; Wang et al., 2010; Cutting, 2011; Bajagai et al., 2016; Dumitru et al., 2018; Dumitru et al., 2020), high pressures, and caustic chemicals, making them suitable for distribution and commercialization (Cartman et al., 2007).

Regarding the *Bacillus* group, the bacilli are easy to produce by conventional fermentation and do not involve expensive manufacture to ensure a stable commercial product (Cutting, 2011; Ramlucken et al., 2020b).

The addition of viable probiotics such as DFM, including bacteria from *Bacillus* group, involves beneficial health. Usually, *Bacillus* are examined as probiotic products in monogastric animals: *Bacillus subtilis, Bacillus licheniformis, Bacillus amyloliquefaciens*, *Bacillus coagulants, Bacillus cereus* and *Bacillus megaterium* (Cutting, 2011; Ciurescu et al., 2020; Dumitru et al., 2021; Uraisha et al., 2021; Mushtaq et al., 2022).

The anti-nutritional factor from feed materials raw could potentially be neutralized by using enzymes that occur a vital role in nutrient absorption by diminishing intestinal viscosity through catalyzing undigested starch polysaccharides (Popov et al., 2021).

Bacillus species possess several capacities such as secretion of beneficial enzymes (amylase, protease, cellulase, lipase, xylanase, phytase, and keratinase), antimicrobial molecules production (Ramlucken et al., 2020c; Sumi et al., 2015), and beneficial metabolites through modification of gut microflora (Grant et al., 2018; Dumitru et al., 2019; Shah & Bhatt, 2011; Jani et al., 2012). Further, the capacity of sporulation extends the percentage of survivability (heat tolerance, low pH of the gastric barrier, and longer viability during storage) in several environmental conditions compared to those containing non-spore-forming bacteria (Mingmongkolchai & Panbangred, 2018; Dumitru et al., 2021).

Bacillus improves the intestinal immune system by raising the levels of cytokines and chemokines as interleukin-1β (IL-1β) and interferon-γ (IFNγ) in the chicken gut (Lee et al., 2013).

In the last decade, the use of probiotics in animal feed has occupied significant attention, and the majority of the recent probiotics are represented by lactic acid bacteria, especially *Lactobacillus* spp. and *Enterococcus* spp. (Sorescu et al., 2019).

Based on published studies, this review will focus on the dietary supplementation with *Bacillus*-based probiotic in broiler chickens and the positive traits of this genus that has functional effects on the development of suitable commercial probiotics in poultry nutrition.

MATERIALS AND METHODS

To conduct this review, more than 160 references were necessary based on *in vitro* probiotic properties which establish the desirable *Bacillus* characteristics of several strains for survivability during GIT harvest conditions, and their effect as a probiotic product in poultry nutrition. The electronic search was carried out over the last 25 years from articles published in ISI Journals, Web of Science (WoS), and Scopus. For example, the systematic topic of research data was carried out from Google Scholar (https://scholar.google.com), ScienceDirect (https://www.sciencedirect.com), PubMed (https://pubmed.ncbi.nlm.nih.gov/), and NCBI-PCM (https://www.ncbi.nlm.nih.gov/pmc/). The topic of interest as a strategy for the search was based on probiotics effect on poultry nutrition. In addition, the keywords used for the search were: probiotics, alternative to antibiotics, probiotic properties, pH resistance, bile salts tolerance, immune response, spores viability, enzymatic activity, microflora, intestinal health, broiler performance, *Bacillus* spp., and poultry nutrition.

RESULTS AND DISCUSSIONS

Probiotics history, niche and mode of action

Elie Metchnikoff was the first investigator in the fermentation processes field and probiotic products. He reported that large soured milk

consumption increases human longevity. Furthermore, Metchnikoff affirmed that the lower gut can be affected by microbes, generally bacteria from *Lactobacillus* genus, for instance, *L. bulgaricus* (Ran et al., 2019).

The term probiotic is correlated with "life" being considered "microbial feed supplements that can affect positively the host". Over the years, the meaning of probiotics was changed. Later, in 1953, Werner Kollath gave other terminology as, "probiotika" and defined as "live microorganisms which are essential for the healthy development of the gut for life". The definition of probiotic was in continuous modification. Lilley and Stillwell (1965) defined probiotics as possible microorganisms with the capacity to help the proliferation of another beneficial microorganism. Our days, their definition is opposite to the antibiotic terms (Abd El-Hack et al., 2020). Morelli & Capurso (2012) defined probiotics as the consumption of enough live microorganisms with the capability to contribute health benefits to the host. Also, the authors affirmed that some strains ingested by the host may induce other reactions in the body. An example that can be given is related by *Bifidobacterium* spp. which can produce metabolic end products (acetate and lactate) with the capacity to diminish Gram-positive and Gram-negative pathogenic bacteria (Abd El-Hack et al., 2020).

Bacillus species have been isolated from a diversity of habitats as soil, vegetables, water, animals, and as a transient part of the human gut, contaminants of raw and prepared foods, aviation fuels (Kotb, 2014; Alou et al., 2015), feces from different animals as chickens, pigs, ruminants and aquatic animals (Mingmongkolchai & Panbangred, 2018). Hong et al. (2005) affirmed that species from *Bacillus* are normally allochthonous from GIT due to the ingestion of bacteria from soil and contaminated food. Table 1 is presented *Bacillus* probiotics isolated from different sources and their benefits in the poultry industry.

Table 1. *Bacillus* isolation from diverse sources with applicability in poultry

***Bacillus* designation**	**Sources**	**Benefits**	**Reference**
B. subtilis	Soil	Improve the growth performance, gut, excreta bacterial community, immune system and gut health, regulate intestinal microstructure and digestive enzymes.	Bar & Friedman (2018); Ciurescu et al. (2020); Liu et al. (2020); Oladokun et al. (2021).
B. subtilis CH16	Chicken GIT	Increase in daily weight gain (ADG), body weight (BW) and biofilm formation, reduce feed conversion ratio (FCR).	Nguyen et al. (2015)
B. subtilis (SC2362, 1781, 747, ATCC PTA-673, PB6)	Environment sources, soil	Capacity to germinate in GIT. Increase eggshell thickness, decrease excreta *Salmonella* counts without harmful effect on performance. Beneficial influence on selected performance parameters, egg quality, and the cholesterol content of yolk lipids. Greater resistance to the avian pathogenic *Escherichia coli* O78:K80 with a reduction in the colonization of the spleen, liver, and caeca.	Cartman et al. (2008); Sobczak & Kozłowski (2015); Park et al. (2020); La Ragione et al. (2001); Jayaraman et al. (2017)
B. subtilis fmbJ	Soil	Significantly decreased reactive oxygen species (ROS) contents in liver mitochondria of broilers.	Bai et al. (2017)
B. subtilis	Soil	Release antimicrobial and antibiotic compounds.	Jayaraman et al. (2017)
B. subtilis 1781 (PB1): *B. subtilis* 1104+*B. subtilis* 747	Environment sources	Modify intestinal activity and influence gut barrier integrity through increased tight junction gene expression.	Gadde et al., (2017b)
B. licheniformis	Unknown	Enhance meat, necrotic enteritis, and enhance growth performance.	Liu et al. (2012); Cheng et al. (2017)
B. subtilis	Soil	Improve feed conversion efficiency and diminish abdominal fat. Reduce the intestinal size and promote the growth of several digestive organs.	Samanya et al. (2002); Wang et al. (2018)
B. amyloliquefaciens	Soil	Increase serum immunoglobulin levels, decrease the number of *E. coli*, NH_3, and H_2S emissions. Enhances gut health and growth performance.	Ahmet et al. (2014); Tang et al. (2017); Li et al. (2015)

B. coagulans	Soil	Protective efficacy in *Salmonella enteritidis* infections.	Zhen et al. (2018)
Symbiotic: prebiotic (xylooligosaccharide and yeast) and probiotic (*B. licheniformis*, *B. subtilis* and C. *butyricum)*	Soil	Prevents necrotic enteritis and enhances growth performance.	Li et al. (2019)
B. subtilis KATMIRA1933 and *B. amyloliquefaciens* B-1895	Soil	Positively affected egg production, quality of sperm, quality and hatchery of eggs.	Mazanko et al. (2018)
BioPlus 2B (preparation of *B. subtilis* DSM 5749 and *B. licheniformis* DSM 5750)	Soybean mash and soils	Improve growth performance.	EFSA (2019)
B. subtilis and *B. licheniformis*		Performance improvement and control effects of *Salmonella* infection.	Abudabos et al. (2020)
B. pumilus and *B. subtilis*		Beneficial effects the intestinal and immune activities, specifically in day-14.	Bilal et al. (2021)

The presence of spores makes the *Bacillus* group to resist in extreme conditions (stomach acidity, bile salts concentrations etc.). Besides, during processing and storage, the bacilli spore-formers involve more stability, making them suitable as an ingredient for probiotic formulations (Elshaghabee et al., 2017).

Different supposable mechanisms for probiotic action have been investigated based on inhibition and stimulation of the host immunity (Guo et al., 2020). The main interest in animal nutrition is occurred to the relationship between nutrition and gut health, mainly in the small intestine (Luise et al., 2022). An important characteristic of this genus is its rapidity of growth and capacity for survival during chickens' GIT (Lattore et al., 2014).

The mechanisms of *Bacillus* spp. action in their vegetative state may function as probiotics are similar to those of other probiotic organisms (Ramlucken et al., 2020c). Figure 1 is illustrated the main actions of *Bacillus* strains in the organism host.

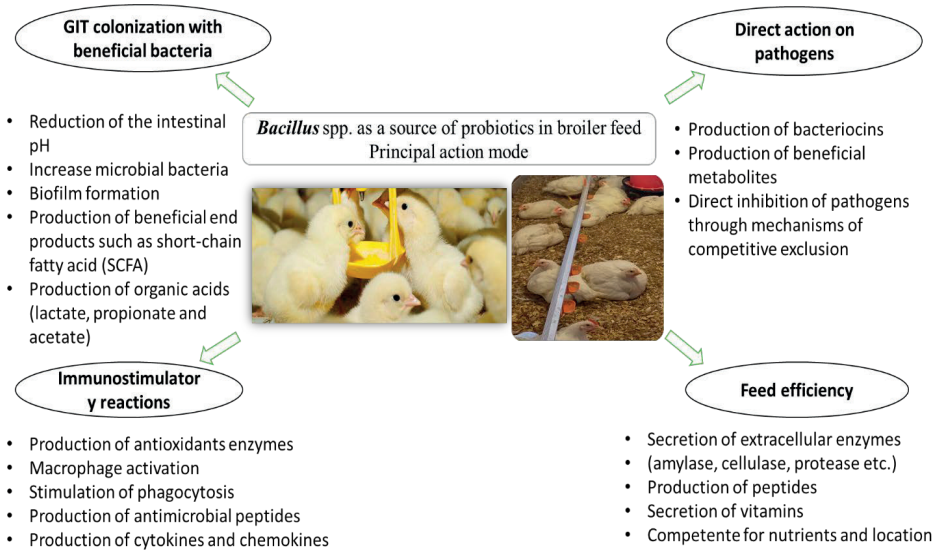

Figure 1. The effects of *Bacillus* as a probiotic source in poultry nutrition

It is known that the oral cavities of birds do not present teeth, compared to the mammals, so, feed going down to the esophagus into the crop (Scanes, 2014). Saliva secretion starts the process of feed humidification in the oral cavity involving a moist and good medium for the progress of bacteria in the crop (Scanes, 2014).

As a group of bacteria *Lactobacillus, Enterobacterium* and *Bifidobacterium* were most representative in the broiler chicken crop (Feye et al., 2020). Additionally, after a review

by Feye et al. (2020) large number of *Bacillus* (more than 70%) can be found in the poultry crop. In this region of the GIT, the feed can remain around 14 h, but many times stay between 1-3 h (Scanes, 2014).

It is very important when using a probiotic product based on *Bacillus* to be resistant to harvest environment conditions. Due to the low pH of the glandular stomach (2.3-4.8) of chickens, the probiotic of interest should not resist (Grist, 2006) which is why most of the microorganisms numbers are lower compared to the crop and intestine. Furthermore, *Bacillus* species have the capacity to produce extracellular enzymes; the first part of the enzymatic process of digestion of feed begins in the gizzard, the organ where feed is broken down and transported to the small intestine in small portions (Scanes, 2014).

The small intestines of poultry (duodenum, jejunum, and ileum) present a gradual pH from 5.0-6.0 (Ciurescu et al., 2020); however, in the small intestine, the feed remains 2 to 8 h, a process that is accompanied by secretion of enzymes and mucin (Scanes, 2014), digestion and absorption of nutrients.

A study by Dumitru et al. (2019) demonstrated the *B. licheniformis* capacity to produce extracellular enzymes by determining the carbohydrate fermentation profile through API 50CHB kits, resistance at low pH (2.0 and 3.0), and bile salts concentrations, properties that are necessary to be evaluated before administration of DFM probiotics in animal nutrition.

In the cecum, feed stays for 12-20 h (Oakley et al., 2014; Clavijo & Flórez, 2018). Gang et al. (2002) affirmed that the first function as a result of fermentation in the cecum is enzymatic activity and detoxification of damaging substances.

As mentioned above, the conditions during GIT are different, and not every bacteria can resist. In this case, the best option is to find significant probiotics through the commensal microorganisms that populate the interest intestine area (Popov et al., 2021).

Competition for adhesion to the intestinal epithelium

The selection of *Bacillus* as DFM candidate or probiotic product is based on adheration to the epithelial surface, colonization, and population of the GIT host, and afterward to form a strong barrier to prevent the adhesion of pathogens (Chauhan & Singh, 2019).

The results obtained by Nishiyama et al. (2020) suggest that *B. subtilis* C-3102 supplementation presented the potential to diminish *S. enterica* infection rates and accelerate the pathogen exclusion from the cecum, spleen, and chickens liver. Another study confirmed the potential of *B. amyloliquefaciens* US573 for exhibiting good adhesion efficacy to chicken enterocytes and the ability to create biofilms that may favour survivability in the animal tract. Moreover, the US573 strain neutralizes the antinutritional factor and maximizes nutrient absorption due to the enzymatic activity (xylanase, β-glucanase, and amylase). Therefore, the mode of action of poultry probiotics is not very clear, for this reason, further studies with *Bacillus* as DFM-probiotics will be in continuous research.

Secretion of inhibitory substances

Bacillus species are well-known producers of antimicrobial peptides such as bacteriocins, small ribosomal peptides with the capacity to inhibit the growth of pathogens. Khalique et al. (2020) showed that *B. subtilis* SP6 exhibits a wide antibacterial spectrum that has antagonist activity against *Clostridium perfringens*, a normal inhabitant in chicken intestinal microflora, usually found in low numbers in the posterior gut section (Arif et al., 2021).

Bacteriocins can attend as colonizing peptides by facilitating the population of probiotic strains into an already employed niche on the intestinal epithelium (Bahaddad et al., 2022). *B. subtilis* KATMIRA1933 and *B. amyloliquefaciens* B-1895 are probiotic strains that secret the bacteriocins subtilisin A and subtilin which have significant potential against *Salmonella* by inhibition of biofilm formation which may serve in decreased the pathogens microorganisms (Tazehabadi et al., 2021).

Modulation of the immune system

It is known that innate immunity is the first line of protection against pathogens. The probiotic spore-forming bacteria have been described for their aptitude to stimulate and/or control the

poultry immune system by secreting cytokines and immune defence substances (Popov et al., 2021).

Probiotic supplementation, as an immunomodulatory answer, positively increased the level of serum immunoglobulin (Paturi et al., 20007). In addition, Mountzouris et al. (2007) affirmed that the inclusion of probiotics stimulates GIT immunity by decreasing the number of pathogens in microflora. Likewise, Xu et al. (2012) reported that *B. subtilis* stimulated the production of cytokines as Il-10 and Il-4. Furthermore, *Bacillus* spp. registered a vital role in cytokines regulation (Mushtaq et al., 2022), immune modulation, and activation of macrophages without cytotoxicity (Popov et al., 2021).

According to Bai et al. (2017), the inclusion of *Bacillus* spp. in broiler diets improves radically the IgA. Al-Khalaifa et al. (2019) reported that the administration of probiotics in broiler production improves significantly the immune globulin. Furthermore, Fathi et al. (2017) described those dietary probiotics in broiler chickens improved the level of immune-globulins (IgA, IgM, and IgG).

Several studies were demonstrated that the inclusion of *B. subtilis* in chickens' diets enhances the innate and acquired immune responses of broiler (Pagnini et al., 2010; Lee et al., 2015; Gadde et al., 2017a; Guo et al., 2020; Tarradas et al, 2020; Sikandar et al., 2020). Besides, *B. subtilis* was shown to modulate the responses of immune protective hosts against potential infections (Rajput et al., 2014; Zhang et al., 2017).

Spore formers as probiotic product

Due to physiological properties, *Bacillus* species have the capacity to produce a multitude of enzymes, metabolites, antibiotics, having thus a high spectrum of utilization in medical and pharmaceutical fields, agricultural and industrial processes, animal nutrition, etc. (Celandroni et al., 2019).

The most common bacteria from the *Bacillus* group used as a probiotic product in animal production including in the poultry industry is the *B. subtilis* strain (Joerger & Ganguly, 2017; Idriceanu et al., 2020).

Efficacy of probiotic inclusion can be ascribed to the species of bacteria and the formula of supplementation used, such as wet (liquid culture) or powdered (lyophilization) (FAO & WHO, 2001). Administration of *Bacillus* as a probiotic product in the poultry diet can be performed orally, directly in water drinker (as liquid inoculum culture), or homogenized along with the feed (Lei et al., 2015; Lattore et al., 2017; Ma et al., 2018; Ciurescu et al., 2021). During the manufacturing including fermentation, drying, freezing, thawing, and rehydration, *Bacillus* spores have the ability to resist passage through the GIT, proliferate and populate the host digestive tract (Elisashvili et al., 2019; Popov et al., 2021).

Bacillus is a group recognized as spore-forming bacteria, known for their capability to germinate, proliferate, and re-sporulate. Due to the production of endospores, Bacilli involve long viability making them more stable and resistant to harvest environmental conditions. Keller et al. (2020) showed that when used *in vitro* human model, the *B. coagulans* GBI-30 can proliferate up to 97% in the GIT with active metabolically cells. Lattore et al. (2014), also, noted that 90% of *Bacillus* spores germinate in the small intestine of chickens within one hour. The spores can adhere to the intestinal walls, germinate and sporulate under anaerobic conditions as commensal to the animal intestines (Hong et al., 2009; Auger et al., 2009).

Probiotic benefits in poultry diets

The administration of probiotics in the poultry field specifically in broiler chickens has positive impacts on growth performance, feed efficiency, gut histomorphology improvement, immunity status, increase diseases resistance, and a beneficial microbiota increment (Simon et al., 2001; Mountzouris et al., 2010; Grant et al., 2018; Zhen et al., 2018).

As a direct effect, *Bacillus* probiotics can act on pathogenic bacteria such as *E. coli, Salmonella, Clostridium,* and *Campylobacter*, ensuing inhibition of their growth and population of the animal gut (Luise et al., 2022), thus preventing or reducing the incidence of infections (Zhang et al., 2014; Ding et al., 2017; Castaneda et al., 2021). For example, necrotic enteritis (NE) in the broiler industry, is produced by *Clostridium perfringens,* which is a digestive tract infection

with negative effects on host profitability (Abd El-Hack et al., 2022) and has conducted over the years to economic losses (Salem and Attia, 2021). As an enteric disorder, *C. perfringens* can be found in air, wastewater, healthy human, and animal GIT (Khelfa et al., 2012). Through 2 and 6 weeks, NE can occur in the broiler chickens tract due to the strong characteristics caused by *C. perfringens* pathogen (anaerobic, Gram-positive, endospore-forming, without motility), which could survive and stay life in extreme environmental conditions such as disintegration of organic matter and soil, due to the capacity to form endospores (Khelfa et al., 2015).

Studies have presented that inclusion of *Bacillus* spp. improves overall intestinal health and performance growth in broiler chickens (Grant et al., 2018). Teo & Tan (2007) showed that two types of *B. subtilis* strains, isolated from the chicken gut, involved antagonistic action against *C. perfirngens* ATCC 13124. Later, in 2010, Knap et al. observed a reduction of *C. perfringens* in chickens at the addition of three levels of *B. licheniformis* s (8×10^5 CFU/g feed, 8×10^6 CFU/g feed, and 8×10^7 CFU/g feed). All three concentrations maintained similar body weight (BW) and feed conversion ratio (FCR) in the chicken's trial. Further, intestinal *Salmonella typhimurium* was significantly decreased in the presence of *B. subtilis* B2A (1×10^4 CFU/g, 1×10^5 CFU/g, and 1×10^6 CFU/g). Park and Kim (2014) reported a better feed conversion ratio (FCR) and a less feed intake (FI) in the experimental chicken group feed with *B. subtilis* B2A. As mentioned, *Bacillus* is in a vegetative state it is possible to not persist in the chicken's intestinal epithelium for a long time (Latorre et al., 2014). However, *Bacillus* once inside, the chicken's GIT germinates rapidly and vegetative cells can outnumber spores within 20 h of oral administration as mentioned by Cartman et al. (2008). The presence of spores could be detected over GIT. So, in the gut wall, bacteria from *Bacillus* group start to colonize these host section which competes with and block the pathogenic bacterial sites (Mushtaq et al., 2022).

The pathogens prevention could be due to the secretion of antimicrobial peptides by *Bacillus* spp. such as amylase and protease enzymes (Dumitru et al., 2018) and metabolites (lipopeptides, surfactins, bacteriocins, inhibitory substances) which involve antagonistic results for microorganisms (Baruzzi et al., 2015; Sumi et al., 2015). It is known that, when an enzymatic bacterium is added to animal feed, the absorption and nutrient availability will improve (Amerah et al., 2017).

During competition with pathogens from the gut, the host can recover a part of the energy lost by captivating nutrients and metabolites such as lactic acid and volatile fatty acids resulting from fermenting bacteria (Grant et al., 2018).

Utilization of nutrients

An effective probiotic for growth and proliferation within the host is necessary to use nutrients and energy (Jha et al., 2020). The effects of the inclusion level of five probiotic bacterial strains (*L. reuteri* DSM 16350, *L. salivarius* DSM 16351, *Enterococcus faecium* DSM 16211, *Bifidobacterium animalis* DSM 16284, and *Pediococcus acidilactici* DSM 16210) were investigated by Mountzouris et al. (2010) as sources of probiotics in broilers feed (hybrid Cobb, male). The author's study found that the higher inclusion level ($> 10^9$ CFU/kg feed) modulates the cecal microflora composition and enhanced the growth performance and nutrient utilization in a corn-soybean diet.

Higher digestive absorption of nutrients in animal nutrition in the presence of probiotic supplementation is followed by an improvement of the intestinal structure and environment gut modulation (Choi et al., 2011). For example, the place of proliferation and differentiation of intestinal epithelial cells that stimulate villus growth is occurred by crypts.

The addition of *B. subtilis* DSM 29784 improved interior eggs quality with a significant increase in nutrient retentions and dietary apparent metabolizable energy (AME) in laying hens during the production cycle (Neijat et al., 2019).

Among of four inclusion levels (100, 150, 200, and 250 mg/kg, time 42 days) of *B. subtilis* improved the apparent metabolism of crude protein, crude fat, dry matter, and organic matter. Overall, Gao et al. (2017)

recommended that the inclusion of *B. subtilis* at 200 mg/kg could improve the broiler's performance.

He et al. (2019) investigated the effect of *B. subtilis, B. licheniformis,* and *S. cerevisiae* as an antibiotic substitute on growth performance and intestinal health status in broilers. The results obtained showed that the inclusion of probiotic complex as an alternative to chlortetracycline could improve performance growth, nutrient digestibility, serum antioxidant capacity, jejunal mucosal barrier function, and intestinal broilers morphology.

Even in unsuitable farming conditions, such as spore-forming bacteria, *B. subtilis* is implied in improving nutrient digestibility (Jha et al., 2020). After intramuscular inoculation with *E. coli,* the control + 0.1% *B. subtilis* increased the digestibility of nutrients ($P < 0.01$) followed by a reduction of *E. coli* respectively broiler colibacillosis disease (Manafi et al., 2017).

A large number of microorganisms are used as probiotics in poultry; the inclusion of *B. subtilis* DSM 17299 in broilers was correlated well with high nutrient digestibility of dry matter (DM), crude protein (CP), and AME (Reis et al., 2017). Due to their capacity to synthesize enzymes, *Bacillus* species occur as an essential solution in animal nutrition. Several studies reported that dietary probiotic supplementation enhanced the ileal digestibility of some nutrients like CP and most amino acids (Apata, 2008; Oso et al., 2019).

Due to their intense activities, digestive enzymes can affect nutrient digestibility (Zaghari et al., 2016). The improvement of digestibility, nutrients absorption, and digesta viscosity may be associated with the production of extracellular enzymes by the vegetative form of *B. subtilis* which secrete protease, amylase, and lipase (Chen et al., 2009) improving, in the end, the productive animal profile (Ravindran, 2013). The addition of *B. subtilis* enhances the feed efficiency, highlighting the production level through oxygen utilization in host GIT and secretion of various enzymes like subtilisin and catalase (Bajagai et al., 2016).

Guo et al. (2020) proved that *B. subtilis* used in their study had the capacity to produce protease. Another study affirmed that *B. licheniformis* ATCC 21424 was found as an effective enzyme producer (amylase and protease) through the submerged fermentation process (Dumitru & Habeanu, 2021). Enzymes supplementation aims to reduce the presence of indigestible components and can subsidize better digestion and therefore intensify the nutritional value of feed and energy in animal nutrition (Hmani et al., 2017).

Health status

According to the literature data, the administration of *Bacillus* diminishes the broiler chickens mortality (Teo and Tan, 2007; Knap et al., 2010; Abdel Baset et al., 2020; Qiu et al., 2021). In addition, *B. subtilis* used as probiotic (8×10^5 CFU/g) in poultry feed decreased mortality by 2.51% compared with non-supplemented groups (Harrington et al., 2016). Indeed, due to the possibility to germinate, with rapid multiplication, *Bacillus* strains have more advantages providing a wide range of health benefits to the host. Sen et al. (2010) reported that the addition of *B. subtilis* LS 1-2 improved the intestinal microbial balance and gut health of broiler with a decrease in cecal *Clostridium* and Coliforms counts. Further, *B. amyloliquefaciens*-based DFM as replace of antibiotics decreased the cecal population of *E. coli* followed by an increment of *Lactobacillus* counts comparatively with the control broiler group (Lei et al., 2014).

Probiotics affect the host (Fuller, 1989) and develop beneficial gut microflora that suppresses the growth of pathogens and modulate intestinal health in broiler (Shim et al., 2010; Reddy et al., 2010; Li et al., 2011; Rajput et al., 2012; Ciurescu et al., 2020).

Growth Performance

Growth performance characteristics (BW, ADG, ADFI, and FCR) are some of the most important aspects used to assess the economic benefits of broiler production (Zhang et al., 2021).

In poultry, the spore-forming probiotics affect positively FCR and are able to improve the growth and productivity of broilers in a variety of ways (Hooge et al., 2004; Jeon et al., 2014; Park et al., 2014; Ciurescu et al., 2020).

Studies from literature data have shown that the administration of *Bacillus* as probiotic product in the poultry industry can significantly

promote the growth performance of broilers (Knarreborg et al., 2008; Zhou et al., 2010; Jeong & Kim, 2014; Park & Kim, 2014; Rhayat et al., 2017). Zhang also reported that ADG was enhanced by the inclusion of *Bacillus*-based probiotics in a dose of 10^5 and 10^8 CFU/kg feed (Zhang et al., 2012, 2013).

Rhayat et al. (2017) study presented that the addition of *B. subtilis* DSM 29784 in broilers significantly improved the FCR ($P < 0.05$). Reis et al. (2017) observed an improvement of FCR ($P = 0.07$) at the addition of *B. subtilis* DSM 17299 (1.6×10^9 CFU/g), results which are in agreement with other data (Jeong & Kim, 2014; Zhang et al., 2021). Bai et al. (2017) investigated the effect of *B. subtilis* fmbJ (2×10^{10} CFU/kg feed) and observed significantly improved ADG, (ADFI), and FCR of broilers ($P < 0.05$) from 21 to 42 d, respectively on the entire period. In addition, the authors noted an increase of BW in experimental groups (**BS-1**: 2519.47 ± 87.59 g; **BS-2**: 2528.10 ± 71.30 g) compared with the control (2287.34 ± 60.88 g). *B. licheniformis* DSM 28710 improved the broiler performance including BW and FCR (Trela et al., 2020). Also, *B. licheniformis* has a beneficial role on performance parameters in poultry nutrition (Lei et al., 2013; Hanuszewska et al.,2018; Musa et al., 2019). The feed supplementation with two strains of *B. subtilis* (DSM 32324 and DSM 32325) involved significantly higher BW with a lower FCR than the control group during the starter phase (Sandvang et al., 2021).

Further, in the last years, spores-forming bacteria have become a topic of great interest. Single or in combination with other types of bacteria with/without the addition of minerals in the diets, probiotic-based on *Bacillus* spp. involve positive results regarding the broiler's growth promoters.

CONCLUSIONS

In summary, the present review founded on literature data shows the *Bacillus* efficacy as a probiotic and potential supplementation product for reducing antibiotics administration in the poultry industry. Based on the references from the literature, this review is focused on the influence of *Bacillus* spp. used as probiotic source in broiler feed. To point out that due to the capacity of sporulation, the *Bacillus* group has an important advantage founded on their stability through host GIT. As an end-product, in terms of inclusion in the diets or drinking water, the use of *Bacillus* strains is easily done. Future knowledge is necessary and more investigations of the probiotic administrations in poultry should focus on *in vitro* tests for determining the action mechanism of *Bacillus*-probiotic and, as well, for clarifying the correlation between bacterial properties, level of inclusion, optimal concentration, and the host profile (age, health and production conditions, hybrid type) which can influence the purpose pursued.

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CURRENT ASPECTS REGARDING THE USE OF ZEOLITES IN THE PROPHYLACTIC-THERAPEUTIC MANAGEMENT OF GASTROINTESTINAL DISORDERS IN POULTRY, SWINE, RUMINANTS AND DOGS (REVIEW)

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Abstract

Among the factors that cause gastrointestinal disorders in animals, along with microbial pathogens (bacteria, viruses), a major impact have the factors related to diet and stress. The use of effective alternative treatments of natural origin has really materialized. In recent decades, natural products have expanded and the overuse of synthetic drugs has been reduced because of their decreased therapeutic efficacy. Zeolites are volcanic compounds with a porous structure and a complex chemical composition. Their mineralogical, structural and physicochemical peculiarities underlie several applications in many fields, of which the biomedical one has a major impact on the prevention and therapy of gastric and intestinal pathologies. In gastrointestinal pathology, zeolites are used for their adsorbent and absorbent properties, which have been shown to be effective in the treatment of oral intoxications, diarrheal syndromes, and other digestive pathologies.. This review is intended to document and deepen the prophylactic-therapeutic actions of zeolites in gastrointestinal disorders in various animal species, their biomedical potential not being fully explored.

Key words*: gastrointestinal disorders, prevention, therapy, zeolites.*

INTRODUCTION

Gastrointestinal disorders in farm animals and small animals can have multiple aetiologies, including various pathogens (such as viruses, bacteria, fungi, parasites, protozoa), stress factors, microbiota dysbiosis, and unbalanced diets.

The high incidence of antibiotic resistance has led to the imperative development of new therapeutic and prophylactic alternatives of natural sources that can improve efficacy and at the same time reduce the pathologic repercussions. In this context, zeolites represent a veritable alternative because of their diverse structure and physicochemical properties.

Zeolites are compounds of volcanic origin that have been discovered in the XVIIIth century by the Swedish mineralogist Axel Fredrik Cronstedt (Mumpton & Fishman, 1977). Zeolites, that are also known as volcanic tuffs, are microporous aluminosilicate minerals that due to their unique structure have three important characteristics, which are the ability to exchange ions, the ability to perform absorption, and adsorption.

In 1977, Mumpton and Fishman describe the structure of zeolites as a three-dimensional network made up of SiO_4 and AlO_4 tetrahedra that are interconnected through oxygen molecules. Another structural feature is the presence of channels and cavities within the structure. Both natural and synthetic zeolites have a porous structure that allows them to dehydrate and rehydrate reversibly, and thus to adsorb molecules acting like true molecular sieves (Papaioannou et al., 2005). The size of the pores varies between 0.3-1 nm (Bogdanov et al., 2009). The inner network has a negative charge which is balanced by the positive charge of the cations within the pores. Each tetrahedral structure of AlO_4 has a negative charge that is balanced by additional cations such as sodium, potassium, and/or calcium (Bogdanov et al., 2009; Nadziakiewicza et al., 2019). These volcanic tuffs are of great medical interest in many parts of the world, most of the zeolites

that are commercially available being of synthetic origin.
Currently, there are over 50 types of known zeolites, such as phillipsite, chabazite, analcime, clinoptilolite, and so on. Among the characteristics that can vary between different types of zeolites, it is important to mention cationic selectivity, particle density, molecular pore size (https://www.lenntech.pl/zeolites-structure-types.htm).
The aim of this review is to create an overview of the studies that have been conducted in the biomedical field of the use of natural zeolites and provide an overall picture of the prophylactic and therapeutic use of these materials.

MATERIALS AND METHODS

The studies used in this review were taken from using databases such as Google Academics, Web of Science, and Science Direct. A set of eight international articles from the past 15 years, two for poultry, swine, ruminants, and dogs respectively, have been analysed and reviewed in order to exemplify the prophylactic and therapeutic potential of zeolite enriched feed to reduce gastrointestinal disorders. The articles featured a well-rounded experimental design with a large number of animals that participated in the studies.

RESULTS AND DISCUSSIONS

The analysed data has shown great improvement in the gastrointestinal health of the study subjects. The incidence of diarrheic episodes in young animals has been significantly reduced due to the in-feed administration of zeolites as can be seen for swine, ruminants, and poultry. The morphology of the intestinal tract of poultry that benefited from zeolite supplemented diets was improved, namely the duodenal, jejunal and ileal villi count. In poultry, zeolite administration has improved some enzymatic activity as well, for example, the maltase. The in-feed administration of zeolites also had a beneficial effect on the microbiome of the digestive tract in poultry, swine and dogs by reducing the population of harmful bacteria such as *Escherichia coli*, *Vibrio cholerae* and *Enterobacteriaceae* spp.
The bifidobacterial population *(Lactobacillus* spp., *Bifidobacterium* spp. etc.) colonies in dogs' intestinal tract were also shown to be improved by the administration of zeolites.

THE MECHANISM OF ACTION OF ZEOLITES IN THE DIGESTIVE TRACT

Zeolites are biomaterials that have been popularly used in animal husbandry technologies starting with the XXth century. In current times, zeolites have become known and preferred alternatives that still require research to further explore their biomedical potential and mechanism of action, which is yet to be fully understood.
There are some scientific hypotheses that state the fact that zeolites can remove some of the predisposing and causal factors of gastrointestinal disorders. It is already known the fact that zeolites such as phillipsite and clinoptilolite are capable of absorbing water from the intestinal contents, retaining it in its endogenous canalicular system, and thus aiding in the result of well-formed faeces (Papaioannou et al., 2005). This effect is due to the three-dimensional structure of aluminosilicate compounds that create a big internal surface of approximately 300 m²/g of interconnected channels that can be occupied by ions and water molecules (Luz, 1995). Zeolites are also believed to reduce intestinal peristalsis (Papaioannou et al., 2005).
After conducting research, Vrzgula et al. (1988) have proposed a different mechanism of action of zeolites in the gastrointestinal tract in the case of post-weaning diarrheic syndrome in cattle which involves the shift in the osmotic pressure in the intestinal lumen, thus preventing the occurrence of metabolic acidosis.
It is also known that zeolites can adsorb enterotoxins produced by *Escherichia coli,* and like other inorganic adsorbents (such as silica and carbon), zeolites can also adsorb heat-labile toxins produced by *Escherichia coli* and *Vibrio cholerae.* Thus, the use of these inorganic adsorbents can have a beneficial role in the treatment of diarrheal syndromes by reducing the activity of the enterotoxins (Thomson et al., 2004).

THE USE OF ZEOLITES IN FARM ANIMALS (POULTRY, SWINE, RUMINANTS)

Zeolites in poultry

In 2017 Wawrzyniak et al. conducted a study to evaluate the effects of zeolites on the morphology and physiology of the gastro-intestinal tract in Broiler chicken from the Ross line. The experiment involved 90 chicks, randomly selected, that were divided into three groups: the first lot was the control group, the second one was given feed that contained zeolite 2% and the last group had 3% zeolite in the feed. The study was conducted over a 40-day period, and at the end of the experiment, 3 chicks from each group were randomly picked and slaughtered, and their digestive tract was collected to do morphometrical and histological analyses. During the morphometric analyses, the dimensions of both the villi and the intestinal crypts were evaluated from 5 different regions of the small intestine (duodenum, the proximal part of the jejunum, the middle part of the jejunum, the distal part of the jejunum, and the ileum). The results stated a drop in the width of the duodenal villi and an increase in the number of villi in the groups that were fed with basal diet and 2% and 3% zeolite addition. On the other hand, the same two groups presented an increase in the depth of the intestinal crypts all over the small intestine.

This study also revealed the fact that the supplementation of the feed with zeolites resulted in an increase in the activity of maltase in the duodenal sector and in the proximal and distal sections of the jejunum in the chicks which received 3% zeolites.

The experiment conducted by Wu et al. (2013) evaluated the effects of the administration of natural and synthetic clinoptilolite on growth performance, morphology, and gut microbiota in Broiler chicks has also proven to be of major interest. The study was conducted on 240 Broiler chicks over a 42-day period. The control group was fed a basal diet while the experimental groups were fed a basal diet that was enriched with natural clinoptilolite 2% respectively 2% modified clinoptilolite.

The experimental groups presented an increase in the production parameters and an increase in the height of the intestinal villi in the jejunum and ileum compared to the control group. The addition of clinoptilolite to the basal diet did not have any effects on the depth of the jejunal and ileal crypts. A decrease in the total viable counts of *Escherichia coli* was noticed on day 21 for both experimental groups and an increase in the total viable counts of *Lactobacillus acidophilus* on day 22 of the study. The pH levels in the cecum for the experimental groups have proven to be lower than the control group. This study ultimately proved the beneficial effects of the administration of both natural and modified clinoptilolite on gut microbiota in Broiler chicks.

Zeolites in swine

Valpotic et al. conducted a study in 2016 in which they evaluated the effects of clinoptilolite administration on growth parameters, gut microbiota, and lymphoid cellular populations in the ileum in weaned piglets. The study was conducted over five weeks and the experimental model included 46 pigs. The animals were divided into two groups, a control group, and an experimental group, the latter being fed a 0.5% clinoptilolite enriched basal diet. The study revealed that the experimental group showed an improvement in the daily weight gain on day 28 of the study, but eventually on day 35 it went down. The experimental group presented a significantly higher feed conversion ratio compared to the control group throughout the whole period of the study. The faecal elimination of *Escherichia coli* viable counts was also monitored, and it proved to be higher in the experimental lot but the severity of the diarrheic score was 12,96% lower than the control group (47 compared to 54).

Another study of great interest is the one conducted on 720 mixed pigs (Large White x Landrace x Belgian Landrace) by Papaioannou et al. (2004) which was centred on the effects of the administration of clinoptilolite by itself and in combination with antimicrobial substances such as enrofloxacin and salinomycin.

The results stated that the symptoms of the post-weaning diarrheal syndrome were lessened in the experimental group compared to the control group. Moreover, there was a 28,7%

decrease in the mortality rate in the experimental group. These results are in direct accordance with other studies conducted in the field by Benatti et al. (1994) and Gunther (1990).

Zeolites in ruminants

In 2010 Norouzian et al. published a study regarding the effects of clinoptilolite enriched diets on general health, growth performance and some haematological parameters in Baluchi lambs. Thirty 3-week-old lambs were randomly selected and divided into three groups, the C0 control group, which was fed a basal diet, the C1 group which was fed a basal diet plus 1.5% clinoptilolite and the C2 group which had a 2% clinoptilolite enriched basal diet. The study was conducted over a 6-week period. At the beginning of the study, the lambs weighed 6.5 ± 1.2 kg. The lambs were given free access to the feed and water. The results of the study stated that the lambs that were in the C1 and C2 groups had a lower diarrheic incidence than the lambs in the C0 group. Another finding of significance was regarding the daily weight gain of the lambs which was higher in the C1 (138.58 g/day) and C2 (170.56 g/day) as opposed to the control group (136.32 g/day).

Zarcula et al. (2010) conducted a study on the effects of the supplementation of clinoptilolite in the colostrum administered to calves. The study included 26 newborn Romanian Black and White calves which were divided into three groups, the control group, the experimental group E1 which received colostrum with 5 g/L of clinoptilolite and the E2 experimental group which was fed colostrum and clinoptilolite 20 g/L. The study was conducted over a 90-day period. The administration of colostrum enriched with clinoptilolite started immediately after parturition. The calves were fed twice a day every twelve hours. The early incidence of diarrhoea was observed, and the conclusions stated that the animals which were included in the experimental groups had a lower incidence compared to the control group.

ZEOLITES IN SMALL ANIMALS (DOGS)

Zeolites in dogs

To comprehend the gastrointestinal effects of zeolites in the digestive tract, it is important to mention the fact that both human and animal organisms alike have a neurohormonal system that is also known as the brain-gut axis that integrates the intestinal activity with the cerebral one (Superchi et al., 2017).

It is also important to mention that both physical activity and stress have an impact on the permeability, motility, the secretion of mucus, gut microbiota, and implicitly gastrointestinal digestion. The zeolite dietary supplementation can thus modulate the gut microbiota and generate a favourable environment in terms of pH levels and oxidative status (Superchi et al., 2017).

In 2017 Superchi et al. conducted a study in which the effects of the administration of chabazite and phillipsite on the gut microbiota and oxidative status were evaluated. The study was conducted on 40 English Setters over a 28-day period. The experimental groups were given a 5 g/animal/day dosage of zeolites. It is important to mention the fact that the bodyweight of the experimental group did not suffer any changes in the experimental groups. The faeces of the experimental groups were more compact compared to the control group. On day 29 the faecal *Lactobacilli* spp. and *Enterobacteriaceae* spp. faecal colonies were quantified for both control and experimental groups. The reports stated a rise of the number of lactobacilli colonies and a drop in the *Enterobacteriaceae* spp. colonies in the experimental groups compared to the control group. The rise in the number of lactobacilli colonies was associated with a decrease in bacteria with pathogenic potential in the faeces (Grieshop et al., 2002, Superchi et al., 2017). Moreover, it is a known fact that *Lactobacillus* spp. is categorized as probiotic bacteria that have been studied for their potential in the improvement of general health and their beneficial effect on neuronal activity in dogs (Grieshop et al., 2002; Superchi et al., 2017).

The analysis of the potential of chabazite to modulate the intestinal bifidobacterial population in dogs has been the object of an important study conducted by Sabbioni et al. in 2016. The study was conducted on 20 English Setters over 28 days. The conclusions of this study stated that chabazite has a favourable effect on the faecal microbial populations. In return, there were not any changes in the consistency of the faeces, which maintained a

normal consistency throughout the study. The microbiological analysis of the faeces revealed a rise in the number of *Lactobacillus* spp. and *Bifidobacterium* spp. colonies and a drop in the *Enterobacteriaceae* spp. colonies. Chabazite also proved to have a good affinity towards the adsorption of *Escherichia coli* and *Clostridium perfringens.*

CONCLUSIONS

Even though there are a lot of studies that show promising results of zeolite administration in animal models, it is imperative that more research is conducted in this matter to fully investigate the biomedical potential of zeolites. The overall analysis of this review shows that the medical-veterinary research of zeolites is focused less on the implementation of prophylactic-therapeutic remedies than on the formulation of feed supplements that improve the living conditions of animals and facilitate the obtaining of effective combinations with other medicinal products. Out of the many biomedical applications of natural zeolites, the detoxifying potential of this compound involved in the elimination of many toxic metabolites and drug residues is of major importance.

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THE BIOCHEMICAL COMPOSITION AND THE NUTRITIVE VALUE OF FODDERS FROM SOYBEAN, *GLYCINE MAX*, IN MOLDOVA

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Abstract

Soybean, Glycine max, is grown primarily for seed production and has a long history of being grown as a forage crop. The main objective of this research was to evaluate the quality of green mass, prepared hay and haylage from soybean Glycine max cv. 'CLAVERA', cultivated in the experimental plot of the National Botanical Garden (Institute) "Alexandru Ciubotaru", Chisinau, Republic of Moldova. The results revealed that the harvested soybean whole plants contained 26.5 % dry matter. The concentration of nutrients in the dry matter of green mass was: 178 g/kg CP, 286 g/kg CF, 94 g/kg ash, 310 g/kg ADF, 484 g/kg NDF, 49 g/kg ADL, 142 g/kg TSS, 261 g/kg Cel, 174 g/kg HC, with nutritive and energy value 68.6% DMD, 63.4% DOM, RFV=124, 12.73 MJ/kg DE, 10.48 MJ/kg ME and 6.46 MJ/kg NEl. The biochemical composition, nutritive and energy value of prepared hay: 173 g/kg CP, 303 g/kg CF, 105 g/kg ash, 331 g/kg ADF, 504 g/kg NDF, 53 g/kg ADL, 110 g/kg TSS, 278 g/kg Cel and 173 g/kg HC, 64.6% DMD, 57.8% DOM, RFV=116, 12.42 MJ/kg DE, 10.20 MJ/kg ME and 6.22 MJ/kg NEl. The haylage is characterized by pH = 4.69, 13.4 g/kg acetic acid, 69.3 g/kg lactic acid, 181 g/kg CP, 319 g/kg CF, 126 g/kg ash, 334 g/kg ADF, 510 g/kg NDF, 42 g/kg ADL, 71 g/kg TSS, 292 g/kg Cel, 176 g/kg HC, with nutritive and energy value 63.0% DMD, 55.8% DOM, RFV=115, 12.40 MJ/kg DE, 10.18 MJ/kg ME and 6.19 MJ/kg NEl. We consider that soybean forage may be used as multi-purpose feed for livestock.

Key words: *biochemical composition, Glycine max, green mass, hay, haylage, nutritive value, soybean.*

INTRODUCTION

The current global challenges in the agricultural and food sectors, regarding the shortage of natural protein in the human diet and animal feed, as well as the lower possibilities to supply local organic fertilizers and the rising prices of mineral fertilizers and pesticides are problems that can be partially solved by the introduction and large-scale cultivation of protein-rich crops and legumes. Legume plants play a major role in developing sustainable agriculture, both globally and on national level. Legume crops are known to have a positive influence on the physical and chemical properties of the soil and on the yield and quality of the plants cultivated after them on the same land, as well as providing fodder, which is one of the most important inputs of animal production.

Soybean *Glycine max* L. Merr. a member of the *Fabaceae* family, is an annual thermophyte, C_3 photosynthesis type plant, native to Asia. Soybean has been cultivated as a food crop in Asia since 2800 BC, however, in Europe, the first references to this species in literature date back to the 17th century, and only at the beginning of the 18th century soybean began to be sown in botanical gardens, thus becoming known to a wider audience. In 1898, the U.S. Department of Agriculture began to promote actively the cultivation of soybean. In Europe, however, the interest in soybeans began to grow only with the significant population growth in the 20th century. Last but not least, the intensification of livestock production after the World War II was one of the main reasons that contributed to the import of soybean into Europe from the USA and South America. These factors led to the first efforts to breed and grow soybean cultivars adapted to the climatic conditions of Europe, thus, in the late 1980s, the development of the large-scale cultivation of soybean in Europe began, Romania being the most important producer of soybean in Europe. Since 2000, Ukraine has

become the most important soybean growing country, accounting for almost half of the production of soybean grown in Europe. Soybean is one of the most important crops worldwide for producing oil and protein. The total world production of soybeans in 2020-2021 was 383 million metric tonnes, Brazil and the United States were the leading global producers of soybeans. Due to the sharp increase in the demand for soybeans from the industry producing concentrated feeds - which was caused, among other things, by the ban on the use of flour of animal origin as a feed component - Europe currently imports about 90% of soybean.

Soybean may also be grown to be used as forage for grazing, haying or ensiling, either alone or in mixtures, because of its high protein content. Compared to other forage legumes, soybean may reliably be included into crop rotations as a forage crop, since it can be sown as the first or second crop, it can enrich the organic matter content of soils and it is highly nutritious (Medvedev & Smetannikova, 1981; Undersander et al., 2017; Casper et al., 2013; Heuzé, et al., 2016; Kulkarni et al., 2018; Peiretti et al., 2018).

The soil and climate conditions, the temperature and photoperiod regimes, especially in the central and northern areas of the Republic of Moldova, are favourable for soybean cultivation. According to the data of the National Bureau of Statistics, in 2000-2020, in the Republic of Moldova, the highest yield – of 111 thousand tons of soybeans – was harvested in 2010, the area of land sown with soybeans being 59.1 thousand hectares or 4% of the total area cultivated with herbaceous crops. In the 80's of the last century, about 20 thousand ha were sown with soybean monoculture or mixed with maize, Sudan grass, the harvested crop being fed to animals fresh or used to produce flour fortified with vitamins. But, because of the socio-economic changes that affected agriculture, the livestock numbers considerably reduced over time, and the areas sown with fodder crops were also reduced. In recent years, agricultural producers have realized that animal husbandry cannot be efficiently organized without ensuring a sustainable and balanced supply of protein-rich feed, and lately we have noticed, fortunately, a growing interest of local farmers in this crop.

The main objective of this research was to evaluate the quality of green mass, prepared hay and haylage from soybean, *Glycine max*.

MATERIALS AND METHODS

The local cultivar 'Clavera' of soybean, *Glycine max*, created at the Institute of Genetics, Physiology and Plant Protection and grown in monoculture on the experimental land of National Botanical Garden (Institute) Chişinău, N 46°58′25.7″ latitude and E 28°52′57.8″ longitude, served as subject of the research, the fodder crop - common sainfoin, *Onobrychis viciifolia* cv. 'Anamaria', was used as control variant. The experimental design was a randomised complete block design with four replications, and the experimental plots measured 10 m^2. Soybean was sown on 7 May at a depth of 4.0 cm on rows at a distance of 45 cm. The green mass was harvested manually. The soybean samples were collected in early pod stage, on 3 August. The leaf/stem ratio was determined by separating the leaves from the stem, weighing them separately and establishing the ratios for these quantities (leaves/stems). The prepared hay was dried directly in the field. The haylage was prepared from wilted mass. For ensiling, the wilted mass was cut into 1.5-2.0 cm pieces by using a forage chopping unit, shredded and compressed in well-sealed glass containers. The dry matter content was detected by drying samples up to constant weight at 105°C. After 45 days, the containers were opened and the sensorial and fermented indices of conserved forage were determined in accordance with standard laboratory procedures - the Moldavian standard SM 108 for forage quality analysis. For biochemical analysis, the plant samples were dried in a forced air oven at 60°C, milled in a beater mill equipped with a sieve with diameter of openings of 1 mm and some assessments of the main biochemical parameters: crude protein (CP), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), total soluble sugars (TSS), digestible dry matter (DDM), digestible organic matter (DOM) have been determined by near infrared spectroscopy (NIRS) technique PERTEN DA 7200. The concentration of hemicellulose (HC), cellulose (Cel), digestible energy (DE),

metabolizable energy (ME), net energy for lactation (NEl) and relative feed value (RFV) were calculated according to standard procedures.

RESULTS AND DISCUSSIONS

Analysing the results of the assessment of bio-morphological peculiarities of the studied legume crops, it can be noted that the harvested soybean whole plants contained 26.5% dry matter, 57.8% leaves + pods, but common sainfoin - 23.7% dry matter and 53.5% leaves + flowers.

The biochemical composition, nutritive and energy value of the green mass and hay from the studied legume crops: soybean 'Clavera' and common sainfoin 'Anamaria' are presented in Table 1. Analysing the results of the biochemical composition of green mass, we found that the dry matter of the studied legume crops has similar concentration of crude protein, acid detergent fibre, acid detergent lignin, cellulose and energy. The soybean fodder is characterized by lower level of crude fibre, minerals, but high amount of total soluble sugars and hemicellulose which have a positive effect on digestibility.

Table 1. The biochemical composition and nutritive value of green mass and hay from studied legume crops

Indices	*Glycine max*		*Onobrychis viciifolia*	
	green mass	hay	green mass	hay
Crude protein, g/kg DM	178	173	177	163
Crude fibre, g/kg DM	286	303	293	338
Minerals, g/kg DM	94	105	96	99
Acid detergent fibre, g/kg DM	310	331	309	350
Neutral detergent fibre, g/kg DM	484	504	447	496
Acid detergent lignin, g/kg DM	49	53	49	52
Total soluble sugars, g/kg DM	142	110	114	63
Cellulose, g/kg DM	261	278	260	298
Hemicellulose, g/kg DM	174	173	138	146
Digestible dry matter, g/kg DM	686	646	669	625
Digestible organic matter, g/kg DM	634	578	615	560
Relative feed value	124	116	135	115
Digestible energy, MJ/kg	12.73	12.42	12.73	12.17
Metabolizable energy, MJ/kg	10.45	10.20	10.45	9.99
Net energy for lactation, MJ/kg	6.46	6.22	6.48	6.01

Literature sources indicate considerable variation in the chemical composition and nutritional value of whole plants of soybean. Undersander et al. (2007) remarked that soybean forage contained 11.4-19.5% CP, 3.3-4.8% EE, 21.4-30.0% CF, 43.8-47.5% NFE, 59.6-64.1% NDF, 39.6% ADF, 3.8-4.7% ADL, 31.5-37.2% Cel 16.0% HC 18.3-18.4 MJ/kg GE. Blount et al. (2013) reported that the dry matter content and nutrients value of soybean forage harvested mass were: 240-560 g/kg DM, 167-246 g/kg CP, 21-92 g/kg EE, 419-567 g/kg NDF, 58.2-61.4% IVDOM. Heuze et al. (2016) remarked that the average feed value of soybean fresh mass was: 24.0% DM, 15.7% CP, 4.4% EE, 31.2% CF, 48.1% NDF, 31.2% ADF, 5.8% lignin, 9.3% ash, 14.8 g/kg Ca, 2.7 g/kg P, 64% DOM, 18.9 MJ/kg GE, 11.6 MJ/kg DE and 9.2 MJ/kg ME. Tabacco et al. (2018) found that whole soybean plants, depending on the stage of maturity, contained 22.0-37.4% DM, 16.7-25.0% CP, 3.1-6.8% fats, 35.4-47.0% NDF, 26.7-38.3% ADF, 5.7-9.1% ADL, 3.6-7.6% WSC, 1.2-8.1% starch, 8.0-11.6% ash. Peiretti et al. (2015) remarked that herbage quality of soybean plant in the vegetative stage was 185.1-190.5 g/kg DM, 14.26-14.77% ash, 25.77-30.11% CP, 1.29-1.58% EE, 45.32-50.80% NDF, 32.77-35.65% ADF, 5.80-6.47% ADL, 876.5-880.7 g/kg IVTD, 17.5-18.1 MJ/kg GE, but in the generative stage: 181.9-204.4 g/kg DM, 9.25-10.15% ash, 15.38-22.85% CP, 1.06-1.53% EE, 45.42-66.27% NDF, 37.18-42.54% ADF, 6.95-8.12% ADL, 775.9-842.1 g/kg IVTD, 18.0-18.5 MJ/kg GE. Zanine et al. (2020) reported that the harvested soybean genotypes contained 44.30-54.13% stems, 28.20-48.12% leaves; 0-25.6% pods, 156.9-180.8 g/kg DM and their biochemical composition was: 14.45-

16.09% CP, 8.30-14.00% ash, 48.44-59.79% NDF, 41.74-49.60% ADF, 3.18-2.64% HC. Iqbal et al. (2021) mentioned that the nutritional quality of forage soybean as influenced by different mineral and organic fertilization regimes, on irrigated land, under the climatic conditions of Faisalabad, Pakistan was 18.21-21.9% CP, 1.70-1.97% EE, 23.0-26.2% CF and 9.3-11.2% ash.

Hay plays an important role in the animal husbandry feeding system, representing a low-cost and abundant source of nutrients, it is vital to keep animals healthy and productive. We would like to mention that in the process of producing hay, in the studied legume crops, we noticed an increase in the concentration of neutral detergent fibre, acid detergent fibre, acid detergent lignin, cellulose, minerals and a decrease in the content of crude protein, total soluble sugars, matter digestibility, relative feed value and energy concentration as compared with the harvested green mass. The hay prepared from the studied legume crops (Table 1) contained 163-173 g/kg CP, 303-338 g/kg CF, 99-105 g/kg ash, 331-350 g/kg ADF, 496-504 g/kg NDF, 52-53 g/kg ADL, 63-110 g/kg TSS, 278-298g/kg Cel and 146-173 g/kg HC. The nutritive value and the energy value of prepared hays were 62.5-64.6% DMD, 56.0-57.8% DOM, RFV=115-116, 12.17-12.42 MJ/kg DE 9.99-10.20 MJ/kg ME and 6.01-6.22 MJ/kg NEl. The soybean hay is characterized by high amount of crude protein, total soluble sugars and hemicellulose, but lower level of crude fibre, cellulose, which have a positive effect on the nutritive and energy value.

Some authors mentioned various findings about the quality of soybean hay. According to Medvedev & Smetannikova (1981), the chemical composition of hay from soybean plants was 15.4% CP, 5.2% EE, 22.8% CF, 38.6 % NFE and 7.2% ash. Kökten et al. (2014), reported that the hay from the soybean variety tested in Bingol Province of Turkey contained: 7.16-10.13% ash, 10.8-13.2% CP, 48.5-54.9% NDF, 33.3-44.1% ADF with 54.6-62.9% DMD and RFV=96.6-118.2. Heuze et al. (2016) revealed that soybean hay contained 91.5% DM, 1.9% CP, 5.4% EE, 33.8% CF, 47.0% NDF, 4.9% ADF, 7.0% lignin, 7.6% ash, 0.86% Ca, 0.19%P, 59.0% ODM, 19.6 MJ/kg GE, 10.9 MJ/kg DE, 8.6 MJ/kg ME. Başaran et al. (2017) found that the chemical composition and nutritive value of pure soybean hay were 10.55-14.94% CP, 1.10-1.41% Ca, 0.25-0.27% P, 0.87-1.59% K, 0.28-0.40% Mg and RFV = 94.02-152.02, but the hays from binary mixtures with different seed ratio of sorghum-Sudan grass hybrid - 8.15-12.31% CP, 0.3-0.64% Ca, 0.25-0.34% P, 0.85-1.83% K, 0.13-0.34% Mg and RFV = 78.38-92.22, respectively. Sürmen & Kara (2017) reported that the hay prepared from pure soybean plants harvested in full flowering stage contained 19.74% CP, 38.19% NDF, 31.32% ADF, 3.59% ADL with 644.9 g/kg DDM and RFV = 157.14, but the hay from different seed ratio mixtures of buckwheat and soybean contained 14.18-16.20% CP, 40.68-43.78% NDF, 34.94-38.11.36% ADF, 2.55-3.10% ADL, 59.20-61.68 g/kg DDM and RFV = 125.80-141.00.

The production of fermented fodder minimizes the risk associated with field losses, which can be incurred under rainy conditions during hay making. Wilting herbage prior to ensiling has many advantages including reducing effluent production and fuel consumption, improved ensilibility characteristics and reduced quantities of forage for transport. Haylage is an important source of nutrients for the dairy production sector, it is a great way to preserve nutrients for the autumn - middle spring period. When opening the glass vessels with haylage prepared from *Glycine max*, there was no gas or juice leakage from the preserved mass. The prepared soybean haylage had agreeable colour, olive leaves and light-yellow stems with pleasant smell specific to pickled watermelon, the consistency was retained, in comparison with the initial green mass, without mould and mucus. The fermentation profile of the prepared soybean haylage was as follows: pH 4.69, content of organic acids 82.7 g/kg DM, including 4.7 g/kg free acetic acid, 3.5 g/kg free lactic acid, 8.7 g/kg fixed acetic acid, 65.8 g/kg fixed lactic acid, the butyric acid not was detected. It has been determined that the concentrations of nutrients in the dry matter of soybean haylage reached 181 g/kg CP, 319 g/kg CF, 334g/kg ADF, 510 g/kg NDF, 42 g/kg ADL, 71 g/kg TSS, 292 g/kg Cel, 176 g/kg HC, 126 g/kg ash, 12.6 g/kg Ca and 2.1 g/kg P. The

nutritive and energy values of the prepared soybean haylage were 63.0% DMD, 55.8% DOM, RFV=115, 12.40MJ/kg DE 10.18 MJ/kg ME and 6.19 MJ/kg NEl.

Several studies have evaluated the quality of soybean fermented fodder (silage, haylage) as feed for ruminants. Garcia (2006) reported that the nutrient composition of silage from direct-cut soybean ranged from 16.0 to 20.6% CP, 38.3 to 48.3% NDF, 27.3 to 37.3% ADF, 6.0-7.4% ADL, 1.36-1.49% Ca, 0.26-0.31% P. Mustafa et al. (2007) found that the dry matter content and the chemical composition of soybean silage, after 45 days of ensiling, were 527-542 g/kg DM, 14.9-20.8% CP, 44.4-49.0% NDF, 35.3-37.1% ADF, 6.4-8.1% ADL, 9.5-9.7% ash. Vargas-Bello-Pérez et al. (2008) reported that the dry matter content and the chemical composition of silages from soybean were 409 g/kg DM, pH=5.29, 18.4% CP, 12.6% ash, 1.5 % fats, 46.9% NDF, 37.7% ADF, 11% ADL, 1.1 Mcal/kg NEl., alfalfa silage contained 459 g/kg DM, pH=4.89, 24.4% CP, 10.0% ash, 2.6.5% fats, 42.5% NDF, 32.4% ADF, 7.6% ADL, 1.44 Mcal/kg Nel, respectively. According to Ayaşan (2011) the nutrient concentration soybean silage was 350 g/kg DM, 18.3% CP, 43.3% NDF, 32.3% ADF, 6.7% ADL. Casper et al. (2013) reported that the nutrient composition of 20 soybean haylage samples was as follows: pH = 3.48-5.10, 0.5-8.5% lactic acid, 0.8-0.6.1% acetic acid, 14.5-22.9% CP, 2.3-6.6% EE, 30.6-42.6% ADF, 30.6-42.6% NDF, 6.9-11.5% ADL, 17.0-32.0% NFC, 61.1-77.1% IVDMD, 4.6-10.3% ash, 0.67-1.39% Ca, 0.19-0.43% P, 0.25-0.44% Mg, 1.37-2.48% K, 0.12-0.24% S, 0.07-0.89% Cl. Tabacco et al. (2018) remarked the fermentative and chemical characteristics of silage prepared from soybean plants harvested at two stages of growth were: 22.0-37.4% DM, pH = 4.23-5.15, 1.47-11.63% lactic acid, 4.63-7.09% acetic acid, 0.38-1.13 % propionic acid, 0-3.87% butyric acid, 1.00-3.53% ethanol, 18.0-24.40% CP, 8.0-13.4% ash, 36.8-50.6% NDF, 31.3-41.9% ADF, 6.3-9.7% ADL. In their study conducted to determine the effect of wilting times of harvested mass on haylage quality, Sahar et al. (2020) found that soybean haylage had pH = 4.52-5.44 and contained 272.7-648.7 g/kg DM, 8.60-12.93% CP, 47.86-54.46% NDF, 38.00-47.07% ADF, 56.12-57.80% DDM with RFV = 92.58-111.04. Zanine et al. (2020) mentioned that the nutrient concentration and fermentation characteristics of soybean silages were 150.6-193.0 g/kg DM, 8.72-10.48% ash, 6.53-10.54% CP, 42.26-50.05% NDF, 3.78-5.01% WSC with 6I.78-64.48% IVDMD, pH=5.23-5.66, 0.02-3.71% lactic acid, 1.47-3.67% acetic acid, 0.10-5.47% butyric acid. Homan et al. (2021) revealed that pure soybean silage was characterized by 26.54% DM, pH 4.94, 1.04% lactic acid, 0.17% acetic acid, 1.85% propionic acid, 0.36% butyric acid 15.56% CP, 4.22% EE, 38.68% NDF, 33.3% ADF and 8.61% ash, but the silage made from mixtures of soybean and corn contained 27.51-29.03% DM, pH 4.22-4.35, 1.01-2.29% lactic acid, 0.17-0.44% acetic acid, 0.49-1.19% propionic acid, 0.18-0.39% butyric acid 10.20-11.76% CP, 3.96-4.73% EE, 41.05-44.73% NDF, 23.28-28.77% ADF and 5.46-5.91% ash.

CONCLUSIONS

The concentration of nutrients in the dry matter of the green mass of *Glycine max* cv. 'CLAVERA' reached 178 g/kg CP, 286 g/kg CF, 94 g/kg ash, 310 g/kg ADF, 484 g/kg NDF, 49 g/kg ADL, 142 g/kg TSS, 261 g/kg Cel, 174 g/kg HC, with nutritive and energy value 68.6% DMD, 63.4% DOM, RFV = 124, 12.73 MJ/kg DE, 10.48 MJ/kg ME and 6.46 MJ/kg NEl.

The biochemical composition, nutritive and energy value of the prepared hay are characterized by the following indices: 173 g/kg CP, 303 g/kg CF, 105 g/kg ash, 331 g/kg ADF, 504 g/kg NDF, 53 g/kg ADL, 110 g/kg TSS, 278 g/kg Cel and 173 g/kg HC, 64.6% DMD, 57.8% DOM, RFV = 116, 12.42 MJ/kg DE, 10.20 MJ/kg ME and 6.22 MJ/kg NEl.

The haylage is characterized by pH = 4.69, 13.4 g/kg acetic acid, 69.3 g/kg lactic acid, 181 g/kg CP, 319 g/kg CF, 126 g/kg ash, 334 g/kg ADF, 510 g/kg NDF, 42 g/kg ADL, 71 g/kg TSS, 292 g/kg Cel, 176 g/kg HC, 126 g/kg ash, 12.6 g/kg Ca and 2.1 g/kg P, with nutritive and energy value 63.0% DMD, 55.8% DOM, RFV = 115, 12.40 MJ/kg DE, 10.18 MJ/kg ME and 6.19 MJ/kg NEl.

The plants of *Glycine max* cv. 'Clavera' may be used to prepare different types of feed for livestock.

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PROBIOTIC CHARACTERIZATION OF *LACTOBACILLUS* SP. IN VARIOUS ENCAPSULATION FORMULA

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Abstract

Research has been carried out on the characterization of the properties of L. paracasei and L. curvatus in several encapsulation formulas. This study aims to obtain the kind of encapsulation material formulation that maintains the viability and probiotic properties of both Lactobacillus. The method used in this research is a laboratory experiment using a completely randomized factorial design. The probiotic characteristics of the Lactobacillus sp. that have been encapsulated with several formulas were carried out by observing cell viability, acid resistance, bile salt resistance, and antimicrobial activity against pathogenic bacteria. The results showed that the bacteria L. paracasei in the cassava flour-alginate and L. curvatus in the alginate - skimmed milk had high viability for three weeks, same as the initial time, with the population reaching 3.52 x 10^{10} CFU/ml and 3,96 x 10^{10} CFU/ml, respectively. Furthermore, Lactobacillus bacteria encapsulated in alginate-skimmed milk formula have high resistance to acidic environments, high bile salt levels, and antimicrobial activity against E. coli and S. typhimurium. Therefore, alginate and skim milk as an encapsulant can protect probiotics survive longer and maintain their probiotics.

Key words*: encapsulation, Lactobacillus, probiotics characterization.*

INTRODUCTION

Probiotics are microorganisms that are beneficial to humans, especially in maintaining health and preventing disease. These microorganisms can enter the digestive tract and regulate the balance of microbes in the intestine, because they have special characteristics such as: non-pathogenic, resistant to gastric acid conditions, resistant to bile salt concentrations in the intestine, producing organic acids, and having antimicrobial properties against pathogenic intestinal bacteria (Markowiak & Śliżewska, 2017). One of the genera the probiotic found in cow colostrum is Lactobacillus. These bacteria have the ability to attach to host cells, remove or reduce pathogenic bacteria, produce acids, hydrogen peroxide, and bacteriocins that can inhibit the growth of pathogenic bacteria (Vieco-Saiz et al., 2019).

The viability of bacterial cells in probiotic products should be in the range of 10^8 – 10^9 CFU/g. The range will assure that probiotic bacteria survive the upper ingestion to exert their positive physiological functions in the human body. For instance, to ensure that it has been stated that the so-called "minimum therapeutic" level of viable probiotic microorganisms should be at least 10^6 CFU/g of viable cells throughout the product shelf-life because this viability will decrease during storage and while in the digestive tract (Terpou et al., 2019). Due to environmental factors that are less supportive to the survival of probiotic bacteria, namely a low gastric acid environment with a pH between 1.5-2.0 in an empty stomach and a pH between 4.5-5.0 in a filled stomach.

Also, the presence of the content of bile salts in the small intestine, which bacteria must pass while in the digestive tract. The concentration of bile salts equivalent to the physiological concentration of bile salts in the duodenum is 0.5% (Terpou et al., 2019; Oberoi et al., 2021; Puspawati, 2010). In addition, that all microbes that managed to live in a 0.3% bile salt content were declared to be resistant to bile salts in the small intestine (Wijayanto, 2009).

Among the several criteria for selecting candidate probiotic strains of Lactobacillus spp., bile salt resistance is one of the most important selective criteria, since bile salts are well known as strong surfactants and bile exposure in gastrointestinal tract is intensely toxic for probiotic Lactobacillus species to survive and retain activity in human intestine (Kusada et al., 2021). Microencapsulation is a technology of packing liquids, solids, and gaseous materials into tiny capsules that release those contents at controlled rates over long periods of time (Oberoi et al., 2021).

Encapsulation is the process of coating a core material, in this case, probiotic bacteria. This coating is carried out using certain encapsulating materials that are useful for maintaining viability, their characterization, and protecting probiotics from damage due to unfavorable environmental conditions. The viability of probiotic bacteria needs to be maintained during processing, food shelf life, supplements, and in the digestive tract. The purpose of encapsulation techniques is to stabilize cells increase the survival and stability of bacteria in production, storage, and digestion. Polysaccharides like alginate, gelan, carrageenan, chitosan, and starch are the most commonly used materials in the microencapsulation of bifidobacteria and lactobacilli. Techniques commonly applied for probiotic microencapsulation are emulsion, extrusion, spray drying, and adhesion to starch (Das et al., 2014).

Encapsulation materials often used are alginate, which is used as a protector or coating the core material; the advantage of using alginate is it is easy to form a gel matrix that coats bacteria. Most commonly employed polymer for immobilizing viable cells, due to its strong capacity to be cross-linked, easy to obtain, and release trapped cells, as a food additive, its lack of toxicity, low cost, simplicity, and biocompatibility. However, alginate is also weak and vulnerable in an acidic environment. Therefore, for the alginate encapsulation process to be carried out optimally, it is necessary to combine alginate with various other polymer compounds.

The addition of starch, skim milk, gelatine, and chitosan mixed with alginate can give better results after encapsulation. Blending alginate with other biopolymers could serve as a useful approach in strengthening the microcapsule structure (Mahmoud et al., 2020). Milk proteins are one of the candidates that can be combined with alginate to improve the structural characteristics of the microcapsules that envelope probiotic bacteria (Abd El-Salam & El-Shibiny, 2015). Milk proteins are caseins and whey proteins; whey protein induces gelation through heating (Abd El-Salam & El-Shibiny, 2015) and can be used as a coat or in combination with alginate for encapsulation probiotics (Mahmoud et al., 2020).

In this study, we aimed to characterize the probiotic properties of Lactobacillus which have been encapsulated with several types of encapsulation formulas against high acid and bile salt conditions, to determine the material formula that can be selected. The characteristics of the probiotic Lactobacillus sp. that has been encapsulated will be evaluated: cell viability, acid resistance, high bile salt resistance, and antimicrobial activity against pathogenic intestinal bacteria.

MATERIALS AND METHODS

The method used in this study was experimental in a laboratory with a completely randomized design (CRD) with a factorial pattern consisting of four tests. The first test was the storage of the viability of probiotic bacteria in an encapsulated formula with two factors consisting of *Lactobacillus paracasei* and *Lactobacillus curvatus* encapsulated in Alginate-skimmed, Tapioca Alginate. All treatments were stored for 1, 2, 3 and 4 weeks. The second test is the viability test of *Lactobacillus paracasei* and *Lactobacillus curvatus* which are encapsulated against the acidity of pH 2, 4, and 6. The third stage test is the viability of *Lactobacillus paracasei* and

Lactobacillus curvatus, encapsulated to bile salt concentrations of 0.3% and 0.5% (bile salt). The fourth stage of the test is a test of the antimicrobial activity of probiotic bacteria in the encapsulated formula against pathogenic digestive bacteria *E. coli* and *Salmonella typhimurium*. The data observed were the viability and the zone of inhibition.

Inoculum preparation

Lactobacillus paracasei and *Lactobacillus curvatus* were grown on MRS agar slant media for 24 hours at 37ºC. In sterile MRSB medium, 10% fresh bacterial culture was inoculated and incubated at 37ºC for 18-20 hours. The working cultures were then harvested and centrifuged at 5000 rpm for 10 minutes. The supernatant was separated from the filtrate to obtain biomass.

Alginate preparation

The bacterial biomass was suspended in physiological NaCl at MacFarland 3 turbidity, and TPC analyzed the population. Furthermore, 3 ml of the isolates of *Lactobacillus paracasei* and *Lactobacillus curvatus* were resuspended in 10 ml of sterile NaCl, then 60 ml of Sodium Alginate 3% (w/v) was added. After mixing, it was then dripped into a beaker glass containing 200 ml $CaCl_2$ 0.1 while stirring with a magnetic stirrer. Subsequently, it was washed with 0.8% sterile NaCl, then dried.

Skim alginate

Lactobacillus paracasei and *Lactobacillus curvatus* isolates that had been cultured were subcultured singly on 2.5 ml in MRSB and incubated for 9 hours. Then it was subcultured again in a consortium on 25 ml of skim milk and then set for 9 hours. Then it was subcultured again in 250 ml skim milk and then incubated again for 9 hours. After incubation, the bacterial inoculum in skim milk was centrifuged at 5000 rpm for 10 minutes at 4ºC. The obtained biomass was suspended in 100 ml of distilled water. A total of 100 ml of bacterial biomass suspension was then mixed with 100 ml of 3% alginate as a carrier. The mixture is then put into a syringe and dripped into a beaker glass containing 0.1 mol/l of $CaCl_2*2H_2O$ with a distance of 10 cm between the tip of the syringe to the surface of $CaCl_2*2H_2O$, then allowed to stand for 1 hour so that the encapsulated granules hardened while being stored on a shaker. Finally, the encapsulated granules were rinsed with sterile NaCl 0.8% and sterile distilled water, then dried.

Tapioca alginate

A sterile coating material, 1% (w/v) sodium alginate, dissolved in 90 ml of distilled water followed by 3% (w/v) tapioca flour, was prepared. First, inoculation of isolates biomass on the coating material was carried out by aseptically mixing 10% of the consortium biomass into 90 ml of the coating material formula solution until smooth. Then, the mixture of consortium biomass and the coating material is put into a dropper to be dripped on 0.1 M $CaCl_2*2H_2O$ solution, after which dripping Ca-alginate capsules will be formed. The capsules formed were separated from the $CaCl_2*2H_2O$ solution and washed twice using sterile distilled water. The washed Ca-alginate capsules were then put into NB medium and incubated for 24-30 hours in a rotary shaker at 150 rpm at room temperature to regrow the consortium of bacteria contained in the capsules (secondary multiplication) then dried.

Antimicrobial Activity Test

The method used to test the antimicrobial activity is the agar diffusion method, namely in a well. The tests for antimicrobial activity were carried out by taking 1 gram of microcapsules and putting it in 9 ml of NB, then vortexing. Then it was incubated for 24 hours at 37ºC. *Lactobacillus paracasei* and *Lactobacillus curvatus* cell-free supernatants were obtained using a centrifuge for 20 minutes and sterilized. A total of 20 L of cell-free supernatant and pellet were each put into wells on nutrient-dense agar (NA) media that had been inoculated with the test bacteria. The media was incubated for 24 hours at 37oC. The clear zone formed indicated an inhibition of the growth of the test bacteria by the supernatant. The diameter of inhibition (clear zone) (mm) around the hole was measured using a caliper. The provisions of the potential for bacteria can be seen by measuring the inhibition area, which is classified as Stout (2001).

Data analysis

The data obtained were analyzed using analysis of variance with a 95% confidence level and if there is a significant effect of treatment, it will be continued with Duncan's Multiple Distance test.

RESULTS AND DISCUSSIONS

Viability of *L. paracasei* and *L. curvatus* in the encapsulated formula at various storage times

Probiotic encapsulation is a process of coating probiotic bacterial cells using a protective material. The capsules formed immediately by contacting the cell-polymer droplet with the crosslinking solution. The formulations used as encapsulation materials in this study were alginate, skim alginate, and alginate cassava starch.

Tabel 1. Viability of *L. paracasei* and *L. curvatus* in Various Encapsulated Formulas Against Storage Time (CFU/ml)

Density (10^{10}) Cfu/ml						
Storage time	*L. paracasei* in Alginat	*L. paracasei* Alginate Skim	*L. paracasei* Alginat Cassava starch	*L. curvatus* Alginate	*L. curvatus* Alginate Skim	*L. curvatus* Alginat Cassava starch
t_0	1,06 D c	3,39 C b	3,72 C a	1,03 C c	3,73 C a	3,75 C a
t_1	3,17 A d	4,58 A c	4,91 A b	3,39 A d	4,91 A b	5,21 B a
t_2	3,00 AB e	3,86 B c	4,14 B b	3,26 A d	4,45 B b	5,55 A a
t_3	2,83 B c	3,65 B b	3,52 C b	2,82 B c	3,96 C a	3,75 C ab
t_4	2,13 C b	2,1 D b	2,98 D a	1,06 C d	2,25 D b	1,79 D c

Duncan's Multiple Range Test (MRT) (Table 1) shows that the number or density of bacteria until the third-week *Lactobacillus paracasei* and *Lactobacillus curvatus* encapsulated in the alginate skim matrix can maintain their viability with the bacterial cell density equal to the initial encapsulation time. The population reached 3.65 x 10^{10} and 3.96 x 10^{10} CFU/ml and began to decrease in the fourth week. The number of bacterial colonies that still reached 10^{10} CFU/ml showed that the alginate skimmed matrix as an effective encapsulation was used as a protective material.

Some works mention that alginate microbeads can protect probiotics during food storage, but not upon exposure to low-pH solutions, such as gastrointestinal conditions. However, mixing alginate with other polymers, such as chitosan and starch can enhance microcapsules' resistance to acidic media (Oberoi et al., 2021). An increase in microcapsule alginate resistance was also seen in the encapsulation of probiotics with alginate cassava starch which resulted in a higher probiotic population than only alginate.

Mahmoud et al. (2020) reported that Alginate-Skim proved to be the most promising encapsulating combination that maintains the survivability of *L. plantarum* to the recommended dose level under almost all the stress conditions. The results also revealed that microencapsulation does not affect the metabolic activity of the entrapped cells. Furthermore, there was no significant difference in the production of bioactive compounds between the encapsulated and the unencapsulated cells. Blending alginate with other biopolymers could strengthen the microcapsule structure. Milk proteins are also candidates for can be incorporated with alginate to improve the structure characteristic of the microcapsules as envelope probiotic bacteria. Milk proteins are caseins and whey proteins; whey protein induces gelation through heating (Abd El-Salam & El-Shibiny, 2015) and can use as a coat.

Tolerance of *L. paracasei* and *L. curvatus* in Encapsulated Formula Against Acidic

Duncan's Multiple Range Test (MRT) (Table 2) shows that the higher the pH the higher the number of probiotic in microencapsulated. At pH 6 *L. paracasei* and *L. curvatus* in all encapsulated formula of alginate, alginate-Skim, and alginate-cassava starch grew optimally, because the optimum growth of Lactobacillus bacteria was at the optimal pH range of 5.5-6.2.

Tabel 2. Tolerance of *L. paracasei* and *L. curvatus* in Various Formulas Against Acidic (CFU/ml)

Encapsulation Formula	Number of Population		
	pH 2 (10^2)	pH 4 (10^5)	pH 6 (10^8)
L. paracasei in Alginat	5.05 C b	7.5 B c	4.03 A A
L. paracasei Alginate Skim	4.33 C B	2.84 B Ab	3.93 A A
L. paracasei Alginat Cassava starch	6.12 C ab	1.86 B b	4.57 A a
L. curvatus Alginate	1.81 C c	2.17 B ab	3.26 A a
L. curvatus Alginate Skim	1.02 C a	3.69 B a	3.71 A a
L. curvatus Alginat Cassava starch	1.08 C d	2.08 B b	3.86 A a

The probiotic response in various formulas did not show a significant difference. However, up to pH 4 both *L paracasei* and *L. curvatus* which

were encapsulated by alginate skim had a higher bacterial population than the other formulas. Skim milk is composed of various complex ingredients such as lactose, casein protein, citrate, and phosphate which can act as a buffer so as to protect bacteria exposed to acids and bile salts.

Tolerance of *L. paracasei* and *L. curvatus* Encapsulated Against Bile Salt

Tolerance test of probiotic bacteria in the encapsulated formula to the concentration of bile salts was carried out to determine the ability of the bacteria to survive in an environment with high concentrations of bile salts, on 0.3% and 0.5% concentrations**.** One of criteria as candidate probiotic strains of Lactobacillus spp., is resistance bile salt since bile salts are well known as strong surfactants and bile exposure in gastrointestinal tract is intensely toxic for probiotic Lactobacillus to survive and retain activity in human intestine.

The analysis of variance showed that the bacteria *L. paracasei* and *L. curvatus* in the encapsulated formula had high resistance to bile salts of 0.3% and 0.5% after incubation for 6 hours. The number of colonies of probiotic bacteria in this encapsulated material shows a fairly high number, which is around 10^{10} CFU/ml. Although these results were not significantly different from the mean number of bacterial cells, the result showed that skim incorporated into alginate gave the population of bacteria higher than another formula. Lactobacillus resistance to high concentrations of bile salts is due to the presence of the enzyme bile salt hydrolase (BSH) which can hydrolyze bile salts. This enzyme is able to change the physico-chemical ability of bile salts to be non-toxic to lactic acid bacteria.

Antimicrobial Activity of *L. paracasei* and *L. curvatus* Encapsulated Against Digestive Pathogenic Bacteria

Based on the analysis of variance, there was no interaction between pathogenic bacteria and *L. paracasei* and *L. curvatus* encapsulated. However, the two pathogenic bacteria showed significantly different responses to each probiotic species. Based on Table 3, the pathogenic bacteria *E. coli* has a higher sensitivity than the bacteria *Salmonella typhimurium*.

Table 3. Duncan's Multiple Range test inhibition zone of *L. paracasei* and *L. curvatus* encapsulated against *E. coli* and *Salmonella typhimurium*

Pathogen	Zone Inhibition (mm)
E. coli	15.22 a
Salmonella	9.19 b

The antimicrobial activity test of probiotic bacteria in the encapsulated formula against pathogenic digestive bacteria to determine the ability of probiotics that have been encapsulated in the various coating in inhibiting pathogenic digestive bacteria such as *E. coli* and *Salmonella typhimurium*. Based on Duncan's Multiple Range test inhibition zone, it is known that *E. coli* has a higher sensitivity to *L. paracasei* and *L. curvatus* encapsulated than *Salmonella typhimurium*. Lactobacillus, including *L. paracasei*, produce bacteriocin, which is antimicrobial against Gram-negative bacteria *E. coli* and *Salmonella enterica* and Gram-positive bacteria *S. aureus* and *Bacillus thuringiensis*. However, bacteriocins from LAB with a broad antimicrobial spectrum against Gram-negative and Gram-positive bacteria and fungi are still not frequently reported. Bacteriocins are a group of antimicrobial compounds, which are ribosomally synthesized peptides produced by bacteria to inhibit the growth of similar or closely related bacterial strains either in the same species, or across genera (Todorov, 2019).

L. paracasei and *L. curvatus* bacteria produce organic acids in the form of short chain fatty acids which are antimicrobial against pathogenic bacteria, so that they can inhibit the growth of these two pathogenic bacteria. According to Brink et al. (2005), the antibacterial and inhibitory activity against *E. coli* and other enterobacteria was caused by the increased production of short-chain fatty acids and acetic acid, which caused a decrease in pH in the digestive tract.

The low pH condition is the optimum pH for the growth of lactic acid bacteria, resulting in an increase in the population of lactic acid bacteria that will compete with pathogens for nutrients. Short-chain fatty acids such as formic, acetic, propionic, butyric, and lactic acids are produced during the anaerobic metabolism of carbohydrates and have an important role in decreasing pH. The microbial

growth inhibition by organics may be due to the ability of these acids to pass across the cell membranes, dissociate in the more alkaline environment of the cell interior, and acidify the cytoplasm (Gilor et al., 2008). The sensitivity of each pathogenic bacteria to probiotic bacteria in the encapsulated material can be seen in Table 4.

Table 4. Sensitivity of Pathogenic Bacteria *E. coli* and *Salmonella typhimurium* to encapsulated *L. paracasei* and *L. curvatus*

Probiotic Bacteria	Supernatan / Pellet	Pathogenic Bacteria	Inhibition Diameter (mm)	Note
L. paracasei Alginat	Supernatan	*E. coli*	13.17	Sensitive
	Pellet		14.67	Sensitive
	Supernatan	*S. typhimurium*	9.67	Less Sensitive
	Pellet		7.17	Less Sensitive
L. paracasei Skim Alginate	Supernatan	*E. coli*	11.33	Sensitive
	Pellet		13.33	Sensitive
	Supernatan	*S. typhimurium*	8.00	Less Sensitive
	Pellet		8.83	Less Sensitive
L. paracasei Tapioca Alginate	Supernatan	*E. coli*	13.17	Sensitive
	Pellet		14.17	Sensitive
	Supernatan	*S. typhimurium*	7.50	Less Sensitive
	Pellet		9.83	Less Sensitive
L. curvatus Alginate	Supernatan	*E. coli*	18.83	Sensitive
	Pellet		18.33	Sensitive
	Supernatan	*S. typhimurium*	9.33	Less Sensitive
	Pellet		9.50	Less Sensitive
L. curvatus Skim Alginate	Supernatan	*E. coli*	20.83	Sensitive
	Pellet		14.83	Sensitive
	Supernatan	*S. typhimurium*	12.67	Sensitive
	Pellet		11.33	Sensitive
L. curvatus Tapioca Alginate	Supernatan	*E. coli*	15.00	Sensitive
	Pellet		15.00	Sensitive
	Supernatan	*S. typhimurium*	10.00	Sensitive
	Pellet		6.50	Less Sensitive

Based on the results in Table 4, supernatants and pellets of *L. paracasei* and *L. curvatus* bacteria in the encapsulated formula produced the same inhibitory power against *E. coli* and *S. typhimurium* bacteria. Both have high enough antimicrobial activity to inhibit the growth of pathogenic bacteria *E. coli* and *S. typhimurium*. The mean inhibition diameter of the supernatant and pellet of probiotic bacteria encapsulated against *E. coli* and *S. typhimurium* was the highest produced by *L. curvatus* in alginate-skim 14.83 mm 20.83 mm against *E. coli*, and the inhibition zone was 11.33 mm and 12.67 mm against *S. typhimurium*.

The results showed that using Alginate-skimmed milk as an encapsulant was effective as a coating so that the inhibitory activity was more optimum. The results of this antimicrobial activity test showed that *E. coli* was more sensitive to both *Lactobacillus paracasei* and *L. curvatus* species than *Salmonella typhimurium.* Meanwhile, alginate and alginate-skim as the best probiotic protectors and produce high sensitivity to pathogens, especially *E. coli.*

CONCLUSIONS

Based on the analysis results, the conclusion is as follows:

1. The bacteria *Lactobacillus paracasei* and *Lactobacillus curvatus* encapsulated in various formulas can withstand acidic conditions and high bile salt levels.
2. Skim alginate is the best encapsulation material that can maintain probiotic viability against acidic conditions and high bile salt levels.
3. The skim alginate is the encapsulation material that can maintain the characteristics of probiotics, having antimicrobial activity against pathogenic bacteria in the form of supernatants and pellets.

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THE USE OF NUT KERNEL CAKE IN THE FEEDING OF YOUNG PIGS

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Abstract

The aim of the research was directed to: assess the nutritional potential and the impact on production indices, blood and economic, using in the food of the young pig breeding, the nut kernel cake in different proportions. The results of the analysis of the chemical composition showed that the nut kernel cake contains: 4.19% nitrogen, 26.2% crude protein, 9.34% crude cellulose, 13.6% fat, 12.69 Mj/kg metabolizable energy, 0.33% calcium and 0.44% phosphorus. The results of this study indicate that nut kernel cake represents a viable solution for the partial replacement of soybean meal in the food of the young pigs, because the use of the amount of 4%/t and 8%/t of compound fodder, reduces feed consumption by 3.8% and 5.2%, increases feed conversion rate by 5.3%-4.5%, the average daily increase in the experiment increases by 1.4%, does not negatively affect the state of health and blood indices reduces the cost price of 1kg of compound fodder on average by 1.3 and 2.2 cents.

Key words*: blood index, compound fodder, chemical composition, nut kernel cake, nutritional value.*

INTRODUCTION

Obtaining pigs with a high quality carcass represents an essential condition for all pig farmers in the Republic of Moldova.

From the point of view of the use of pig feed, it converts concentrated fodder into meat and fat better than other animals, and there is currently a tendency to reduce the proportion of cereals in pig rations, which is possible primarily by diversifying feed sources and using non-traditional fodder and waste from the manufacturing industry.

The identification of effective alternative nutritional solutions for the partial replacement, total or complementary substitution of some ingredients from pig rations with new feed resources, would allow not only the considerable supplementation of the nutritional value but also the reduction of the cost price of nutrition rations (Danilov & Donica, 2020). That is why the complex use of new or lesser-known feed resources in pig feed is an ever-present problem.

After the processing of agricultural products, impressive quantities of precious fodder waste are obtained, and walnut cultivation occupies an important place in the country's agriculture, because the annual production of nuts is on average over 4.0-4.5 thousand tons per year.

Data on the chemical composition of walnut kernels indicate that it contains: fatty acids of which more than 50% linoleic acid, proteins, lipids, vitamins A, B1, B2, B6, C, E, F, tyrosine, gallic acid, ellagic acid, calcium, copper, zinc, fluorine, inositol, flavonoids, juglon, hydrogelone, extractive substances, magnesium, potassium, carbohydrates, etc.

After extracting the oil from the nut kernel, the nut kernel cake is obtained by pressing, and some of these valuable nutrients remain in this by-product. The cake resulting from the extraction of the oil is not further processed. Unfortunately, in the scientific and specialized literature, the information on the use of nut kernel cake in animal feed is very limited or totally missing. This product is a real storehouse of essential vitamins and other useful components. Research in this direction is up-to-date as diversifying and expanding the range of protein feed sources for the pig farming sector is an ongoing issue.

Data from the literature (Dinu et al., 2002; Kalashnikov et al., 2003) show that the practical application of new or less studied feeds requires thorough complex investigations

of the chemical composition and nutritional value of compatibility with other ingredients and the impact on productivity and economic indices.
Based on the above data, the purpose of the research was aimed at: assessing the nutritional potential and the impact on production and economic indices, using in the feed of young pig breeding, nut kernel cake in different proportions.

MATERIALS AND METHODS

The research was carried out in the laboratories of SPIBZVM and in the precinct of the State Enterprise for Research in the Breeding and Hybridization of Pigs "Moldsuinhibrid", Orhei district, within the project: 20.8009.5107.12 "Strengthening the food-animal-production chain by using new feed resources, innovative sanitation methods and schemes". The object of the research was served with nut kernel cake and biracial pig youth. Walnut kernel cake (residue obtained from extracting nut kernel oil) was purchased from JSC "Azamet" PRO Ciadâr-Lunga district.
The biological testing of the nut kernel cake was carried out on a herd of 15 biracial sows (Yorkshire x Landrace) which were divided into three lots of 5 heads each, with the same level of development, constitution, health, with evidence growth energy during the leveling period according to classical methods (Ovsyannikov, 1976). The experimental period was 101 days, of which the leveling period lasted 15 days and the evidence period 86 days.
The main indicators that were studied were: the chemical composition of the nut kernel cake and the combined fodder used in the experiment, the edibility and daily consumption of fodder in groups, the average daily increase, the specific consumption, the blood indices.
The determination of the chemical composition of the nut kernel cake and of the combined fodder used in the experiment was carried out according to the classical methods (Pochernyaev et al., 1977): total humidity - according to the general methods by drying, crude ash - by calcination, crude protein - after Kjeldahl, crude fat - after Soxhlet, crude fiber - after Kirchner, calcium and phosphorus by titration.
The assessment of the average daily increase and the specific consumption for 1 kg of increase was performed according to known techniques (Cuckoo et al., 2004). For the study of hematological indices, at the beginning and at the end of the experiment, blood samples were taken from three sows in each lot.
The analysis of the hematological parameters of the blood was performed using the biochemical analyzer STAT FAX-3300.
Economic efficiency was calculated using the VNIIPI method (Loza et al., 1983).
By means of the computer program Microsoft EXCEL, using the classical methods (Plokhinsky, 1978), the biometric processing of the experimental data and the testing of the significance of the differences were performed.

Table 1 Scheme of experience

Lot	Livestock (n)	Average weight (kg)/ head	The mode of nutrition
Control	5	37.0	BCF (recipe 1)
Experimental I	5	37.4	ECF* (recipe 2)
Experimental II	5	38.0	ECF* (recipe 3)

Note: BCF- basic compound feed, ECF* - experimental compound feed

In the structure of the combined fodder recipe for pigs in the control lot was used (BCF), and in the experimental lots combined fodder was used, with the inclusion in the recipe of nut kernel cake in different proportions: in experimental lot I the cake was used from nut kernel in a proportion of 4% / t, recipe no. 2, and for the animals from the experimental lot II, the nut kernel cake was used in a proportion of 8% / t, recipe no.3 (Table 1).

RESULTS AND DISCUSSIONS

The assessment of the sensory qualities of feed sources is of particular interest, as it is an important tool for making important decisions in accepting them for use in animal feed. As a result of the analysis of the conventional profile of the nut kernel cake according to four descriptors: appearance, color, taste and smell, we can mention that it has a compact structure, relatively crumbly, consisting of pieces or powder of different sizes, without the presence

of foreign particles or impurities and mold, dark gray to dark brown in color (Figure 1), has a sweet bitter taste and a nutty odor, with no hints of foreign taste. We could mention that consuming the nut cake after a while, the feeling of bitter taste disappears.

Figure 1. Nut kernel cake

The results of the analysis of the chemical composition showed that the fresh nut kernel cake has a moisture content of 10.82%, a nitrogen content of 4.19%, crude protein 26.21%, crude fat 13.61%, crude cellulose 9.34%, metabolizable energy 12.69 Mj/kg, calcium 0.33% and 0.44% phosphorus. Based on the received results, we find that nut kernel cake, obtained by cold pressing, as a nutritional value can be placed in an intermediate position between soybean meal and cakes and sunflower and is a valuable fodder. Using the native ingredients and using the computer program "HYBRIMIN", according to the nutrition rules in force, 3 recipes of combined fodder were developed for each growing period (Table 2).

Table 2. The structure of the combined fodder recipes used in the experiment

Ingredients ,%	Lot					
	Control		Experimental I		Experimental II	
	Growth periods, kg					
	40-70	71-115	40-70	71-115	40-70	71-115
Maize	29.8	34.9	23.8	29.4	21.8	27.4
Barley	32.3	39.0	32.3	39.0	32.3	39.0
Wheat	23.1	10.0	29.1	15.0	29.1	15.0
Wheat bran	-	7.0	-	7.0	-	7.0
Soybean meal	12.0	6.5	8.0	3.0	6.0	1.0
Nut kernel cake	-	-	4.0	4.0	8.0	8.0
Chalk	1.0	1.2	1.0	1.2	1.0	1.2
Salt	0.4	0.4	0.4	0.4	0.4	0.4
Premix	1.4	1.0	1.4	1.0	1.4	1.0
Total	100	100	100	100	100	100

Biological testing of nut kernel cake was performed on biracial sows (Yorkshire x Landrace) selected according to the same level of development, constitution, health, with evidence of growth energy during the leveling period.

The nutritional value of the combined fodder recipes used in the feeding of breeding sows during the growing period (40-70 kg) was of 11.08; 10.88; 10.80 Mj metabolizable energy, crude protein 15.30; 15.31; 15.18%, gross fat 3.61; 2.64; 3.87%, crude cellulose 3.63; 5.09; 5.37%, non-nitrogenous extractive substances on average 68.80; 72.44; 65.31%, corresponding to the lots. The feed ration for the growing period (71-115 kg) had, corresponding to the lots, a level of 12.70; 12.62; 12.44 Mj metabolizable energy, crude protein 13.40; 13.1; 13.1%, gross fat 2.94; 3.23; 3.64%, crude cellulose 4.54; 4.62; 4.79%. Analyzing the data of the results of the chemical composition and the nutritional value of the nutrition ration used in the experiment, we can mention that they corresponded to the daily norms of nutrition, provided in the specialized literature (Kalashnikov et al., 2003). Data on the combined fodder consumption of the sows taken into studies are presented in (Table 3, Figure 2).

Table 3. Consumption of combined fodder in the experimental period

Specification	Consumption of combined fodder, kg		
	Control	Experi-mental I	Experi-mental II
In the first period of growth, per lot	494	412	398
In the second period of growth, per lot	566	608	607
In the experiment, on the lot	1060	1020	1005
On average at 1 head	212	204	201
Average daily feed consumption	2.47	2.37	2.34
Feed consumption per 1 kg increase	3.79	3.59	3.62

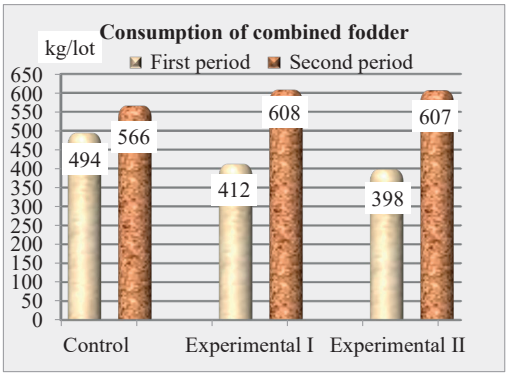

Figure.2. Consumption of combined fodder per lot

As a result of the monitoring carried out during the experiment, it was found that the nutrition rations including nut kernel cake in different proportions were well tolerated by sows, and throughout the experiment, the combined fodder consumption by a sow in experimental lot I of was lower by 3.8% and in experimental lot II by 5.2% compared to the sows in the control lot.

The sows in the control lot had an average daily consumption of 2.47 kg/head, while the sows in the experimental groups consumed an average of 2.37-2.34 kg of fodder. The basic indicator that characterizes the success of efficient pig breeding is the average daily increase which is influenced by the quantity and quality of the administered fodder (Danilov & Donica, 2020; Kalashnikov, 2003).

The data obtained (Table 4) allow us to find that the sows in the experimental lots had a higher growth rate, and at the end of the first growing period, depending on the combined fodder recipe administered, the body mass of the sows in the experimental lots was higher than the sows in the control lot by 0.4 kg and 0.6 kg ($p < 0.05$; $p < 0.01$).

In the first growth period, the sows from the experimental lot II achieved an average daily increase of 695g times by 19 g higher ($p < 0.01$) compared to the average daily increase achieved by the sows from the control lot.

Table 4 Evolution of body mass dynamics

Specification		Lot		
		Control	Experimental I	Experimental II
Live mass, kg	at the beginning of the experience	37.0±1.00	38.0±0.500	37.4±0.570
	end of I growth periods	67.4±0.908	67.8±1.193*	68.0±0.935**
	end of experience	93.0±5.397	94.8±3.130	93.0±3.708
Growth increase, kg	in the first period	30.4±1.351	29.8±0.741	30.6±0.447
	in the second period	25.6±4.698	27.0±2.031*	25.0±3.335
	in the experiment	56.0±5.062	56.8±2.724	55.6±3.492
Increase environment daily, g	in the first period	676±30.021	662±16.480	695±10.163**
	in the second period	612±110.829	658±49.537	595±79.414
	in the experiment	651±58.861	660±31.684	647±40.614
Feed consumption per 1 kg increase, kg		3.79	3.59	3.62

Note: * $p < 0.05$, ** $p < 0.01$

The results of the research showed that, in the second period of the experiment, the administration of nut cake in the amount of 4% of compound fodder (experimental lot I), increases the increase in body weight by 1.4 kg ($p < 0.05$) compared to the increase of sow growth in the control lot. It was found that a higher growth intensity in the experiment was

achieved by the sows in experimental lot I where the average daily increase was 660 g or by 9 g higher than that of the sows in the control lot. Based on fodder consumption data, as well as the absolute weight gain, the feed conversion index for a production unit was calculated. The feed conversion index (kg compound fodder/kg increase) had values between 3.79; 3.59 and 3.62 kg, corresponding to lots. The sows of the experimental lot I had an index of fodder conversion by 5.3% better than in the case of sows in the control lot.

We believe that nut kernel cake, obtained mainly by cold pressing, due to its organoleptic qualities and its rich content in natural proteins, fibers, fats, minerals could be accepted for use in the diet of breeding sows as a nutritional supplement.

Establishing hematological values provides important data on the general health of animals, the nutritional and metabolic status of pigs.

Data on the evolution of some hematological indices of breeding sows at the end of the experiment whose rations were supplemented with nut kernel cake in the proportion of 4%/t and 8%/t of combined fodder, compared to the animals in the control lot are shown in Table 5.

The results of the biochemical analyzes at the beginning and end of the experimental period showed that all the animals were healthy and the blood indices in the animals in all lots showed no changes and were characterized by average values of the limits of the norms provided in the literature.

Table 5. Average values of hematological indices at the end of the experiment

Indices	Unit of measure	Lot			
		norm	control	experimental I	experimental II
Hemoglobin	g/l	90-130	98.3±5.401	95.0±3.535	105±9.354
Erythrocytes	10^{12}g/l	5-7	8.77±0.889	9.47±1.846	8.13±0.864
Leukocyte	10^9 g/l	11-22	21.67±2.265	19.63±0.531	17.53±2.138
ESR*	mm/hour	1.0-9.0	7.66±2.483	8.33±2.857	8.67±2.160
Lymphocytes	%	35-75	49.33±1.080	53.00±6.042	44.33±2.273
Eosinophils	%	0-15	8.0±2.121	14.33±4.021	11.67±1.080
Unsegmented neutrophils	%	20-70	33.0±1.871	22.33±1.453	30.3±1.472
Segmented neutrophils	%	4-8	9.67±1.08	10.33±2.160	13.66±2.160

Note: *ESR - erythrocyte sedimentation rate

The results of the hematological analyzes at the beginning of the experiment showed that the average values of the leukocyte content had individual oscillations ranging from 27.5 * 10^9 g/l in the control lot to 30.4 * 10^9 g/l in the experimental lots (Figure 3).

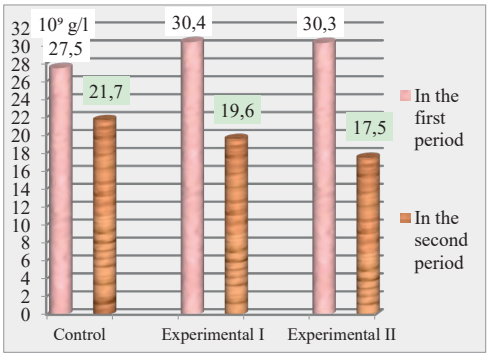

Figure 3. The amount of leukocytes in the experiment

A tendency to decrease of the number of leukocytes at the end of the experiment was reported from 21.67 * 10^9 g/l in the control lot to 19.63 * 10^9 g/l in the experimental lot I and 17.53 * 10^9 g/l in experimental lot II.

In research, the level of uric acid was important, which characterizes the work of renal functions and an important marker of the efficiency of the use of protein from food, which was in the range of 6.43 mol/l, 7.30 mol/l and 4.8 mol/l corresponding to lots. The amount of uric acid was maintained in a range corresponding to the limits of physiological norms (2.8-8.8 mmol/l). It should be noted that the amount of creatinine in all lots taken into studies increased from 87 mmol/l at the beginning of the experiment to 110 mmol/l at the end of the experiment in the control lot, in the experimental lot I from 76 mmol/l at the beginning of the experiment to 195 mmol/l at

the end of the experiment and from 84 mmol/ l to 160 mmol/l in experimental lot II.
We find that the amount of blood creatinine is maintained in a range corresponding to the limits of physiological norms (70-208 mmol/l). The study of the serum alkaline phosphotase content at the end of the experiment revealed a decrease from 130.7 u/ l in the control lot to 101.0 u/l in the experimental lot I and 109.7 u/l in the experimental lot II, but which was within the parameters of the limits of physiological norms (41-176 u/l).
The assessment of the economic efficiency of the use of the nut kernel in recipes of mixed fodder in different proportions was made on the basis of the absolute increase of the animals during the experiment, the cost of 1 kg absolute increase, the consumption of combined fodder, the cost price of the combined fodder and of nut kernel cake. The cost of 1 kg of combined fodder used in the first growing period was 25.3; 23.8; 23.2 cents and in the second period 22.8; 21.5; and 20.8 cents corresponding to the lots. The positive effects of the ratios which contained the nut kernel cake were observed by increasing the daily weight gain during the experiment periods and more importantly, by reducing the cost price of a kg of combined fodder in the first growing period by 1.5 and 2.2 cents, and in the second period respectively with 1.3 and 2.0 cents compared to the cost of 1 kg of compound fodder used in feed of the breeding sows in the control lot.
During the experimental period, the cost of the combined fodder used varied depending on the specific consumption of the animals and the cost of 1 kg of combined fodder, being within the limits of 50.27 $,USD in the control lot and 45.75-43.68 $,USD in the experimental lots.
Feed consumption is a main indicator of economic efficiency because in the experimental lots this index decreased by 8 kg and 11 kg or by 3.8% and 5.2% compared to the control lot. During the experimental period, the average price of achievement of breeding sows was 4.49 USD/kg, the cost of 1 kg of nut kernel cake - 20.9 cents/kg and of soybean meal - 55 cents/kg. According to the results obtained, the use of nut kernel cake in the amount of 4%/t of combined fodder gave the possibility to obtain an economic effect on the animal in the amount of - 7.22 $,USD, and in the amount of 8%/t of combined fodder- 4.38 USD.
Taking into account the current high level of purchase price of soybean meal and other protein ingredients due to their complex chemical composition and, in particular, their high protein and fat content and their unique functional properties, for reducing the cost of food recipes and save of fodder, nut kernel cake could be an attractive ingredient for use in pig feed.

CONCLUSIONS

Nut kernel cake is of economic interest and can be used as part of the daily diet of pigs as an additional protein source, because it has good sensory qualities and contains 4.19% nitrogen, 26.2% crude protein, 9.34% crude cellulose, 13.6% fat, 12.69 Mj metabolizable energy/kg, 0.33% calcium and 0.44% phosphorus.
The results of the experimental investigations show that the use of nut kernel cake in the feed of breeding sows in the amount of 4%/t and 8%/t of compound fodder reduces the cost price of 1 kg of compound fodder, on average, with 1.3 and 2.2 cents, does not negatively influence the state of health, reduces the consumption of fodder by 3.8% and 5.2%, increases the fodder conversion rate by 5.3%-4.5%, in the experimental lot II the average daily increase increases by 1.4%.
The results of this study indicate that nut kernel cake represents a viable solution for the partial replacement of soybean meal in the feeding of breeding sows because under the conditions of an inclusion rate of 4%/t of compound fodder was obtained an economic effect of -7.22 USD/head, and the inclusion of 8%/t ensured an economic effect for each sow in the amount of - 4.38 USD/head.

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AGE-RELATED CHANGES IN PERFORMANCE, PLASMA PROTEINS AND NITROGEN CONTENT OF EXCRETA IN ROSS 308 BREEDERS

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Abstract

The study aimed to evaluate the effect of age on breeders' performance, plasma proteins and total nitrogen content of excreta. The trial was conducted on 150 Ross 308 female breeders' (21-week-old). During 6-weeks, the birds were reared in similar management conditions and fed with standard feeds according to age (21 to 24-weeks, and >25 weeks). At the end of weeks 22, 24 and 26, blood and fresh excreta were sampled for analysis. Plasma proteins were determined by dry chemistry using reagent strips. Total nitrogen from excreta was assessed according to the Kjeldahl method. As expected, age significantly affected body weight, feed intake, protein intake, and nitrogen intake of breeders ($P<0.0001$). A dynamic age-related change was noticed in breeders' plasma proteins by increasing the total protein, albumin, albumin/globulin ratio, uric acid and urea nitrogen concentrations ($P<0.05$). The total nitrogen content of excreta registered a tendency to increase at 26-weeks compared to 22-weeks of age ($P<0.06$). Significant positive correlations were found between certain performance variables (feed intake, protein and nitrogen intake), plasma proteins and excreta total nitrogen.

Key words*: age, excreta nitrogen, performance, plasma proteins, Ross 308 breeders.*

INTRODUCTION

Blood biochemical parameters, as relevant indicators of status health in chickens, are influenced by numerous factors such as species, genotype, age, sex, physiological (breeding, moulting, etc.), nutritional, as well as breeding conditions (Tóthová et al., 2019; Gheorghe et al., 2017; Toghyani et al., 2010; Silva et al., 2007). Blood proteins are an essential indicator of birds' production traits, and their evaluation allows for the identification of metabolic alterations (Filipović et al., 2007).

The assessment of the blood protein concentrations in poultry is also important due to the physiological roles in the body and the maintenance of homeostasis (Piotrowska et al., 2011). Primarily synthesized in the liver, serum proteins control blood volume and pH buffer capacity, transport hormones and medications, helps with cell coagulation, act as enzymes and hormones, and protect the immune system (Melillo, 2013). It was noticed that compared to mammals, the total protein concentrations in birds represent about half of the values (~40 g/l), caused by the higher blood concentrations of osmotically active glucose, which reduce the protein concentrations to maintain the colloid osmotic pressure (Scanes, 2015).

Other important factors that affect the intensity of metabolism and induce changes in poultry blood proteins are age and the associated production (e.g., growing, meat-type) or reproduction processes (Tóthová et al., 2019; Filipović et al., 2007; Szabó et al., 2005).

Pullets' breeders are reared under feed restriction programs to control excessive growth during the rearing phase and avoid compromised health and impaired reproduction (Carneiro et al., 2019; Decuypere et al., 2010).

Birds use approximately 30-50% of the ingested nitrogen during their metabolism, and the excreted part is the potential source of ammonia emission (Such et al., 2021). On the other hand, it is known that poultry farming is responsible for the emission of several air pollutants, including nitrogen (De Sousa et al., 2017; Nahm, 2007). Several factors such as species, age, live weight, dietary composition,

housing conditions or manure management may affect this process (Battye et al., 1994). The current study aimed to evaluate the age-related changes in performance, plasma proteins and total nitrogen content of excreta on Ross 308 breeders.

MATERIALS AND METHODS

Birds and experimental design

The procedures implying live birds and the protocol trial used (no. 366/2021) were allowed by the National Research-Development Institute for Biology and Animal Nutrition (INCDBNA-Balotesti, Romania) Ethical Committee, in line with EU legislation (Directive 2010/63/EU; OJEU, 2010).

The evaluation trial was performed on 150 clinically healthy breeding females (Ross 308) for six weeks (21 to 26 weeks old). The birds were bred in controlled microclimate conditions in an experimental floor hall with wood saving litter, in pens (25 birds/pen), six replicate pens equipped with manual feeders, a nipple drinker line, and nest boxes. The photoperiod and lighting intensity were provided according to the strain management guide (Ross-Aviagen, 2019). The birds were immunized following the specific sanitary-veterinary protocol before the transfer age (20 weeks), and no veterinary treatment was applied during the test period.

The birds were fed with the same standard combined feed according to age: pullets (21 to 24-weeks of age) and breeders (>25 weeks). The feeds were given in the granular form, restricted, daily at 07:30, and water was *ad libitum*. The administered feed was weighed and registered daily. The birds body weight was measured weekly by individually weighing, and weight gain, protein intake (PI) and nitrogen intake (NI) were calculated. Table 1 presents the analyzed nutrient content of the standard feeds used.

Table 1. Analysed composition of breeders' diets

Nutrients (as-fed basis)	Pullets (21-24 weeks)	Breeders (>25 weeks)
Metabolizable energy (MJ/kg) [c]	11.66	11.30
Dry matter	90.35	89.29
Crude protein	14.50	15.30
Total lysine	0.68	0.70
Digestible lysine	0.58	0.60
Total methionine + cysteine	0.64	0.64
Digestible methionine + cysteine	0.56	0.57
Calcium	1.20	2.80
Available phosphorus	0.35	0.40
Crude fibre	6.65	5.30
Neutral detergent fibre	18.33	14.74
Acid detergent fibre	6.86	6.17
Ether extract	4.50	4.10
Ash	7.13	9.91

[c] calculated values

Blood sampling and analysis

Blood was sampled at 22, 24 and 26-weeks of age from 18 birds (3 female/pen of 6 replicates for each age period). Approximately 6 mL of blood per bird was taken via the brachial vein into heparinised tubes in the morning. After blood centrifugation for 15 min. 3000 × g at 4ºC to separate plasma was transferred into 1.5 mL tubes and kept at −20ºC for further analysis. The plasma proteins (total protein, TP; albumin, Alb; uric acid, UA; urea nitrogen, UN) were determined using dry chemistry and reagent strips (Spotchem EZ SP-4430, Arkray Inc., Japan). Each plasma sample was analysed in duplicate for the biochemical variables. Calculated plasma parameters were globulins (Glb) as a difference between TP and Alb and the Alb/Glb ratio.

Excreta sampling and analysis

Excreta samples were collected at 22, 24 and 26-weeks of age from each pen, from 8-10 different places to obtain a pooled sample. After the excreta sub-samples from each pen were homogenised, the representative excreta pooled sample was deposited at −20ºC for N

analysis. The N content was determined according to OJEU (2009) by the Kjeldahl method using a semiautomatic analyser (Kjeltec Auto 1030, Hillerod, Denmark) as described by Hăbeanu et al. (2020).

Statistical analysis

Data were analysed by one-way ANOVA using the GLM procedure (SPSS v.20, 2011). The experimental unit for growth performance parameters was a replicate pen and each sample for the other variables. The responses are presented as means and SEM (standard error of the mean). Pearson's correlation was used to determine the relationship between certain variables. Statistically significant differences were assumed when $P<0.05$.

RESULTS AND DISCUSSIONS

Growth performance

The effect of age on growth performance parameters of Ross 308 female breeders is presented in Table 2. As expected, the results showed significant age-related changes from 21 to 26 weeks in body weight (2240 vs 3206 g/bird), feed intake (126 vs 153 g/bird/day), protein intake (18.27 vs 23.46 g/bird/day) and nitrogen intake (2.92 vs 3.75 g/bird/day) of female breeders ($P<0.0001$).

The breeder's growth performance was comparable with the recommended genetic strain guide (Ross-Aviagen, 2016).

Plasma protein profile

Our results showed a dynamic age-related change in the plasma protein profile of breeders (Table 3).

The relative levels of the total protein, albumin and albumin/globulin ratio showed a gradual and significant increase, with the lowest mean value at 22 weeks (3.22 g/dl TP; 1.59 g/dl Alb; 0.98 Alb/Glb ratio) and the highest at 26 weeks of age (4.23g/dl TP; 2.63 g/dl Alb; 1.65 Alb/Glb ratio; $P<0.05$).

Table 2. Effect of age on growth performance parameters of Ross 308 female breeders

Variables	Age (weeks)						SEM	*P*-value
	21	22	23	24	25	26		
Bodyweight (g/bird)	2240^{f}	2446^{e}	2650^{d}	2846^{c}	3033^{b}	3206^{a}	81.47	*0.0001*
Weight gain (g/bird)	175	206	204	196	188	172	14.86	*0.987*
Feed intake (g/bird/day)	126^{f}	133^{e}	138^{d}	144^{c}	147^{b}	153^{a}	2.22	*0.0001*
Protein intake (g/bird/day)	18.27^{f}	19.24^{e}	20.00^{d}	20.88^{c}	22.49^{b}	23.46^{a}	0.44	*0.0001*
Nitrogen intake (g/bird/day)	2.92^{f}	3.08^{e}	3.20^{d}	3.34^{c}	3.60^{b}	3.75^{a}	0.07	*0.0001*

SEM, standard error of the mean. $^{a\text{-}f}$Means within the row with different superscripts differ significantly (P<0.05).

Table 3. Effect of age on dynamic changes on plasma proteins profile of Ross 308 female breeders

Variables	Age (weeks)			SEM	*P*-value
	22	24	26		
Total protein (g/dl)	3.22^{c}	3.67^{b}	4.23^{a}	0.15	*0.0002*
Albumin (g/dl)	1.59^{c}	2.00^{b}	2.63^{a}	0.15	*0.0001*
Globulin (g/dl)	1.63	1.70	1.60	0.03	*0.290*
Albumin/Globulin ratio	0.98^{c}	1.18^{b}	1.65^{a}	0.10	*0.0001*
Uric acid (g/dl)	0.067^{c}	0.080^{b}	0.092^{a}	0.004	*0.004*
Urea nitrogen (g/dl)	0.021^{b}	0.024^{a}	0.025^{a}	0.001	*0.015*

SEM, standard error of the mean. ^{abc}Means within the row with different superscripts differ significantly (P<0.05).

Uric acid and urea nitrogen concentrations follow the same increasing trend from 0.067 g/dl UA and 0.021 g/dl UN at 22 weeks to 0.092 g/dl UA and 0.025 g/dl UN at 26 weeks of age ($P<0.05$). These variations could be related to the metabolic processes, nutrition changes, and physiological conditions of breeders (Tóthová et al., 2019). Overall, our plasma protein profile results were within normal limits (Ritchie et al., 1994). The blood total protein and albumin are important markers of dietary protein intake and haemoconcentration level (Kraus et al., 2021; Tóthová et al., 2019; Melillo, 2013; Pavlík et al., 2007). A higher total protein value indicates a better health status, and also this might be related to an estrogenic-induced rise in globulin in layers (Marono et al., 2017). Ritchie et al. (1994) stated that proteins are egg yolk precursors (vitellogenin and lipoproteins)

generated in the liver, transferred to the ovary through plasma, and incorporated into egg cells. Evaluating the effects of age, housing system, and genotype on blood parameters and egg quality in Czech and Slovak native hens, Kraus et al. (2021) reported a significant effect of age on total protein concentration. In birds, urea and uric acid are the end-product of protein metabolism, produced by the liver and eliminated by kidneys, considered a biomarker of renal function (Kim et al., 2012; Lumeij, 2008). The uric acid level is influenced by the balance between its production and excretion and is affected by genetic and dietary factors. Our results are in line with other studies (Harlap et al., 2021; Ibrahim et al., 2012) who mentioned that the increased level of uric acid in female birds is related to the ovulatory activities and oviposition, and also genotype had a significant effect (Eleroglu et al., 2015; Isidahomen et al., 2011; Silva et al., 2007).

Excreta nitrogen As shown in Figure 1, the total nitrogen content of excreta registered a tendency to increase at 24- and 26-weeks compared to 22-weeks of age ($P<0.06$), most probably as an effect of dietary protein content. Several studies reported that fed reduced crude protein diets had no adverse impact on the reproductive performance of broiler breeder hens (van Emous et al., 2018; 2015; 2013; Lopez and Leeson, 1995). Previous research reported that uric acid represents 50–60% of poultry excreta's total nitrogen content, which is converted to polluted ammonia (Malomo et al., 2018; Nahm, 2007; Nahm, 2003).

Lopez and Leeson (1995) stated that higher nitrogen excretion is due to ammonia, urea and creatinine elimination, excessive water intake/output, and litter and environmental quality issues in a breeder house. The same authors concluded that nitrogen excretion is directly related to nitrogen intake, so there is the potential to reduce the nitrogen content of manure.

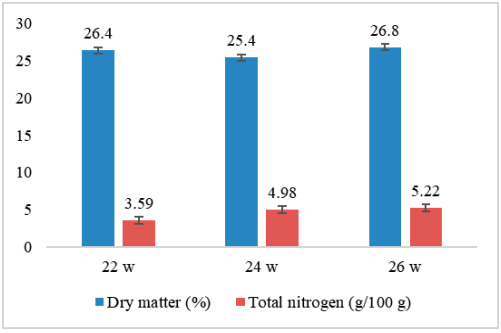

Figure 1. Effect of age on excreta nitrogen of Ross 308 female breeders

Positive significant correlations ($P<0.05$) were found between certain performance variables (feed intake, protein intake, nitrogen intake), plasma proteins and excreta total nitrogen (Table 4).

Table 4. Pearson's correlation between nitrogen excreta, performance and plasma proteins variables

	N g%	FI g/day	PI g/day	NI g/day	TP g/dl	Alb g/dl	Glb g/dl	Alb/Glb	UA g/dl	UN g/dl
N excreta g%	1	0.893** 0.001	0.828** 0.006	0.828** 0.006	0.840** 0.005	0.825** 0.006	0.047 0.905	0.780* 0.013	0.809** 0.008	0.819** 0.007
FI g/day		1	0.983** 0.0001	0.983** 0.0001	0.949** 0.0001	0.966** 0.0001	-0.149 0.702	0.951** 0.0001	0.920** 0.0001	0.890** 0.001
PI g/day			1	1.000** 0.0001	0.952** 0.0001	0.986** 0.0001	-0.245 0.526	0.989** 0.0001	0.907** 0.001	0.837** 0.005
NI g/day				1	0.952** 0.0001	0.986** 0.0001	-0.245 0.526	0.989** 0.0001	0.907** 0.001	0.837** 0.005
TP g/dl					1	0.985** 0.0001	0.038 0.922	0.933** 0.0001	0.827** 0.006	0.748* 0.021
Alb g/dl						1	-0.134 0.730	0.981** 0.0001	0.880** 0.002	0.774* 0.014
Glb g/dl							1	-0.324 0.395	-0.350 0.356	-0.185 0.634
Alb/Glb								1	0.908** 0.001	0.768* 0.016
UA g/dl									1	0.811** 0.008

**Significant correlation at the 0.01 level; *Significant correlation at the 0.05 level.

CONCLUSIONS

The results showed that age-specific changes in Ross 308 breeders' growth performance were positively correlated with protein intake and plasma protein profile, reflecting their good health state and physiological status. Total nitrogen content of excreta registered a tendency to increase with age and reflected the nitrogen intake. Further studies are necessary to develop solutions to reduce nitrogen losses in breeders.

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DESIGNING A METHODOLOGY FOR TRACKING OBESITY CASES IN DOGS AND CATS

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Abstract

Obesity has become one of the main health problems affecting progressively dogs and cats, being one of the essential medical topics in veterinary clinics. Veterinarians and nurses have identified an increase in the frequency of overweight or obese patients presenting at the clinic, and the causes highlighted by them regarding the weight gain of dogs and cats are a sedentary lifestyle, ad libitum food consumption, poor quality food, and associated conditions detected too late, of which the owners are not aware until further medical investigations. Therefore, the creation of a nutritional management plan for patients suffering from obesity or obesity complicated by other conditions was necessary to achieve the goal: a healthy weight for the pet.

Key words*: cats, dogs, nutritional plan, obesity, weight.*

INTRODUCTION

Obesity is defined as an excessive accumulation of triglycerides in adipose tissue due to an imbalance between energy demand and energy consumption, being the most common form of malnutrition in Westernized countries (German, 2006) for both humans and pets, mainly dogs and cats.

Two types of obesity are studied - obesity caused by over-consumption of food also called metabolically healthy obesity in medicine or overweight without associated health conditions (Miyazaki, 2007).

At the opposite end, there is pathological obesity which involves the appearance of associated changes such as metabolic, endocrinological, functional changes, and other diseases exacerbated by weight gain (Laflamme, 2006, 2008).

MATERIALS AND METHODS

In order to develop a monitoring plan for obesity in pets and to differentiate between the two types of obesity, the research focused on the development of the most detailed anamnesis forms and the nutritional consultation and monitoring form.

Inclusion criteria for pets, dogs and cats, are: body weight, body score (>4 or >5 - ideal body score according to WSAVA - Global Nutrition Committee), a body score scale from 1-9 being used (Lund, 2005), screening for health conditions by biochemical, endocrinological, pancreatic, urinary analysis of nutritional or metabolic conditions associated with overweight or obesity. The conditions associated with obesity are shown in Table 1.

RESULTS AND DISCUSSIONS

The subjects studied are dogs and cats that can be classified as overweight or obese according to their body weight at the time they are weighed compared to the breed-specific and official body weights and according to the body score published by the WSAVA-Global Nutrition Committee and used internationally in the pet obesity prevention programme.

In the literature we find three types of scales that characterize the body score of dogs and cats to determine the degree of overweight.

Two of these scales characterise body score with values between 1 and 5, for each value the animal's body parts and fat deposits in specific body regions are explained, the most helpful scale is the one with values between 1 and 9

with the body regions better described and with precise details about fat deposits.
Veterinarians at the University of Tennessee developed a scale expressing body fat score in percentages, with values ranging from 20% body fat, a value found in the literature as the "optimal" percentage of body fat in dogs and cats (Bjørnvad et al., 2011, Witzel et al., 2014) to 70% body fat associated with morbid obesity.
The animal will be approached by visual inspection and fitting into a body score, by palpation the ratio of bone, muscle and fat mass will be made and the weighing scale will be used as a measuring instrument.
This will compare the weight of the animal at that time with the ideal weight of the species and breed, and also the medical tests of interest before and at the end of the diet program would provide reliable information about the health status of the animal and possible diseases associated with obesity.
The medical history of the patient should be obtained by evaluating the individual health booklet, the information provided by the owner or even the medical record of the animal, these are obligatory steps in order to know as much as possible about the health status of the patient to be studied.
The research will focus on the creation of detailed history sheets of each patient to be studied, thus observing the state of health and body maintenance with the help:
- observation of clinical aspects of interest (appearance of fur, skin and teeth, appearance of fat deposits, listening to breathing sounds, heart rate, etc.);
- ultrasound comparison of areas of fat before and after participation in a diet programme;
- comparison of biochemical profiles of patients in the study before and after the start of the diet programme;
- the owners will be offered to change the diet before the start of the weight loss programme to a veterinary diet specialised for the health conditions the patient is suffering from and also to a diet specially designed for overweight patients.
The nutritional factsheet as presented in Table 2 is structured in the following sections: essential identification data of the dog/cat and owner (name, species, breed, age, gender), current medical data of the patient (hormonal status, weight, body score), medical and dietary history of the patient (conditions diagnosed so far at other hospitals or veterinary practices - are called conditions, current medication treatments or receipt of dietary or vitamin-mineral supplements, allergies to medication, food or food types), the patient's feeding history (type of food - dry food - brand, wet food - brand, home-cooked food - type, preferred type of food - commercial - dry or wet food, cooked or mixed food, amount of food - number of meals per day, treats - type and number of treats per day), data on the patient's environment (activity level of the dog/cat, space available, other animals in the house, possibility of being fed individually).
The information and communication stage with the owner involves informing the owner of the pet's current body score and the target weight that the patient should reach within a certain period of time.

Table 1. Obesity-related or exacerbated conditions

Metabolic changes	Endocrinopathies	Functional changes	Other diseases
Anaesthetic complications Dyslipidemia or hyperlipidemia Glucose intolerance Hepatic lipidosis (cats) Insulin resistance	Diabetes mellitus Hyperadrenocorticism Hypopituitarism Hypothalamic lesions Hypothyroidism Insulinoma Pituitary chromophobe adenoma	Decreased immune function Dystocia Heat intolerance Hypertension Osteoarthritis/joint stress/musculoskeletal pain Respiratory stress or dyspnoea	Impaired kidney function Cardiovascular disease Dermatopathies Neoplasia Oral (dental) diseases Pancreatitis Transitional cell carcinoma (bladder) Urinary tract disease (cats)

Laflamme, 2006

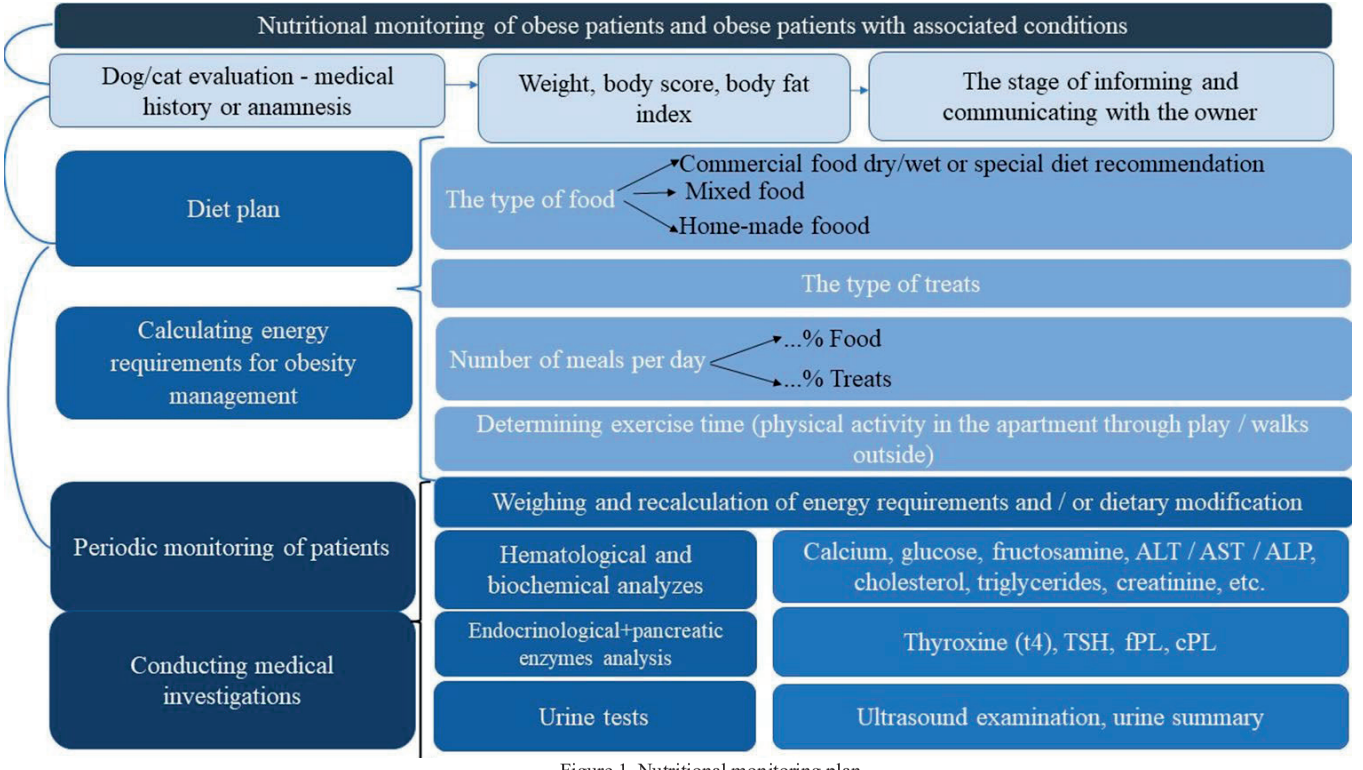

Figure 1. Nutritional monitoring plan

Table 2. Nutritional consultation factsheet for dogs and cats

I.	**Basic animal identification data:**	
Essential data for the identification of the animal and current data of the body condition of the dog or cat.	Date of arrival at the clinic: Name: Name of owner: Species: Breed: Age: Sex: Hormonal status: Weight: Body Score:	
II.	**Patient's medical and dietary history**	The owner's reply
The medical history of the animal provided by the owner from the animal's medical records tests/investigations performed at other veterinary clinics or hospitals.	From your knowledge (previous visits to the veterinary clinic), does your pet suffer from any chronic condition/illness/autoimmune disease? (cardiological, orthopaedic, urinary, renal, digestive, respiratory)	
	Is the animal currently on medication?	Yes/ No What drugs?
	From your knowledge, is the animal allergic to any food/medication or type of food?	Yes/No Allergens:
	Does the animal receive food supplements / vitamins / minerals? What type? How often?	
Feeding dietary history of the animal provided by the owner or sometimes several family members.	What kind of food does the animal currently receive? (home-cooked food and/or dry food - brand / wet food - brand)	
	Does the animal prefer cooked or commercial dry/wet feed?	
	How much feed does the animal get? How many times a day? Does the animal receive treats? What type? How often?	
Data about the dog or cat's environment.	How active is the dog/cat? (how much time the pet spends outside or inside playing/how often is the pet taken for walks)	
	Are there other animals in the apartment/house? Is it possible to feed the animal individually?	
The willingness of the owner to invest in additional medical investigations for the companion animal.	Are biochemical, haematological, pancreatic and/or endocrinological tests desired to be performed?	Yes/No

Figure 2. Veterinary weight scale - Hong Kong Metal for dogs

In order to minimise variability factors, constant communication is made with the owner and written recommendations are given at the beginning of the diet such as:

- a phased transition to the new food involving gradual habituation (over 7 or 10 days increasing the amount of the new food daily to replace the old one) of the dog or cat to the recommended food for weight loss and/or other health conditions;
- adhering to a feeding schedule for both dogs and cats by appointing times of day or even fixed times;
- compliance with certain quantities of the recommended food to be weighed using a kitchen scale;
- feeding the dog or cat individually to minimise the risk of the pet eating from other animals in the household.

A simplified weight monitoring sheet is also provided for the animal owner so that the nutritional programme sheet is easy to understand and follow.

The weight monitoring sheet contains: the current weight of the pet (dog or cat) and the target weight the pet should reach within a set time. The second element of the sheet is the kilocalorie/day or energy requirement calculated for obesity management in the dog or cat.

Figure 3. Veterinary weight scale - U-grow for cats and small dogs

Also integrated into the sheet is the nutritional programme itself displayed as a table divided into columns containing: "times of day - morning, noon and evening", which can be converted into fixed times if they wish and manage to follow as fixed a feeding schedule as possible; "type of food/feed to be given at one of the times of day"; "grammage/quantities in grams of dry or wet food to be given", "number of kilocalories for each type of food/feed".

At the end a total will be made of the amount of food and kilocalories consumed.

A table for owners who have the possibility to weigh the animal is also integrated in the monitoring form for overweight patients, containing: date of weighing, weight and possible observations (changes in diet, possible behavioural changes of the animal, etc.).

CONCLUSIONS

A first recommendation of nutritionists, specialists in canine and feline nutrition, refers to the determination of the degree of body maintenance of the animal by consulting the visual physical assessment tables of the risk indicators of overweight or obesity, a recommendation that comes to the assistance of pet owners and veterinarians.

The key points that bring success in treating obesity are:

- setting the ideal (or target) weight;
- a fixed programme and a set amount of food to reach the target weight;
- appropriate low-calorie but nutritionally balanced feed;
- all these, combined with gradually increased physical activity, are practical ways of managing being overweight in dogs and cats.

In addition, regular monitoring of patients through weighing, recalculation of energy requirements, dietary modification and a review of the medical test plan provides the success of an appropriate nutritional programme.

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MONITORING OF DAIRY FARMS TO ASSESS THE POTENTIAL LEVEL OF POLLUTION OF ANIMAL FEED AND ANIMAL PRODUCTION

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Abstract

The over pollution in recent years has meant that the relationship between animal husbandry and the environment to be approached in the light of a sustainable vision, focused on animal welfare and ensuring the safety of feed and animal production. Given the influence of pollutants on the environment, this paper aims to outline the relationship between animals and environmental pollution, for assessing the potential level of pollution of feed and animal production. Thus, by correlating with the data from the literature, for three dairy farms, located in different geographical areas, was assessed, by observation and questionnaires, the specificity of activities in relation to monitoring feed and milk pollution. Following the monitoring and application of the evaluation questionnaire, the particularities of each farm and also the specifics of feed within them were highlighted, obtaining important information which allowed the assessment of the relationship between environment and animal husbandry, all of this for evaluating the potential level of pollution of feed and animal production and for classification of the studied farms by expected level of pollution: S - low; M - medium; R - high.

Key words*: animal production, environment, feed, pollutants.*

INTRODUCTION

The current situation regarding the level of global pollution has emphasized more than ever the interest for the environment, for human health and also for animal welfare. Despite numerous global measures to reduce pollution, harmful emissions released into the environment continue to cause important damages to soil, crops, animals or people (EEA, 2017).

Raising animals according to rational principles, in optimal and effective conditions, as well as the level of animal productivity, highlights particularly the influence of feed in their development (Mitchell, 2007). However, the process of rational animal husbandry involves a combination of some complex actions, focused on the growth and on the development of animals as a result of ensuring a nutritionally balanced diet and on ensuring the safety of feed and maintaining a harmonious relationship between the animals and the environment, which needs to be kept balanced and healthy.

As for the other trophic elements, the environmental pollutants can be harmful for the feed and dangerous for the animal body and also for the animal production. Despite many efforts to reduce pollution, however, pollutants remain dynamic compounds in the environment and can be a real threat to all the elements of the environment, forming a continuous cycle of contamination, from the soil, to vegetal products (animal feed), to animals and their productions and to human body (Nica et al., 2012; Manciulea & Dumitrescu, 2016).

In relation to the environment, pollutants can have important negative effects on crops (inhibiting the development of plants), on animals (metabolic disorders, decreased productivity, qualitative degradation of animal production) (Wielsoe et al., 2017), while on humans, pollutants can have a strong toxicity (Desiato et al., 2014).

In the production chain, the vegetal products used as feed may be exposed to contamination as a result of the absorption of harmful compounds from the soil or as a consequence of various human activities, such as industrial production, transport or agricultural activities (Rychen et al., 2008; Ukaogo et al., 2020). In terms of of animal production, the contamination may be a consequence of consumption of contaminated feed (EFSA,

2005; Rychen et al., 2008; Tao et al., 2009) and of metabolic transfer of pollutants stored in the animal body to the production obtained (Aytenfsu et al., 2016).
In the literature, different proportions of pollutants have been identified by studies, both in vegetal products used as feed (Albu et al., 2007; Dai et al., 2016; Piskorska-Pliszczynska et al., 2017; Tahir et al., 2017; Bedi et al., 2018; Miclean et al., 2019), as well as in the animal production obtained (Ahmadkhaniha et al., 2017; Chen et al., 2017; Lapole et al., 2017; Marin et al., 2020).
For these reasons, the purpose of this paper is to monitor the activities and the organization of feed bases in some dairy farms, in relation to environmental pollution, in order to assess the potential level of pollution of animal feed and animal production obtained (milk), necessary for a future quantification of the potentially pollutants found.

MATERIALS AND METHODS

According the specificity of the study and its associated purpose, the assessment of the potential level of pollution of feed and milk was carried out by monitoring, during 2021, three dairy farms (A, B, C), of different sizes (between 40-390 animals), selected depending on the level of pollution expected in their geographical area: A - mountain area; B - rural area; C - urban area (Figure 1).

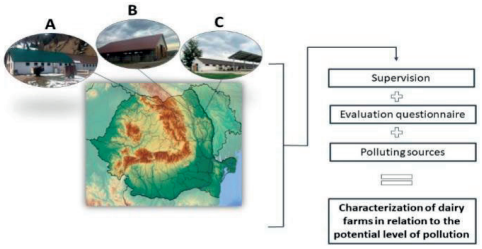

Figure 1. Farm monitoring stages

The characterization of the farms and the identification of the most relevant aspects in terms of production activity and ensuring the fodder base, in relation to monitoring feed pollution and animal production pollution (Table 1), was achieved through a combination of three actions which included:

- direct supervision and observation of the specific activities carried out on the farm;

Table 1. Farm monitoring questionnaire

Farm monitoring		Relevant information obtained
The specifics of the farm and livestock	Geographical location (type of settlement*, geographical coordinates, climate)	Correlation between geographical location and the climatic conditions (temperature, wind direction)
	Polluting activities in the vicinity of the farm	Identification of the main sources of pollution
	Herds, breeds and daily milk production	Assessment contaminated milk production
	Raising system; type of animal shelter; the milking operation	Assessment of direct exposure to pollution and of the possibilities of accidental contamination
Specific alimentation	Types of feed and structure of ration	Identification of the administered feed
	Free grazing	Assessment of direct exposure to pollution
	Number of meals administered	Frequency of possible contamination
	Feed administration	Assessment of the possibilities of accidental contamination
	Adaptation of the ration according to the physiological status of the animals	Correlation of physiological status with the action mechanisms of pollutants
The feed base	Feed origin (own production)	Assessment of pollutant traceability
	Quality analysis of feeds	Characteristics of feeds
	Harvesting, processing	Assessment of the possibilities of accidental contamination
	Crops: applied treatments and rotation	Possible contamination in terms of used fertilizers
	Feed storage	Assessment of direct exposure to pollution
Water	Unconventional water sources	Assessment of external pollution sources
Other information	Animal and shelter care / Substances used	Assessment of the possibilities of accidental contamination

**rural / urban*

- taking specific information on the activity of the farm, the feed base and the specifics of the feed using evaluation questionnaires developed in accordance with the data found in the literature;
- identification of pollution sources in the vicinity of farms and correlation of information found with data from the literature.

RESULTS AND DISCUSSIONS

Following the monitoring and application of the evaluation questionnaire, the particularities of each farm studied were highlighted, as well as the specificity of the animal feed within them (Table 2). Were obtained valuable information to assess the level of potential pollution of feed and animal production and for the classification of the three farms by pollution levels.

The information about the breeding system of animals and also the organization of the activity of the farms highlighted in general important differences between the three farms analyzed, especially in terms of the complexity of the activities developed in the farm. The differences highlighted focused on the characterization of farm A as having a lower pollution potential because, compared to farms B and C, where animal husbandry is carried out at an intensive system, in farm A a semi-intensive breeding and maintenance system was highlighted, which showed that it has the lowest operating capacity and the lowest degree of technology, therefore lower pollution risk.

Table 2. Information obtained by assessing the specificity and organization of the feed base in the monitored farms

	Farms							
	A			B		C		
Geographical location (type of settlement*, geographical coordinates, climate)	Mountain* area			Rural* area		Urban* area		
	47°34′N - 25°19′E			47.2134° N - 27.5066° E		47°09′11″N - 27°39′41″E		
	temperate, baltic and east-continental influences wind direction - variables			temperate - continental wind direction: NW – SE humidity: 63-84%		temperate - continental wind direction: WNW humidity: 60-81%		
Polluting activities in the vicinity of the farm / Generated pollutants	Stationary sources: former mining area			Mobile sources: air pollution		Stationary sources: chemical and steel industry		
						Mobile and surface sources: road and air transport/ POPs		
	Agricultural activities: chemical treatments applied to crops / OCP							
Herds, breeds and daily milk production	40			55		390		
	Brown Swiss; Romanian Black Pied			Fleckvieh		Holstein Friesian; German Black Pied		
	12 L / day			20 L / day		25 L / day		
Raising system; type of animal shelter; the milking operation	Stall–related maintenance in closed shelter; Pasture - free maintenance in closed shelter Milking system: mechanical, individual			Stall - free maintenance in closed shelter				
				Milking system: Mechanical, 8 seats /2 rows		Milking system: Mechanical, 32 seats /2 rows		
Types of feed and structure of administered ration	Type of feed		% of ration	Type of feed	% of ration	Type of feed	% of ration	
	Cold season ration	Natural hay	50	Corn silage	56	Corn silage	45.45	
						Alfalfa silage	10.9	
		Corn silage	50	Alfalfa hay	22.4	Alfalfa hay	5.45	
						Brewers grain	18.18	
				Corn grain	11.20	Corn grain	6.35	
	Warm season ration	Natural pastures	100	Soybean meal	8.40	Triticale grains	4.54	
						Soybean meal	7.26	
				Supplements	2	Supplements	1.87	
Free grazing	In warm season			Not		Not		
Number of meals	2			2		2		
Feed administration	Manually			Technological trailer		Technological trailer		

Table 2. Information obtained by assessing the specificity and organization of the feed base in the monitored farms (continuation)

	Farms		
	A	B	C
Adaptation of the ration according to the physiological status of the animals	Yes - Gestation - elimination of CS	Yes - Gestation - elimination of CS - reduction to ½ of the proportion of concentrates	Yes - Ration administered on productive levels (preparation, lactation/parturition, breast rest) - changing the proportions of concentrates and silage
Feed origin (own production)	Cold season ration - 50% Warm season ration - 100%	75%	71%
Quality analysis of feed	To third parties	Own laboratory	Own laboratory
Harvesting, processing	May - August Manually + Mechanized	May - October Mechanized	May - October Mechanized
Crops: applied treatments and rotation	3-11 ha 700-1100 m altitude Natural fertilizer Monoculture	8-25 ha Monoculture ** at 4 years – alfalfa Fertilization: N;S,P/ Crop protection – herbicides	20-100 ha Monoculture ** at 4 years – alfalfa Fertilization: N; P, Cu, Mn, Zn / Crop protection – herbicides, pesticides, fungicides, insecticides
Feed storage	Type of storage (closed wood storage type and open cell storage type)	Type of storage (open type warehouses, open cell storage type, closed type deposits)	
Unconventional water sources	Warm season (grazing)	Not	Not
Animal and shelter care / Substances used	Current sanitation actions	Current sanitation actions: Milking parlors: substances based on $C_3H_6O_3$, CH_3COOH, $C_2H_4O_3$, H_2O_2, NaOH, KOH, NaClO Animals: substances based on $C_3H_6O_3$	

***crop rotation for alfalfa.*

In the literature, Aytenfsu et al. (2016) mentioned the importance of the breeding system for assessing the possibility of exogenous contamination of feed and milk, therefore, highlighting the system of breeding and maintenance of animals in relation to the study of pollutants, found its applicability for assessing the possibility of accidental contamination of feed and animal production, in the shelter area, in the food storage area or during transport.

The indications regarding the geographical area of each farm, correlated with the environmental conditions in the targeted area (temperature, wind direction) highlighted the position of farm B and of farm C on the dominant wind direction, which can be an additional way of contamination with pollutants generated from neighborhood sources.

The evaluation of the feed base of each farm showed that all three farms have a feed base provided mainly from their own vegetable production, which, in the context of the study, highlights the possibility of a more rigorous control of feed production and allows further study of the traceability of pollutants.

At the same time, the evaluation of the farms showed that the complexity of the feed bases of the three farms analyzed and the specificity of the animal feed (Figure 2) changed in proportion to the intensification of the farming system applied. In the analyzed context, for farm A a less developed feed base was highlighted, with seasonal ration and the majority of feed obtained on its own, which, in the context of the study, shows that the risk of contamination within it is lower compared to other two farms analyzed (B and C). The diversification of feed bases, specific to farm B (rural area) and farm C (urban area), as well as the predominant mechanization of operations, bring to the fore the possibility of a higher level of pollution than in the case of farm A (rural area, mountain); these details are also relevant for the study of the traceability of pollutants, because, within the same farms (B; C), a higher proportion of feed is obtained from external sources, thus being more difficult to assess in terms of monitoring pollutants.

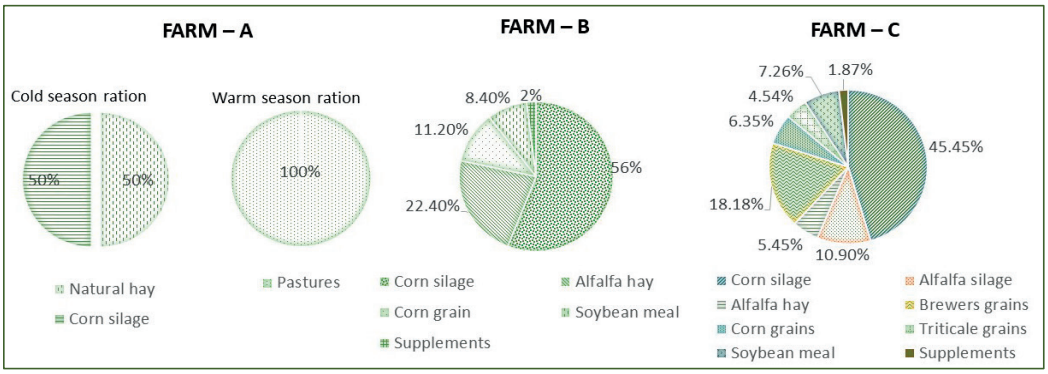

Figure 2. Type of feed and proportion of ration

Particularly important in determining the incidence of feed and milk pollution, the identification of the main sources of pollution for all the farms analyzed allowed the monitoring of certain types of pollutants whose presence is predominant, in accordance with the identified issues in Table 3. The forms of pollution found has included stationary sources, such as positioning in a former mining area, as is the case of farm A, important, given the persistence of some of the pollutants in the environment; mobile sources such as transport or industrial activities, common to the farm B and C, but also various agricultural activities with potential pollutants (application of potentially polluting substances), especially for the farms B and C.

Table 3. Sources of pollution identified in the vicinity of the farms

FARM			A	B	C	GENERATED POLLUTANTS
Location and expected level of pollution			*Mountain* 47°34′N, 25°19′E	*Rural* 47.21° N, 27.50° E	*Urban* 47°09′N, 27°39′E	
POLLUTION SOURCES				-		
Stationary sources	Waste combustion				●	PCB, HCB, PCDD/F, PAH, Cd, Cr, Cu, Hg, Zn
	Chemical and steel industry				●	As, Cd, Cr, Cu, Fe, Mo, Ni, Pb
	Mining activity		●			Cd, Cu, Fe, Hg, Mn, Ni, Pb, Zn
Mobile and surface sources	Road transport (car aerosols, tire wire)			●	●	As, Cd, Co, Mn, Ni, Pb, Zn
	Airline (landing-take-off cycles)				●	Cd, Mn, Ni, PCDD/F
	Construction industry				●	Cd, HCB, Pb, PCB
	Wastewater treatment plant				●	Cd, Cu, Fe
Agricultural activities	Organic fertilizers / Compost		●	●	●	Cd, Cr, Cu, Fe, Hg, Mn, Mo,Ni, Pb, Zn
	Treatments applied to crops	Mineral fertilizers		●	●	Cd, Cu, Mo, Pb, Zn
		Pesticides			●	Cu, Hg, OCP, Pb, Zn
CONTAMINATION FACTORS				-		
Free grazing			●			-
Unconventional sources of water			●			
Fedd from third parties				●	●	
Chemicals for the care of animale, shelters and facilities				●	●	

***HCB**=hexachlorobenzene; **OCP**=organochlorine pesticides; **PAH**=polycyclic aromatic hydrocarbons; **PCDD/F**=polychlorinated dibenzo-p-dioxins **(D)**/furans **(F)**; **PCB**=polychlorinated biphenylene.*

Consistent with the issues highlighted by Ukaogo et al. (2020), the sources mentioned in Table 3 reported as main potential pollutants generated the persistent organic compounds and heavy metals, which subscribes to other research in the same field (Shafy & Mansour, 2016; Kulkarni et al., 2019; Senthikumar & Naven Kumar, 2020).

Table 4. Chemical treatments applied to vegetable crops

	Vegetal product	Type of treatment	Commercial formula	Quantity/ha	Active substance
B	Alfalfa	Weed control (herbicide)	Pulsar 40	1.1 L	40 g/L Imazamox
	Corn for silage	Fertilization	Sulfammo–25–MPPA–1	170 kg	25% N (18 % N amoniacal; 7% N nitric); 31% SO_3; 2% MgO
		Weed control (herbicide)	Principal Plus	440 g	9.2% Nicosulfuron; 55% Dicamba; 2.3% Rimsulfuron
	Corn for grain	Fertilization	DAP 18–46–0	250 kg	18% NH_4; 46% P_2O_5
			Sulfammo–25–MPPA–1	250 kg	25% N (18% N amoniacal; 7% N nitric); 31% SO_3; 2% MgO
		Weed control (herbicide)	Principal Plus	440 g	9.2% Nicosulfuron; 55% Dicamba; 2.3% Rimsulfuron
C	Corn for silage *and* Corn for grain	Fertilization	Uree	100 kg	$CO(NH_2)_2$
			Complex Azomures NPK 20–20–0 vq	100 kg	20% N total; 20% P_2O_5 total; 60% P_2O_5 water soluble; 98% P_2O_5 soluble in citric acid 2%; max. 0,6% water
			Nitrocalcar	150 kg	27% N; 7% CaO; 5% MgO
		Weed control (herbicide)	Henik	1.5 L	40 g/L Nicosulfuron
			Mustang	0.6 L	6.25% Florasulfam; 30% Acid 2,4D EHE
			Adengo	0.4 L	225 g/L Isoxaflutole; 90 g/L Tiencarbazon-methyl; 150 g/L Cyprosulfamide (safener)
	Alfalfa	Fertilization	Complex 16–16–16	250 kg	16:16:16 N:P:K
		Weed control (herbicide)	Corum	1.2 L	480 g/L Bentazon 22.4 g/L Imazamox
	Triticale	Fertilization	Uree	150 kg	$CO(NH_2)_2$
			Nitrocalcar	150 kg	27 % N; 7 % CaO; 5 % MgO
		Fertilization	Lebosol – Mix Cereale	1.5 L	1.6% Cu – $Cu_2Cl(OH)_3$ 25 g/L; 11.5% Mn – MnO_2 183 g/L; 4.9% Zn – ZnO 78 g/L.
		Weed control (herbicide)	Pixxaro Super	0.3 L	12 g/L Halauxifen-methyl; 280 g/L Fluroxipir mepthyl; 12 g/L Cloquintocet-mexil
		Fungi control (fungicide)	Orius	0.5 L	250 g/L Tebuconazol
			Falcon Pro	0.5 L	53 g/L Protioconazol; 224 g/L Spiro-xamină;148 g/L Tebuconazol
		Insect control (insecticide)	Mospilan	0.15 L	20% Acetamiprid

Relevant for the study of pollutants, variable factors for contamination were identified (Figure 3): the presence of free grazing and unconventional water sources in the vicinity of pastures (farm A); intensification of agricultural activities; an important percentage of feed from external sources or use of chemical substances for the care of animals or for shelters (farm B and C).

Given that the modernization of agriculture has led to an increase in chemical treatments applied to crops (Chavoshani et al., 2020) and, taking into account the particularities related to the possibility of accumulation and persistence of various pollutants in the soil, monitoring the potential level of feed pollution aimed how to obtain crop production in terms of treatments applied or crop rotation, highlighting in general as potentially harmful actions the use of natural

fertilizer-farm A, dangerous due to the fact that there is no control over their components or the application of various fertilizers on base of N, P, K and various chemical treatments to control weeds or pests (Table 4).

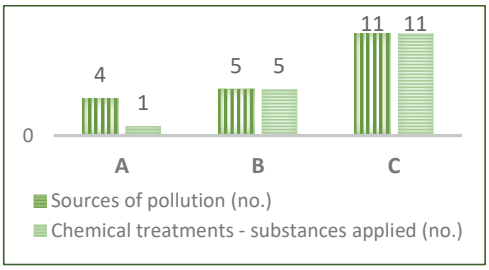

Figure 3. Evaluation of potential polluting level

Following the general characterization and assessment of the analyzed farms, but also following the identification of the main sources of pollution in their vicinity, the farms were grouped according to potential levels of pollution. Given the application of a semi-intensive growth system and also the location in an area devoid of important sources of pollution, farm A was considered to have a low potential level of pollution. Regarding farm B, given its position in the vicinity of one of the most polluted cities in the country, but also the location of the main pollution sources from the urban area on the dominant wind direction (NW-SE), the farm was classified as having a medium level of potential pollution.

In contrast to the other two farms, for farm C, the expected pollutant level is not only given by the positioning in the vicinity of the city, but is amplified by the concentration of many industrial factories in the vicinity of the farm, as well as the existence of intense air transport activities, activities in the field of construction or road infrastructure, which means that farm C is considered to have the highest potential level of pollution.

CONCLUSIONS

Following the monitoring of the specific activity of the analyzed farms, focused on the study of feed bases in relation to the assessment of the potential pollution level of feed and milk, it was highlighted that all actions carried out on a farm are relevant in the study of feed pollution and animal production pollution, offering as a whole, the possibility of a continuous assessment of the relationship between the environment, animal husbandry, feed safety and animal production.

The information obtained allowed grouping the studied farms into categories, depending on the expected level of exposure to pollution: L–low level; M–medium level; H–high level.

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EFFECTS OF DIETS WITH INACTIVE DRY YEAST ADDITION ON PRODUCTIVITY AND HEALTH STATUS IN DAIRY COWS

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Abstract

This study evaluated the influence of inactive dry yeast in diets of dairy cows on productivity, blood-urine parameters, and faecal score. The research was carried out on a number of 30 Romanian Black and Spotted lactating dairy cows, with 583±16.99 kg live weight, divided into three groups (n=10), two experimental E_1 and E_2 groups, and one control group (C). The experimental group E_1 received 120g inactive dry yeast/head/day, while the experimental group E_2 received 150g inactive dry yeast/head/day during a 28 days trial. Dietary treatment effects were determined using analysis of variance (ANOVA) for repeated measures (mixed model). Supplementation of the diet with inactive dry yeast generated an increase of milk yield ($P<0.10$) for the E_1 and the E_2 groups, compared with the C group. For the milk lactose percentage, a treatment influence ($P<0.05$) and period influence ($P<0.10$) between the studied groups were observed. Also, for some blood indicators, a dietary treatment influence was recorded. There were no significant effects of the treatments on the other studied parameters. Dry yeast addition in dairy cows diets can have a positive effect on milk yield performance.

Key words*: dairy cows, dry yeast, health, productivity.*

INTRODUCTION

Using yeasts as feed additives for ruminants represents a global trend, with a high number of body literature showing beneficial positive effects on productive parameters (Amin & Mao, 2021). Therefore, yeasts (*Saccharomyces cerevisiae* sp.) through their high nutrient content, represent a potential feed resource that can be used in dairy cows diets, to optimize the structure of the concentrates. Yeasts promote livestock productivity, being beneficial to animal health and are being used as ruminal enhancer (Lopreiato et al., 2020; Faccio-Demarco et al., 2019). In the rumen, yeasts can utilize the remaining dissolved oxygen, protecting anaerobic microbes from the damaging effects of oxygen. Yeasts can increase rumen motility and regulate ruminal pH, minimizing the danger of acidosis in dairy cattle (Chaucheyras-Durand et al., 2016). Yeasts improve cattle feed digestion and metabolism in a variety of ways, including increasing nutritional digestibility, optimizing volatile fatty acid proportions, decreasing ammonia–nitrogen levels, lowering pH fluctuation, and stimulating microbial communities in the rumen (Cagle et al., 2020; Perdomo et al., 2020; Dias et al., 2018a). In ruminants, the beneficial effects of yeasts as microbial feed additives are represented by better ruminal digestion, improved nutrient utilization, higher feed intake, improved milk yields and weight gains (Ogunade et al., 2019; Habeeb, 2017; Sartori et al., 2017). The aim of this study was to evaluate the influence of inactive dry yeast in the diets of dairy cows on productivity, blood-urine parameters and faecal score.

MATERIALS AND METHODS

The study was carried out at the Experimental Farm of the Research and Development Institute for Bovine Balotesti (44°36′46″N 26°4′43″E), Romania, in accordance with the *Romanian Law no. 43/2014* and the *Council Directive 2010/63/EU* on the protection and handling of animals used for scientific purposes. The design trial used 30 multiparous Romanian Black and Spotted dairy cows, clinically healthy, that were divided in three homogenous groups (n = 10/group), balanced for age, milk yield and body weight (group C: 5.6 years old, 18 kg of milk/day/head, 600 live

weight; group E_1: 5 years old, 18 kg of milk/day/head, 590 live weight; group E_2: 5 years old, 18 kg of milk/day/head, 560 live weight). Diets were supplemented with either 0 g inactive dry yeast/head/day (C), 120 g inactive dry yeast/head/day (E_1) and 150 g inactive dry yeast/head/day (E_2) for 28 days. The inactive dry yeast used in this study was procured from certified suppliers. The cows were housed in a tie-stall stanchion barn. The diet consisted of 6 kg alfalfa hay, 25 kg corn silage, and 6 kg concentrates/head/day. Cows had mineral licks and water available for *ad libitum* consumption. The nutritional value of the analyzed feeds and the nutrient composition of the diets used in the study are presented in Tables 1 and 2.

Table 1. The nutritional value of the feeds used during the trial (g/kg forage)

Nutritive crude value	DM, kg	MNU	DIPN, g	DIPE, g	Ca, g	P, g
Alfalfa hay	0.87	0.69	106	82	10	1.9
Corn silage	0.26	0.21	13	17	1.2	0.5
Concentrates	0.90	1.00	120	82	9.6	6.9
Dry yeast	0.90	1.05	315	178	5.3	16

DM=dry mater; MNU=milk nutrition units; DIPN=digestible intestine protein allowed by the nitrogen content of the fodder; DIPE=digestible intestine protein allowed by the energy content of the fodder; Ca=calcium; P=phosphorus.

Table 2. The diet used during the trial

Feed	kg	15-18 DM, kg	13-15 MNU	1200-1350 DIPN, g	1200-1350 DIPE, g	90 Ca, g	54-60 P, g
Alfalfa hay	6	5.22	4.14	636	492	60.0	11.40
Corn silage	25	6.50	5.25	325	425	7.2	12.50
Basic ratio input	**31.00**	**11.72**	**9.39**	**961**	**917**	**67.2**	**23.9**
Concentrates	6.0	5.40	6	720	492	57.60	41.40
Total basic ratio input (C)	**37.00**	**17.12**	**15.39**	**1.681**	**1.409**	**124.80**	**65.30**
Inactive dry yeast	0.12	0.11	0.13	36.60	21.36	0.64	1.92
Total (C + E_1)	**37.12**	**17.23**	**15.52**	**1717.60**	**1430.36**	**125.44**	**67.22**
Inactive dry yeast	0.15	0.14	0.16	47.25	26.70	0.80	2.40
Total (C + E_2)	**37.15**	**17.26**	**15.55**	**1728.25**	**1435.70**	**125.60**	**67.70**

C=diet supplemented with 0 g inactive dry yeast/had/day; E_1=diet supplemented with 120 g inactive dry yeast/had/day; E_2=diet supplemented with 150 g inactive dry yeast/had/day; DM=dry mater; MNU=milk nutrition units; DIPN=digestible intestine protein allowed by the nitrogen content of the fodder; DIPE=digestible intestine protein allowed by the energy content of the fodder; Ca=calcium; P=phosphorus.

Cows were fed and milked twice daily (5AM and 5PM). The assessment of the productive performances and health status of the animals used in this study, such as milk yield, milk composition (fat %, protein %, lactose %), hematological, serum biochemical and urinary parameters were performed. Milk samples (30 ml) were collected during morning and evening milking before feeding and kept at 4°C for analysis. The milk composition was evaluated using the Ekomilk 120 ultrasonic analyzer. The milk/blood/urinary samples and faecal score were evaluated at following intervals: day 0 (start of the trial), at 12 days, and 28 days, for all three treatments. The Abacus Junior Vet 5 automatic analyzer was used for performing hematological examinations (red blood cells, hemoglobin, hematocrit, total white blood cells, lymphocytes, monocytes, neutrophils). The serum biochemical parameters (total proteins, asparagine aminotransferase, alkaline phosphatase, total calcium, inorganic phosphorus) were analyzed using a semiautomated biochemical analyzer StarDust MC 15.

Urine samples were collected in 50 ml sterilized vials, as free catch during micturition. Urine examination, with the following parameters: bilirubin, urobilinogen, ketones, ascorbic acid, glucose, protein, blood, pH, nitrites, leukocytes and specific gravity, were determined with the DocUReader urine analyzer. Evaluation of the faeces was performed on the basis of a scale numbered from 1 to 5, taking into account the '3C': colour, consistency and content, before the morning feeding. Results were expressed as a mean (± standard error of the mean). Dietary treatment effects were determined using analysis of variance

(ANOVA) for repeated measures mixed model. The statistical model was:

$$Y_{ijk} = \mu + T_i + P_j + C_k + (T \times P)_{ij} + e_{ijk}$$

where: Y_{ijk}=dependent variable; μ=overall mean; T_i=effect of treatment i (fixed effect); P_j=effect of time j (fixed effect); C_k=effect of animal k (random effect); $(T \times P)_{ij}$= interaction treatment i and time j; e_{ijk}=residual error term. Significance level was considered when $P \leq 0.05$ and trends when $P < 0.10$.

RESULTS AND DISCUSSIONS

The studied animals had an average daily consumption (depending on the treatments), between 17.12-17.26 kg DM/kg, 15.39-15.55 MNU, 1681-1728.25 g PDIN, 1409-1434.70 g PDIE, 124.80-125.60 g Ca, and 65.30-67.70 g P, values that fall within the standard norms in relation to body weight and milk production (Table 3).

Table 3. The average daily intake of the dairy cow's diets

Season/Group	DM, kg	MNU	PDIN, g	PDIE, g	Ca, g	P, g
C	17.12	15.39	1681	1409	124.80	65.30
E_1	17.23	15.52	1717.60	1430.30	125.44	67.22
E_2	17.26	15.55	1728.25	1435.70	125.60	67.70

Note: C=diet supplemented with 0 g inactive dry yeast/had/day; E_1=diet supplemented with 120 g inactive dry yeast /had/day; E_2=diet supplemented with 150 g inactive dry yeast/had/day. DM=dry mater; UNL=milk nutrition units; PDIN=digestible intestine protein allowed by the nitrogen content of the fodder; PDIE=digestible intestine protein allowed by the energy content of the fodder; Ca=calcium; P=phosphorus.

During the studied period, supplemental dry yeast tended to influence milk yield (C 15.75 kg vs. E_1 18.15 kg vs. E_2 16.51 kg, SEM=0.415, P=0.078), results are being presented in Table 4. Interactions between dietary treatment x period for milk yield, milk fat, and milk protein were not found (P=0.529). However, for lactose percentage, an influence for treatment (P=0.042) and period (P=0.079) among the studied groups were observed. Many research studies showed that supplementation of lactating cows diets with yeasts improved milk yield, milk lactose, milk protein and health status (Enculescu, 2021; Zhu et al., 2017). On the contrary, research published by Fereira, 2019 and Ambriz-Vilchis et al., 2017 found no effects of yeasts administration on performances and health.

Table 4. Effects of inactive dry yeast on milk yield and milk composition in lactating dairy cows

Parameters	Treatment			SEM	Effect*		
	C	E_1	E_2		T	P	(T x P)
Milk yield, kg/day	15.75	18.15	16.51	0.415	**0.078**	0.154	0.529
Fat, %	4.58	4.68	4.62	0.080	0.823	0.159	0.201
Protein, %	3.35	3.36	3.27	0.029	0.410	0.109	0.689
Lactose, %	4.22	4.59	4.28	0.045	**0.042**	**0.079**	0.207

C=diet supplemented with 0 g inactive dry yeast/had/day; E_1=diet supplemented with 120 g inactive dry yeast/had/day; E_2=diet supplemented with 150 g inactive dry yeast/had/day; SEM=standard error of the mean; Effect*: T=treatment; P=period; TxP=interaction between treatment x period.

Moreover, an important aspect was to evaluate the effects of inactive dry yeast addition in the diets on the health status of cows. During our trial, tendencies for treatment influence between the E_1, E_2 and the C groups for HTC (P=0.078), WBC (P=0.092), MO (P=0.060) were observed (Table 5). The effect of the period (P) on dietary treatments was observed for HGB (P=0.028), HTC (P=0.005), WBC (P=0.039), LY (P=0.001), MO (P=0.000) and NE (P=0.000).

Furthermore, an interaction between dietary treatment x period for concentrations of HTC was detected (P=0.093).

Table 5. Effects of inactive dry yeast on haematological and biochemical parameters in lactating dairy cows

	Treatment			SEM	Effect*		
Parameters	**C**	**E_1**	**E_2**		**T**	**P**	**(T x P)**
RBC, 10^6/µl	6.46	6.14	6.08	0.121	0.380	0.375	0.407
HGB, g/dl	9.42	9.46	9.22	0.073	0.637	**0.001**	0.225
HCT, %	26.74	28.58	29.26	0.366	**0.078**	**0.005**	**0.093**
WBC, 10^3/µl	8.41	7.42	8.24	0.305	**0.092**	**0.039**	0.535
LY, %	58.37	59.89	58.06	0.565	0.717	**0.028**	0.297
MO,%	4.49	4.12	6.18	0.634	**0.060**	**0.000**	0.301
NE, %	37.62	35.06	34.64	0.931	0.442	**0.000**	0.283
Proteins, mg/dl	7.32	7.64	7.97	0.162	0.191	**0.072**	0.185
AST, U/L	47.10	45.06	43.73	1.477	0.291	0.654	0.792
ALP, U/L	76.83	74.84	67.56	2.497	**0.060**	0.260	0.129
Ca, mg/dl	8.39	8.69	8.79	0.120	0.244	0.653	0.722
P, mg/dl	4.32	4.63	4.57	0.095	0.430	**0.085**	0.981

C=diet supplemented with 0 g inactive dry yeast/had/day; E_1=diet supplemented with 120 g inactive dry yeast/had/day; E_2=diet supplemented with 150 g inactive dry yeast/had/day; SEM=standard error of the mean; Effect*: T=treatment; P=period; TxP=interaction between treatment x period; RBC=red blood cells; HGB=haemoglobin; HCT=hematocrit; WBC=total white blood cells; LY=lymphocytes; MO=monocytes; NE=neutrophils; AST=asparagine aminotransferase; ALP=alkaline phosphatase; Ca=total calcium; P=inorganic phosphorus.

Total protein was not affected by the addition of dry yeast (P=0.191). However, a tendency for period effect was observed (P=0.072). Concerning the enzymatic profile, the activity of the AST (47.10 U/L vs. 45.06 U/L vs. 43.73 U/L, SEM=1.477, P=0.291) was not influenced by the dry yeast addition. Dietary supplementation with dry yeast tended to reduce the activity of ALP (76.83 U/L vs. 74.84 U/L vs. 67.56 U/L, SEM=2.497, P=0.060), which suggests the involvement of the yeast in promoting intestinal metabolism and regenerating the liver tissue.

Also, the mineral profile (Ca, P) was not affected by dry yeast supplementation, nevertheless, a tendency for the period effect (P=0.085) was detected. The results of the overall urinary examination during the studied period are presented in Table 6. The urinalysis indicated that none of the dietary treatments had any effects on the parameters studied. Most parameters analyzed during the studied period (d 28) were negative. For urine proteins, values of 15-30 mg/dl (E_1, E_2), and respectively 30-100 mg/dl (C), were recorded.

Table 6. The results of the overall urinary examination during the study period

	Treatment		
Parameters	**C**	**E_1**	**E_2**
Bilirubin, mg/dl	Negative	Negative	Negative
Urobilinogen, mg/dl	Normally	Normally	Normally
Ketones, mg/dl	Negative	Negative	Negative
Glucose, mg/dl	Negative	Negative	Negative
Protein, mg/dl	15-100	15-30	30
Blood, ery/µl	Negative	Negative	Negative
PH	5-7	5-7	6-8
Nitrite	Negative	Negative	Negative
Leukocytes, leu/µl	Negative	Negative	Negative
Specific gravity	1.010-1.025	1.010-1.025	1.010-1.025

C=diet supplemented with 0 g inactive dry yeast/had/day; E_1=diet supplemented with 120 g inactive dry yeast/had/day; E_2=diet supplemented with 150 g inactive dry yeast/had/day.

Generally, in lactating dairy cattle values of up to 15 mg/dL are indicated. Pathological causes of proteinuria (presence of protein in urine) could indicate renal disease, urinary tract infections and haematuria (Enculescu et al., 2020). Overall intervals for specific gravity were 1.010 to 1.025 for all groups studied, and 5.0 to 8 for pH. The results reported by Herman et al. (2019) showed pH values between 6.6-8.7 in healthy dairy cows. The assessment of faecal score in lactating cows is being presented in Table 7. Faeces colour was olive-brown for all groups studied. Faecal consistency and faecal content scores varied over the different observation time points. On d 0, the faecal consistency was between 2.8-2.9 for all

groups, and the faecal content of 3.1-3.2 scores (undigested feed particles) for the E_1 and the E_2 groups, and 3.3 score for the C group. On d 28, scores were substantially different, dairy cows having consistency scores of 3 (E_1), 3.4 (E_2), 3.7 (C), and faecal content scores of 3 for the E_1 and the E_2 groups, and respectively 3.5 faecal content score (large fragments of undigested feed) for the C group.

Table 7. The assessment of faeces in the lactating cows studied

Scale/groups	Treatment					
	d 0			d 28		
	E_1	E_2	C	E_1	E_2	C
Colour	Olive brown	Olive brown	Olive brown	Olive brown	Olive brown	Olive brown
Consistency	2.8 (3.64 cm)	2.9 (3.50 cm)	2.8 (3.65 cm)	3 (3.55 cm)	3.4(3.80 cm)	3.7 (4.00 cm)
Content	3.2	3.1	3.30	3	3	3.50

C=diet supplemented with 0 g inactive dry yeast/had/day; E_1=diet supplemented with 120 g inactive dry yeast/had/day; E_2=diet supplemented with 150 g inactive dry yeast/had/day.

CONCLUSIONS

Supplementing dairy cows diets with inactive dry yeast increased the milk yield and lactose percentage, without affecting fat and protein content and with a strong tendency for treatment effects on haematological (HTC, WBC, MO, ALP) and biochemical (ALP) parameteres being observed.

The urine parameters studied were not influenced by the inactive dry yeast addition in the dairy cows diets, however, for faecal score, an optimization of the digestion efficiency during the study has been detected.

Factors related to feeding behavior, such as feed and water intake rates, and physiological factors are variable from one animal to another, and might explain the differences in the obtained results of the two experimental groups with the control group.

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EFFECTS OF MULTI-STRAIN PROBIOTICS ADMINISTRATION ON GROWTH PERFORMANCE AND HEALTH STATUS IN DAIRY AND BEEF-DAIRY CROSSBREED CALVES

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Abstract

The aim of the current study was to evaluate the effects of multi-strain probiotic administration on dairy and crossbreed calves' growth performances and health status. The calves were homogeneously assigned into four groups, as follows: dairy experimental (E_d, n=12), dairy control (C_d, n=12), crossbreed experimental (E_c, n=6) and crossbreed control (C_c, n=6). All calves were managed identically, being housed individually, with the experimental groups receiving 2 ml of multi-strain probiotic (Enterococcus faecium and Lactobacillus plantarum) per day. Blood sampling and growth performance were assessed at 1st, 14th and 28th days of the trial. Probiotic supplementation had no significant effects on hematologic profile (P>0.05). However, serum blood glucose and total cholesterol were significantly lowered (P<0.05) in the probiotic treated groups. Multistrain probiotic administration had significant effects on calves (P<0.05), improving the weight gain of both dairy and crossbreed groups (E_d and E_c) compared with control groups (C_d and C_c), at the end of the trial. Current results suggest that multi-strain probiotics support calves early-stage development.

Key words*:* calves, *E. faecium*, health, *L. plantarum*, performance.

INTRODUCTION

In intensive dairy and beef farming, neonatal diarrhoea affects calves world-wide, generating significant production and financial losses (Medrano-Galarza et al., 2018), that are reflected in high rates of morbidity (up to 35%) and mortality (up to 10%) (Urie et al., 2018). Moreover, the calves that overcome the diarrhoeic episodes, present impaired growth and development, negatively affecting their productivity at maturity (Aly et al., 2016). Therefore, new strategies to improve unweaned calves gastrointestinal health are necessary for mitigating their susceptibility to enteric infections.

Supplementing multi-strain probiotics has been reported to improve early development in calf growth rates (Zhang et al., 2016; Guo et al., 2022) and the immune response (Liang et al., 2020), which reduce the pathogenic risks at farm level (Alayande et al., 2020). Probiotic feed supplementation modulates the gastrointestinal development (Sahu et al., 2019) and the immune system response of neonatal and suckling calves (Arshad et al., 2021) during the first weeks of life. Probiotic such as *Enterococcus faecium* had considerable effects on rumen development and growth rates (Arne and Ilgaza, 2021) when supplemented to beef x dairy crossbreed calves. Furthermore, lactic acid bacteria such as *Lactobacillus plantarum,* was widely studied as probiotic in calves nutrition, in order to improve growth performance, feed efficiency and nutrient availability. However, the efficiency of combining both probiotics has rarely been investigated in suckling calves. Therefore, it is essential to determine the application of multistrain probiotic (Wang et al., 2018) on dairy and crossbreed calves' growth rates and health status.

The aim of the current study was to evaluate the effects of multi-strain probiotic administration on dairy and crossbreed calves' growth performances and health status.

MATERIALS AND METHODS

The experimental design and protocol used were in accordance with the EU Directive

2010/63/EU and the Romanian Law on Experimental Animal Protection.

Calves, husbandry and experimental conditions

Twenty-four dairy and twelve crossbreed calves were assigned (half-sib groups, balanced for age and sex) in four experimental groups (Table 1). The calves farm management was identical, being housed in individual hutches (2.0 x 1.2 x 1.4 m), on deep straw bedding. At birth all calves were fed with 4 kg of colostrum per day, in two equal meals at 12 hours intervals, during their first 3 days of life. Suckling calves received a diet consisting out of 6 kg of milk, into two equal meals per day (at 6:00 AM and 6:00 PM). After the 10th day of life, all calves had *ad libidum* access to water, starter concentrates (18.2% CP) and alfalfa hay until the age of 90 day, when weaning took place.

Table1. Experimental design and dietary treatments

Group	Dietary treatments
C_d	Negative control group - fed with milk (n=12);
E_d	Experimental dairy - fed with milk + Pro (n=12);
C_c	Negative control group - fed with milk (n=6);
E_c	Experimental crossbreed - fed with milk + Pro (n=6).

The probiotics administration was performed daily during the first 28th days of the trial, in drinking water: 1 x 10^6 CFU/ml *Enterococcus faecium* + 1 x 10^8 CFU/ml *Lactobacillus plantarum* (commercial probiotic).

Growth parameters and health

The calves body weights were recorded periodically using a weighing scale platform, on days 0, 14 and 28 of the trial, in order to calculate the average daily gain (first 14th days, last 14th days and the overall trial duration of 28 days).

Before morning feed, blood samples were collected from the jugular vein in all calves, on days 0, 14 and 28, using vacutainer tubes with K_3EDTA and clot activator (Kima®, Italy). Complete blood count (CBC) determinations were performed using an automatic analyser (Diatron, Abacus Junior Vet, Hungary). The haematological screened parameters were: red blood cells count (RBC), haemoglobin concentration (HGB), total white blood cells count (WBC), haematocrit (HCT), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC).

Calves serum blood was separated by centrifugation (3000 rpm, 15 min, SIGMA), collected in 1.5 ml Eppendorf tubes and stored at -20°C, until further analysis. Metabolic serum profile determination used a semi-automatic analyser (StarDustMC15, DiaSys, Spain), recording: glucose (Glu), total cholesterol (TCho), total protein (TPro), albumin (Alb), aspartate- aminotransferase (TGO), gamma glutamil-transferase (GGT), alkaline phosphatase (PAL), calcium (Ca), magnesium (Mg) and phosphorus (P).

During the 28th days of the trial, faecal scoring for fluidity and consistency, and the calves scoured days severity and treatment were recorded daily each morning (10:00 AM). Faecal scores (Table 2) based on a five-point scale were recorded daily using the procedure of (Yao et al., 2020).

Table 2. Experimental calves growth parameters

Score	Degrees and appearance
1	Thick in consistency, normal;
2	Thin but not watery, normal;
3	Slight watery, abnormal;
4	Watery with dark colouring, abnormal;
5	Watery with light colouring, abnormal.

Statistical analyses

Data were analysed employing the ANOVA procedure with SPSS software (version 20 Inc. Chicago, IL, USA). When comparing treatments means, Post hoc Tukey's multiple range test was carried out to assess any significant differences for the measured parameters. Differences were considered significant at a level of $P \leq 0.05$, while the tendency was set at $P \leq 0.10$.

RESULTS AND DISCUSSIONS

Results for calf body weight gain (BWG), average daily gain (ADG) and faecal score are presented in Table 3. BWG of suckling calves that received multistrain probiotics treatment had significantly higher values ($P \leq 0.05$) at the end of the trial, compared with the control groups. Moreover, dairy calves BWG showed a tendency ($P=0.090$) towards significance, starting the first 14th day of the trial, compared

with the experimental groups. Furthermore, probiotics influenced positively (P≤0.05) the ADG of both dairy and crossbreed calves groups (E_d and E_c), compared with the control groups. The mean faecal scores registered were significantly lowered (P≤0.05) by the dietary treatments, starting from the 14th day of the trial. Similar to our findings, recent studies regarding dietary supplementation of *Lactobacillus plantarum* reported an improvement on calves growth rates (Jiang et al., 2020) and faecal scores (Casper et al., 2021). Moreover, by using multi-strain probiotic Guo et al. (2022) found an improvement in dairy calves ADGs and lower the faecal scores. Preweaning is known to be a particular difficult stage for the growth and development of the gastrointestinal tractus in dairy breeds. Therefore, early weaning strategies are often adopted in the intensive dairy cattle industry, in order to support the production and reproductive performance of heifers and to increase the farm economic returns. Calves probiotics supplementation had as effect an improvement of the weaning weight, with higher values of ADG, during the 28th days of trial. The calves higher growth rates in the experimental groups could be attributed to the gastric modulative role of the probiotics, that might had established a balanced rate between the enteric benefic and pathogenic populations, enhancing nutrient availability and feeding efficiency.

Table 3. Body weight gains, average daily gains and faecal scores in dairy and crossbreed calves groups

		Dairy calves' groups				Crossbreed calves' groups			
Parameters		**C_d**	**E_d**	**SEM**	**P-value**	**Cc**	**Ec**	**SEM**	**P-value**
BWG	1st day	54.98	60.63	3.769	0.300	54.27	66.87	4.365	0.069
	14th day	63.00	72.34	3.719	0.090	62.80	76.23	5.6689	0.126
	28th day	72.10^{b}	80.96^{a}	3.793	0.038	73.3^{b}	92.17^{a}	5.823	0.045
ADG	first14th days	0.574^{b}	0.837^{a}	0.048	0.001	0.610^{b}	0.699^{a}	0.161	0.799
	last 14th days	0.650^{b}	0.830^{a}	0.060	0.047	0.750^{b}	1.138^{a}	0.130	0.061
	overall trial	0.612^{b}	0.833^{a}	0.033	<0.001	0.680^{b}	0.904^{a}	0.068	0.042
Faecal score	first 14th days	1.964	1.607	0.085	0.007	1.833	1.631	0.110	0.222
	last 14th days	1.872^{a}	1.560^{b}	0.124	0.037	1.952^{a}	1.393^{b}	0.093	0.002
	overall trial	1.896^{a}	1.583^{b}	0.084	0.007	1.893^{a}	1.512^{b}	0.089	0.013

Different superscripts within the same row are different

Diet multi-strain probiotics supplementation had no effects on the calves CBC levels (P>0.05). Furthermore, all experimental calves had similar values on CBC parameters, that fell in between the physiological range for the species and age category, as previously published by the Merck Veterinary Manual (2014) and Weiss and Wardrop (2011).

Serum glucose concentration (Glu) was significantly lowered (P≤0.05) during 14th to 28th day on the probiotics treated groups (E_d and E_c), compared with the control groups (C_d and C_c). Moreover, lowest mean Glu values were observed in the E_d group (59.42 mg/dl), compared with all experimental groups. Calves serum total cholesterol concertation (E_d and E_c) was significantly lower (P≤0.05) in the probiotic supplementation groups at the end of the trial. However, probiotic supplementation had no effect (P>0.05) on serum total protein and albumin concentrations of E_d, E_c, C_d and C_c groups. No differences (P>0.05) were observed on calves serum enzymatic profile (GOT, GGT and PAL).

Probiotic supplementation might have effects on the nutrient availability during suckling and preweaning stages (Salazar et al., 2019). Lower plasma glucose levels are often a consequence of higher levels of insulin secretion that enhances the energetic metabolism facilitating cellular absorption (Stahel et al., 2016). Our findings are in line with Ilgaza and Arne (2020), who found that dietary supplementation of *E. faecium* decreased the calves serum glucose.

The results for serum TCho values in the current paper are similar to those obtained by Jiang et al. (2020), which observed that calves treated with lactic acid bacteria had lower TCho concentrations. Apart from that, lactic acid bacteria such as *L. plantarum* display the ability to absorption the serum TCho into their own cells (Gupta et al., 2020), having implications in the TCho metabolism regulation (Albano et al., 2018).

Table 4. Calves blood constituents and serum metabolic profiles

Blood parameters		Dairy calves				Crossbreed calves			
		C_d	E_d	SEM	P-value	Cc	Ec	SEM	P-value
Complete blood count									
WBC	1st day	9.98	8.32	1.101	0.303	9.25	8.11	0.511	0.154
	14th day	9.23	9.42	0.711	0.851	9.21	6.47	0.924	0.069
	28th day	9.51	8.74	0.613	0.387	9.14	8.00	0.762	0.324
RBC	1st day	8.75	9.45	0.378	0.213	8.99	8.79	0.785	0.866
	14th day	9.08	9.81	0.308	0.115	9.39	9.87	0.602	0.588
	28th day	9.06	9.15	0.354	0.854	8.19	9.31	0.990	0.445
HCT	1st day	24.00	27.18	1.532	0.164	25.45	25.97	2.256	0.876
	14th day	26.72	27.61	1.138	0.586	26.52	28.37	1.235	0.321
	28th day	25.36	28.70	1.407	0.115	24.57	27.90	2.240	0.323
HGB	1st day	8.75	9.35	0.423	0.333	8.44	9.36	0.552	0.273
	14th day	9.51	8.98	0.398	0.356	8.90	9.86	0.474	0.190
	28th day	8.34	9.84	0.346	0.086	8.80	10.00	0.406	0.070
MCV	1st day	29.63	29.34	1.288	0.893	28.40	30.20	2.302	0.595
	14th day	29.50	28.13	0.940	0.318	28.60	29.00	1.371	0.842
	28th day	27.75	28.75	1.048	0.511	28.40	28.40	1.749	1.000
MCHC	1st day	32.91	34.80	0.851	0.139	33.48	36.84	2.288	0.329
	14th day	33.49	34.54	0.466	0.134	33.44	34.76	0.672	0.202
	28th day	35.84	35.03	1.142	0.623	36.82	36.52	2.572	0.936
Serum biochemical profile									
Glu	1st day	79.58	81.40	4.556	0.785	84.94	84.36	3.781	0.917
	14th day	86.60[a]	70.81[b]	3.410	0.011	88.20[a]	63.89[b]	3.729	0.002
	28th day	79.81[a]	59.42[b]	2.362	<0.001	78.56[a]	62.76[b]	3.453	0.120
TCho	1st day	140.20	138.40	5.224	0.813	144.27	145.25	4.116	0.870
	14th day	141.94	148.90	5.325	0.383	144.38	149.15	7.161	0.650
	28th day	148.43[a]	130.66[b]	3.95	0.013	151.75[a]	138.67[b]	3.53	0.031
TPro	1st day	5.59	5.37	0.301	0.616	5.52	5.54	0.307	0.975
	14th day	5.65	5.78	0.169	0.624	5.96	5.82	0.128	0.461
	28th day	5.80	5.78	0.188	0.930	6.02	5.95	0.212	0.796
Alb	1st day	2.45	2.71	0.145	0.234	2.82	2.60	0.137	0.282
	14th day	2.70	2.59	0.185	0.702	2.79	2.61	0.181	0.493
	28th day	2.60	2.28	0.136	0.132	2.40	2.87	0.184	0.108
GGT	1st day	10.60	10.79	0.811	0.866	9.72	11.08	0.772	0.247
	14th day	11.24	11.52	0.671	0.467	11.54	10.70	0.758	0.454
	28th day	11.67	12.04	0.711	0.734	11.35	11.56	0.780	0.850
GOT	1st day	18.78	21.17	1.744	0.360	19.74	19.53	1.771	0.936
	14th day	20.01	21.37	1.399	0.512	19.52	20.62	1.185	0.531
	28th day	20.62	20.56	0.775	0.962	20.62	19.70	0.858	0.465
PAL	1st day	33.50	33.45	3.240	0.992	30.83	36.56	3.072	0.224
	14th day	35.55	36.36	4.377	0.899	38.76	39.65	2.169	0.852
	28th day	37.79	34.53	3.283	0.503	38.02	39.71	3.144	0.715
CA	1st day	8.72	9.96	0.185	0.307	9.21	9.24	0.213	0.343
	14th day	9.77	9.56	0.134	0.663	10.67	10.87	0.080	0.220
	28th day	9.30	9.90	0.089	0.441	9.90	9.46	0.120	0.330
MG	1st day	2.91	2.63	0.440	0.082	2.88	2.58	0.345	0.949
	14th day	2.55	2.46	0.338	0.669	2.43	2.96	0.645	0.528
	28th day	2.58	2.68	0.428	0.356	2.67	2.84	0.467	0.530
P	1st day	7.08	6.56	0.970	0.346	6.41	6.48	0.957	0.344
	14th day	7.18	6.94	0.669	0.809	6.22	6.57	0.661	0.719
	28th day	6.64	6.97	0.708	0.750	5.90	7.00	0.466	0.134

CONCLUSIONS

Probiotics supplementation had no significant effects on hematologic profile of calves. However, serum blood glucose and total cholesterol levels were significantly lower in the probiotics treated groups. Multi-strain probiotic administration had significant effects on calves, improving the weight gain of both dairy and crossbreed groups, compared with the controls. Current results suggest that multi-strain probiotics support calves early-stage development. Early calves ruminal development and health status have a direct influence on the rearing management, lowering the age of weaning and costs related with veterinary treatments. Further studies are needed in order to investigate the mechanisms through which the multi-strain probiotic dietary supplement affect the energetic serum profile in pre-weaned calves.

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GROWTH PERFORMANCE OF 'SUPER NATIVE CHICKEN' TREATED WITH A SUPLEMENTATION OF MAGGOT FLOUR OF *DROSOPHILA MELANOGASTER* IN RATION

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Abstract

The role of insects as natural resources could be used by farmer as animal feed has been starting to get important attention in livestock sciences. This research aimed to study the growth performance of super native chickens that treated with D. melanogaster maggot flour supplementation. A total of 64 super native chickens (DOC) reared until they are eight weeks old. This study used a completely randomized design (CRD). Experimental animals were divided into four groups according to the level supplementation treatment: T0 0%; T1 0.25%; T2 0.5%; and T3 0.75%. Each group divided into four units of replication consisted of 4 chickens. The variables observed were total of feed consumption, body weight and feed conversion ratio. The results showed that the effect of treatment on feed consumption was non-significant (P>0.05) while the effect on body weight and FCR value were significant (P<0.05).

Key words*: growth performance, insect, maggot, native chickens, natural resource.*

INTRODUCTION

Insects are abundantly available in nature. Many of insects have great potential to be used as animal feed (Chia et al., 2019) and van Huis (2016) because of they contain nutrients that are important for the development and production of livestock, especially for poultry (Toar et al., 2015).

Utilization of insects as feed could reduce the use of food that is needed directly by humans globally, especially as the population continues to increase.

The potential of insects to be used in livestock improvement (Rumokoy et al., 2020; Toar et al., 2021) especially to be applied in animal feed production (Chaalala et al., 2018) is inseparable from various factors such as: socio-cultural aspects (Hartman et al., 2015), ecological sustainability (Chavez, 2021; Premalatha et al., 2011), regulations in the countries concerned, human skills, and awareness of farmers to reduce the use of human food as animal feed.

Foodstuffs are more suitable for use only for humans, especially in difficult times where the distribution of food for the world's population is experiencing obstacles both in terms of quantity and other aspects. Rumokoy et al. (2019) stated that this effort was wise in suppressing competition between humans and livestock in using food.

Various scientific reports indicated the role of insects in the development of poultry farming: Allegretti et al. (2018) reported the use of insects in the chicken farming industry, as stated by Toar et al. (2020) that during a pandemic the use of insects can act as animal feed.

The use should be aligned with efforts to consider its impact on the environment in a sustainable manner. This is in line with the various opinions expressed by Tomberlin et al. (2015), Ratnadass et al. (2012), that linked to

Yen (2009) that concerned with environmental protection when carrying out insect production activities as a source of livestock nutrients. In other side Abro et al. (2020) and Smetana et al. (2016) considered the importance connected to socio-economic and environmental impacts of the application of insects as animal feed. Beside that Rumokoy et al. (2018) reported that certain proteins from insects play a role in livestock health

In a study of bio-chemical compounds conducted by Church & Robertson (1966) showed the nutrient content of *D. melanogaster* larvae in several life cycles.

In essence, many of insect species are easy to breed and spread throughout the world.

Based on the above background, we have conducted a study concerning use of *D. melanogaster* larva flour (DMF) as feed supplement in ration on growth performance of chickens.

MATERIALS AND METHODS

This study used a total of 64 day-old-chick of 'super native chicken'. The chickens were cultivated until 8 weeks old in a battery-system cage, formed of 16 units where 4 chickens placed in each unit. The chickens were divided in four groups according to the *D. melanogaster* larva flour (DMF) treatment levels in ration: T0 0.0%; T10 0.25%; T2 0.5%; T3 0.75% which was added in a 100% of ration formed.

Insect larvae come from the eggs of wild mother insects. Larvae were cultured in transparent plastic cylindrical containers with a diameter of 8.5 cm and a height of 14 cm.

Wild adults come from the environment and are allowed to lay eggs in cylindrical containers and are equipped with food media and ovipositing. Each container was equipped with an attractant from fresh ripe papaya flesh which is left for three days and placed in a shady open space, on the fifth day the larvae begin to grow.

Each group constituted with 4 chickens. The experiment was designed by using a CRD (completely randomized design). The parameters measured were total of feed consumption, final body weight, and feed conversion ratio. The ration including drink water were *ad libitum* distributed to the animals.

RESULTS AND DISCUSSIONS

The results of the study regarding the consumption of rations, as shown in Figure 1, showed that the treatment of supplementation with DMF up to 0.75% into the ration had no significant effect ($P>0.05$) on the accumulation of feed consumption of experimental chickens.

The application of supplementation to this level had no impact on the palatability of the chickens so the amount of feed consumed was not significantly different in the four experimental groups. These results are in line with various scientific reports regarding the use of insect larvae meal in diets.

This result is in line with the study consumption (Allama et al., 2012) of using insect meal of *Alphitobius diaperinus* larvae into rations up to 2% which did not have a significant effect on broiler.

Supplementation had a significant effect on body weight of experimental chickens ($P<0.05$). The test between treatments showed that the body weight of the T3 treatment group was significantly higher than that of the T0, T1 and T2 treatments.

These results indicate that the DMF supplementation level of up to 0.75% (T3) in ration increased the body weight of experimental chickens by an average of 688 g. This supplementation supports livestock health because there have not any experimental chickens were sick during the observations carried out in this study. Rumokoy et al. (2021) indicated a potential immunity of insect to animal studied.

This performance was most likely conducted by the nutrient compounds in DMF such as methionine and other specific amino acids. This is related to the report of Parkhitko et al. (2016) and Cox et al. (2017) which shows the metabolic role of various chemical compounds, including the role of nutrigenomics in the body of *D. melanogaster* (Baenas & Wagner, 2019).

The application of DMF as feed supplement in chicken ration caused a significant effect on feed conversion ratio value ($P<0.05$).

The test between treatments showed that the FCR value in the T3 (2.03) treatment group was significantly better than the other treatments in the T0 (2.23), T1 (2.24) and T2 (2.21) groups.

Bovera et al. (2015) showed a positive role in broiler FCR by using a feed additive made from several insect species. This role is related to the research results of Oonincx et al. (2015).

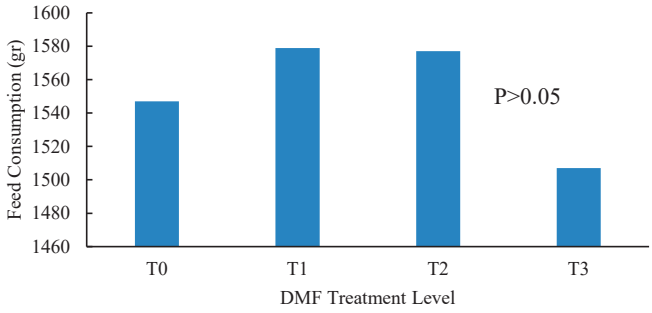

Figure 1. Accumulative of feed consumption of chickens

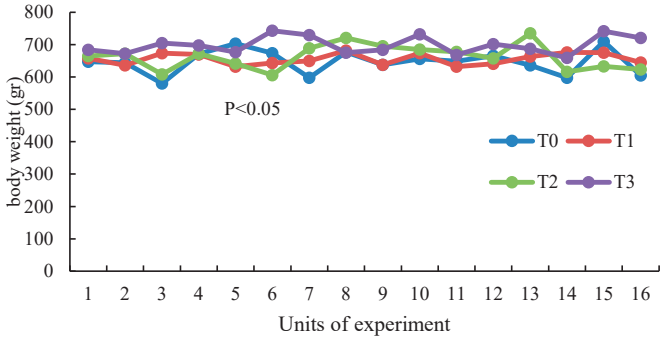

Figure 2. Body weight of experiment chickens

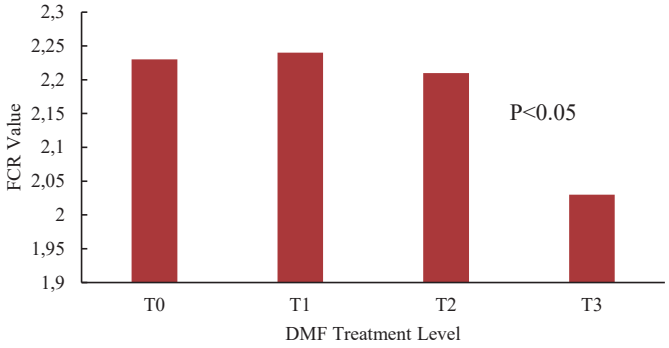

Figure 3. Feed Conversion Ration value of experiment chickens

CONCLUSIONS

Insects as natural resources can play an important role in the development of livestock production, especially poultry. The distribution of insects that are spread in a cosmopolitan manner that is able to adapt to various environmental conditions that make it easy for insects to be cultivated, especially *Drosophila melanogaster* insects.

RECOMMENDATIONS

The results of this basic research support efforts to utilize natural resources from insect organisms, but in practice it must always be aligned with attention to maintaining environmental sustainability. The next step of this research could be to explore the important and essential chemical components of D. melanogaster larvae that affect the growth performance of chickens.

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THE EFFECT OF SUPPLEMENTATION OF *PATANGA SUCCINCTA* FLOUR IN RATION ON INDIGENOUS CHICKENS MEAT PRODUCTION

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Abstract

This study aimed to observed the effect of supplementation of Patanga succincta flour in ration on meat production of local chickens. A total of 48 indigenous chickens were used until eight weeks old. The animals were divided in a same number into two groups: a group as control (CG) and the other group (TG) received a supplementation of P. succincta flour with a concentration of 0.5kg supplemented in 100 kg of ration. The variables observed were: body weight, feed consumption, FCR and carcass percentage. The results indicated that the supplementation of P. succincta flour in ration gave a significant effect (P<0.05) on FCR and body weight, while there was a non-significant effect on carcass percentage and feed intake between chickens in control group and treatment group. We concluded that the supplementation of P. succincta flour up to 0.05% into the basal diet could have a positive effect on a FCR value and carcass percentage of native chickens reared in closed cages

Key words*: indigenous chickens, insect, meat production, Patanga succincta.*

INTRODUCTION

The type of native chickens is a type of livestock that is still cultivated by the farmers, especially in rural areas having biodiversity as a natural source for livestock feed.
The native chickens have fond of hunting various types of insects and other animals as their natural source of feed.
This livestock utilize also fruits and some by-product materials as feed. The insects are scattered and can be found cosmopolitan and to be considered as feed for poultry (Kawasaki et al., 2019). Insects are abundantly available in nature. The use of insects in animal husbandry can be seen as a wise action in anticipating problems in the distribution of animal feed ingredients that compete with human needs for food as linked to the scientific report of Ordoñez-Araque & Egas-Montenegro (2021).
Many of the insects have a great potential to be oriented as animal feed (Toar & Rumokoy, 2021) because their nutrient compounds which are important for livestock production especially in poultry feeding as reported by Sogari et al. (2019), Rumokoy et al. (2020), Van Huis et al. (2013).
The act of using insects for the development of chicken production today is starting to get quite a positive response.
Jagtap et al. (2021) put forward the importance of insects to be used as animal feed while paying attention to the role of the economy and the environment impact.

MATERIALS AND METHODS

Fresh adults of *P. succinta* were obtained from the agriculture environment in Minahasa area.
Swing-Net-trap was used in collecting these insects, and then dried in direct sunlight from 9 AM until 3 PM for five days and then proceed with grinding this material to produce the *P. succincta* flour (PSF). The basal ration was 10% yellow corn of commercial ration The concentration of PSF as 5% mixed in basal ration of native chicken. The basal ration was composed by 90% of commercial ration and 10% of yellow corn.

A total of forty-eight of day-old native chickens were reared until eight weeks in this experiment. The animals were placed in a battery cage with a dimension of (60*60*40) cm. Each unit (a pen) was occupied by six chickens at the beginning until 5th week, after that a pen was placed by four chickens only. The chickens were fed *ad libitum* including their drink water.

Table 1. Nutrien Composition of Basal Ration

Nutrient	comercial ration (90%)	yellow corn (10%)
Protein (%)	20	10.68
Lipid (%)	5	1.78
Fiber (%)	5	0.26
Energy (kcal)	3200	3613
Calsium (%)	0.9	0.02
Phospor (%)	0.5	0.28

The chickens were divided in two groups: control group (Po) and treatment group (P1).
The parameters in this study were: accumulation of feed intake, body weight, FCR and percentage of carcass as a parameter of chicken meat production.
The data obtained were analyse by using t-test to determine the significance different between control group and treatment group by using Microsoft Office Excel software.

RESULTS AND DISCUSSIONS

The average feed intake accumulation of the chickens in control group (Po) was 440 gr as shown in Figure1, tended to be lower consumption then in treatment group (P1) which reached 456 gr although it has recorded a non-significant difference ($P>0.05$) feed consumption between the evaluated groups. The results showed that 0.05% supplementation of PSF in ration could not yet affects the feed consumption of chickens. The use of natural resources in livestock can be provided without having a negative impact on livestock. This is in line with various opinions from various parties that have been previously reported. Thomas et al. (2000) stated that the use of natural resources must be concerned to various aspects for the benefit of life related to natural environment, including the livestock itself (Barlow et al., 2003).
The average body weight shown in Figure 2 has recorded a significant difference ($P<0.05$) between group Po and group P1. These results indicated that the supplementation of *P. succincta* flour up to 0.05% into basal ration did not adversely affect the body weight of the chickens evaluated. Insect flour as feed could be related to some factors that can play a role and affect chicken weight gain: chicken type, sex, level of insect flour applied. The study of Pietras et al. (2021) used different insect larvae meals as protein sources did not significance influenced the body weight of chickens while the results differed to its FCR value as related to the feed conversion ratio which has shown a significant difference ($P<0.05$) between group Po and group P1 as presented in Figure 3.
According Toar and Rumokoy (2020) the interference of the nutrient compound in insect flour could influence the body weight of the chickens.
The average comparison of chicken carcass percentage of the two groups indicated a non-significant difference ($P>0.05$).
Even though the level of *P. succincta* flour supplementation up to 0.05% did not give a different impact, Figure 4 shows that the P1 sample in general tended to have a higher carcass presentation than the control group Po. It is possible to increase the production of meat as measured by carcass percentage by increasing the level of supplementation of this insect meal in the ration.
These results support the expectation of using insect products for livestock development utilizing surrounding natural resources, in addition to contributing to the development of chicken farms which various countries have obtained legality to apply insect as animal feed (Rumokoy et al., 2022).

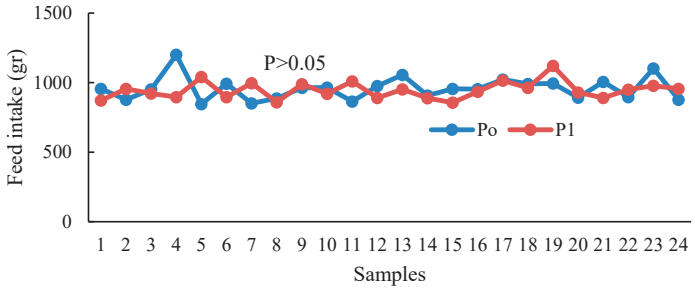

Figure 1. Accumulative of feed consumption of chickens

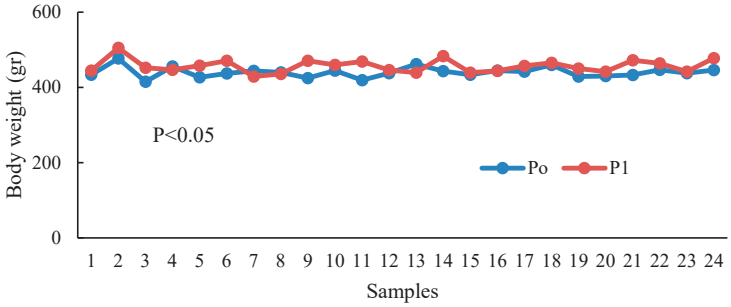

Figure 2. Body weight of experiment chickens

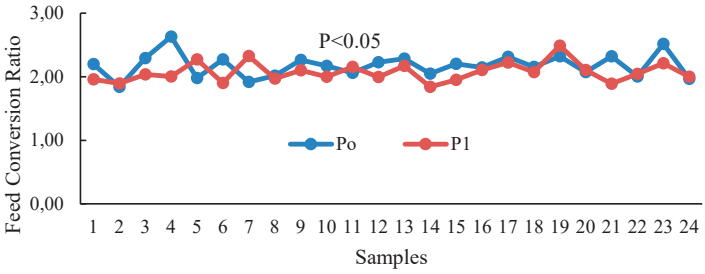

Figure 3. Feed Conversion Ratio value of experiment chickens

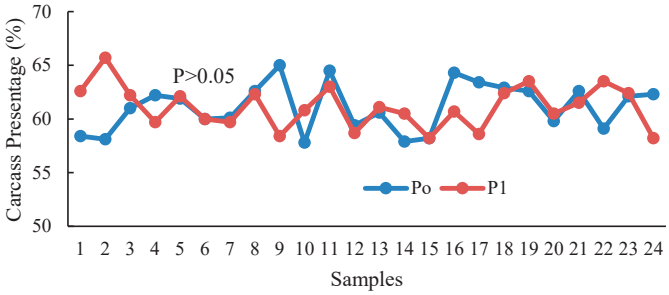

Figure 4. Meat Production of The Evaluated Chickens

CONCLUSIONS

We concluded that the supplementation of *Patanga succincta* insect meal up to 0.05% into the basal diet could have a positive effect on a FCR value and carcass percentage of native chickens reared in closed cages.

RECOMENDATION

Based on the results of this study, we are interested to disclose a possibility next step in exploring the role of this insect substances on the metabolism and immunity effect of chickens.

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EFFECT OF PARSLEY AND INULIN ON BIOPRODUCTIVE PARAMETERS AND ANTIOXIDANT NUTRIENTS OF EGGS PROVIDED BY LAYING HENS REARED UNDER HEAT STRESS CONDITIONS

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Abstract

The objective of this study was to examine the effects of dietary inclusion of parsley and inulin, as sources of natural antioxidants in poultry diets, on the enrichment of antioxidant nutrients in the egg yolk and on the susceptibility of the yolk to lipid peroxidation during storage. The experiment was conducted on 47-week-old TETRA SL LL laying hens, reared in high temperature (30°C). Experimental dietary treatments differed from control diet (C) by addition of 2% parsley or 2% inulin. The addition of parsley and inulin in laying hens' diets significantly decreased the iron content in the yolk eggs and increase the total polyphenol content, vitamin E, lutein and zeaxanthin and concentrations in the egg yolks. In regards to the oxidative stability parameters, a significant decrease in the concentrations of primary oxidation products formed in the egg yolk of experimental groups was seen, proving an efficient inhibition effect of the phytoadditives on peroxyl radical formation. A significant correlation was observed between oxidation products and total polyphenol content of the egg yolks, where lutein and zeaxanthin inhibit the formation oxidation products.

Key words*: antioxidants, heat stress, hens, polyphenols.*

INTRODUCTION

In recent years, heat stress has become a major environmental stressor that harms animals worldwide (Renaudeau et al., 2012). In the case of poultry, the thermal stress is much higher than in other animals, because modern genotypes of poultry have been suggested to produce more body heat due to their higher metabolic activity (Deeb & Cahaner, 2002).

The optimum environmental temperature for performance of adult laying hens is between 19 and 22°C, with temperatures above and below this range requiring thermoregulation (Lin et al., 2006). It is well known that exposure to temperatures above 30°C feed intake decrease (Xing et al., 2019). The decrease in feed consumption seems to be the origin of the most harmful effects caused by thermal stress in egg production. Consequently, this influences the endocrine system, resulting in acid-base imbalance and organ dysfunction, which leads to increased mortality, depressed food efficiency and reduced egg production and quality (Mashaly et al., 2004).

While poultry are exposed to high temperatures, they increase their breathing rate. Increased respiration rate reduces the partial pressure of carbon dioxide in the blood, changing the ratio of bicarbonate to carbon dioxide and eventually resulting in an increase in blood pH, a process known as respiratory alkalosis (Franco-Jimenez et al., 2007).

The increase in blood pH leads to a decrease in the bioavailability of calcium, by forming bonds with the proteins. By default, with a lower availability of calcium, the formation of the eggshell is affected (Etches et al., 2008). It has been observed that when poultry are subjected to high temperatures they have a lower density of red blood cells, this is due to increased water consumption and thus their dilution in the blood. According to some studies, the use of plants with antioxidant potential in laying hens' feed can increase the stability of eggs over the time (Untea et al., 2020).

This study was carried out of determine the effect of utilization of dry parsley (*Petroselinum crispum*) and yeast enriched with zinc in the diets on egg quality, feed consumption, feed conversion ratio, egg production, egg quality, hatchability blood parameters in the laying hens and egg stability.

MATERIALS AND METHODS

The feeding trial was conducted in an experimental hall at the Laboratory of Chemistry and Nutrition Physiology of the National Research Development Institute for Animal Biology and Nutrition (IBNA-Balotesti, Romania) according to an experimental protocol. This protocol was approved by the Ethics Commission of the Institute.

A three-week experiment was conducted on 47-week-old TETRA SL laying hens, which were assigned to three dietary treatments with 20 birds each. They were sheltered in an environmentally verified space (temperature, humidity, ventilation, and light program).

At the age of 47 weeks, the hens were and assigned to 3 groups, control group (C), experimental group 1 (P), experimental group 2 (I). The hens were housed in an experimental hall with 30°C constant temperature, humidity 60% and 16 h light/8 h darkness.

Eggs were collected and weighed between 10:00 and 11:00 am each day. Egg production and egg mass, were all recorded individually on a daily basis and summarized over a 3-wk period.

After 3 weeks of the feeding trial, 6 hens form each group where randomly selected and blood samples were aseptically collected into 9-mL Vacutainer containing 14.3 U/mL of lithium heparin (Vacutest®, Arzergrande, Italy) for serum biochemical assessment on an automatic BS-130 Chemistry analyser (Bio-Medical Electronics Co., LTD, China).

Blood samples were prepared by centrifugation at 775 × g for 25 min at 4°C. The supernatant obtained was employed to analyse the following serum markers: glucose, cholesterol, triglyceride, total bilirubin, total protein, calcium, iron). The biochemical parameters were analysed using an automatic BS-130 Chemistry analyzer (Bio-Medical Electronics Co., Ltd., Shenzhen, China).

Lutein and zeaxanthin were analyzed using a high performance liquid chromatograph (Perkin Elmer 200 series, Shelton, CT, USA) with a UV detector (445 nm). A stationary phase of 5 µm C18 reversed-phase column (250 × 4.60 mm i.d.) (Nucleodur, Macherey-Nagel, Germany) was used. Chromatographic analysis was carried out under isocratic conditions at a flow rate of 1.0 mL/min and a mobile phase of 13% water and 87% acetone was used.

Vitamin E determination was performed according to the method described in EC Regulation no. 152/2009, using a high performance liquid chromatograph and a PDA-UV detector (HPLC Finningan Surveyor Plus, Thermo-Electron Corporation, Waltham, MA) at a wavelength of 292 nm. A HyperSil BDS C18 column, with silica gel, dimensions of 250 × 4.6 mm, and a particle size of 5 µm (Thermo Electron Corporation, Waltham, MA), was used. Chromatographic analysis was carried out under isocratic conditions at a flow rate of 1.5 mL/min and a mobile phase of 4% water, using 96% methanol.

The total phenol content of all extracts was measured spectrophotometrically according to the Folin–Ciocalteu method, as described by Conrad et al., 2001, with slight modifications. Briefly, the extract samples (0.5 mL of different dilutions) were mixed with 0.5 mL Folin-Ciocalteu reagent and 7 mL water, and then homogenized. The solution was kept at room temperature for 3 min before adding 2 mL of 20% sodium carbonate solution. After an hour in the dark, the absorbance was measured at 732 nm against a blank (solution with no extract added). The calibration curve of gallic acid was used to determine the total phenol content, and the results were reported as mg gallic acid equivalents per gram of dried sample (mg GAE/g).

RESULTS AND DISCUSSIONS

The usage of parsley and inulin diets had no effect ($p > 0.05$) on laying hens' performances compared to control group (Table 1).

Table 1. The effect of supplemental parsley and inulin on the productive parameters of the laying hens

Parameter/group	Control	Parsley	Inulin	SEM	p Value
Average daily feed intake (g)	81.05	78.93	81.97	6.3003	0.2797
Laying percentage (%)	85.71	84.21	89.42	10.8282	0.6147
Egg weight (g)	59.13	59.95	59.99	1.0974	0.014
Feed conversion rate (kg feed/kg egg)	1.69	1.77	1.64	0.2703	0.2891

The laying percentage, egg weight and feed conversion rate were not influenced by the dietary treatments. Other studies on broiler chickens show that the use of parsley in diet leads to an increase in feed consumption (Mohammed, 2010).

Table 2 shows the chemical composition of the feed diets used in this experiment. The additive inclusion rate was 2% for the parsley group (P) and 2% for the inulin group (I).

Table 2. Chemical analysis of feed

Parameter	Group	Control	Parsley	Inulin
DM		90.5	90.63	90.5
OM		75.97	75.86	75.81
CP		17.74	20.13	18.34
CF	%	4.23	3.83	3.99
Cel		3.75	3.72	4.51
NES		50.25	48.18	48.97
Ash		14.52	14.77	14.7
Xanthophyll	ppm	7.135	11.017	8.505
Vitamin E	ppm	67.941	65.382	95.317

DPPH is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecules do not dimerise, like most other free radicals (Kedare & Sing, 2011).

Antioxidants are the compounds, which combat the free radicals by intervening at any one of the three major steps of the free radical mediated oxidative process (Sindhi et al., 2013).

These antioxidants are also produced by biological system and occur naturally in many foods and the balance between oxidants and antioxidants decides the health (Kuźma et al., 2014).

Table 4 shows the physical parameters of hens eggs after 21 days from the administration of additives in their feed. Significant differences ($p<0.05$) were recorded for the weight of the yolk, where in group P it was lower than in group C and group I. Also, significant differences ($p<0.05$) were recorded for the weight of the albumen where the groups P and I had higher values compared to group C.

Table 3. DPPH analysis of feed and feed additives

Group	mg/g GAE	mM echiv trolox
Control	1.601	8.024
Feed (P)	1.947	8.457
Feed (I)	1.744	8.202
Parsley	7.71	13.05
Inulin	0.4	1.25

In the case of the amount of xanthophylls present in the yolk (Table 7), group P had a higher concentration but the difference is not statistically supported ($p>0.05$). An oxidized version of carotenoid called xantophyll constitutes a major part of carotenoids in nature. Xanthophylls are yellow pigments that are widely available in nature. Xanthophylls are well known for their benefits in human nutrition (Ribaya-Mercado et al., 2004). Also, in the case of vitamin E, a difference can be observed in the case of the experimental groups, being a larger amount in the group supplemented with inulin, but even here the difference is not statistically significant. The addition of parsley and inulin in laying hens' diets significantly decreased the iron content in the yolk eggs. Also, the addition of inulin in hens diets lead to decrease content of zinc in egg yolk.

Results presented in this study indicate that supplements added in diets of goups P and I were effective in increasing quality in 3 weeks of feeding by increasing polyphenols and antioxidant capacity in eggs. Moreover, it was clear that the supplements used showed high antioxidant properties by manipulating the poultry feed, which further promoted a significant increase in polyphenols and antioxidant compounds in the eggs of the laying hen in the experimental group compared to the eggs in the control group. Polyphenol concentration increased in both experimental eggs, in case of P group the increase was 27% and in group I was 9.5%.

Table 4. Physical and chemical (at the end of experiment) parameters of the egg after 21days on heat stress

	Control	Parsley	Inulin	SEM	p Value
Egg weight (g)	60.60	60.14	61.02	2.4168	0.5568
Egg white (g)	37.40	38.25	38.14	2.3313	0.4988
Egg yolk (g)	15.55^{b}	14.56^{a}	15.30	1.2245	0.0375
Eggshell (g)	7.65	7.33	7.58	0.5511	0.1823
Egg white pH	9.00^{bc}	9.25^{a}	9.27^{a}	0.2710	0.0180
Eggyolk pH	6.58	6.59	6.58	0.1415	0.9679
HU unit	97.52^{c}	97.63^{c}	93.38^{ab}	5.5348	0.0286

*where $^{a, b, c}$ show significant (P≤0.05) differences from C, P and I

Table 5. Physical parameters of the egg after 14 days of storage

Parameter	Control	Parsley	Inulin	SEM	p Value
Egg mass (g)	60.10	62.14	59.62	3.4273	0.4735
Egg white (g)	34.82	38.45	35.76	2.8247	0.0848
Egg yolk (g)	17.56	16.31	16.35	1.2295	0.1433
Eggshell (g)	7.72	7.38	7.51	0.6323	0.6837
Egg white pH	8.36	8.55	8.55	0.2959	0.4822
Eggyolk pH	6.00	5.90	5.96	0.3045	0.8729
HU unit	84.57	81.12	84.01	5.5149	0.2890

Table 6. Physical parameters of the egg after 28 days of storage

Parameter	Control	Parsley	Inulin	SEM	p Value
Egg weight (g)	59.17	56.09	59.22	3.0364	0.1202
Egg white (g)	36.17	33.86	35.26	2.7382	0.3600
Egg yolk (g)	15.45	15.32	16.69	0.9479	0.0118
Eggshell (g)	7.55	6.91	7.27	0.6418	0.2329
Egg white pH	9.69	9.63	9.68	0.1457	0.7534
Eggyolk pH	6.68	6.73	6.62	0.1627	0.5312
HU unit	78.00	77.25	79.56	5.9843	0.1107

Table 7. Polyphenols in egg yolk after 21 days of treatment

Parameter	Control	Parsley	Inulin	SEM	P value
mg/g GAE	0.715	0.908	0.783	0.1339	0.0532
mM echiv trolox	2.500^{bc}	3.296^{a}	3.386^{a}	0.4924	0.0141
Xanthophylls (ppm)	4.2430	6.9900	4.2333	1.5646	0.1160
Vitamin E (ppm)	80.212	90.347^{c}	110.9677	19.2069	0.1255
Iron in yolk (ppm)	154.88^{c}	153.10^{c}	146.01^{ab}	5.4645	0.0041
Zinc in yolk (ppm)	75.02	75.36^{c}	74.55^{b}	0.6371	0.0818

$^{a, b, c}$ show significant (P≤0.05) differences from C, P and I

Table 8. Plasma blood parameter after 21 days of heat stress

Parameter/Group	Control	Parsley	Inulin	SEM	P value
Hematocrit	25.6	26.83	27.67	2.0775	0.2729
Leukocyte (WBC) K/µL	18.92	14.77	16.7	4.01	0.2432
Heterophiles, %	47.4	45	44.33	5.9068	0.7003
Lymphocytes, %	47	51.83	52.5	5.1228	0.1641
Monocytes, %	5.5	3	2.17	2.8718	0.1975
Eosinophils, %	1.5	1.4	1.25	0.6504	0.8811
Heterophiles K/µL	8.97	6.69	7.43	2.2562	0.2525
Lymphocytes K/µL	8.86^{b}	7.6	8.7	1.9168	0.5058
Monocytes K/µL	1.05	0.45	0.4	0.6013	0.217
Eosinophils K/µL	0.28	0.2	0.19	0.0943	0.4069
Uric acid (mg/dL)	4.9	4.93	4.02	1.4354	0.4825
Urea (mg/dL)	1.67^{b}	1.5	1.67	0.6077	0.8476
Urea nitrogen (mg/dL)	0.79^{bc}	0.66^{ac}	0.89^{ab}	0.2405	0.2543

[a, b, c] show significant (P≤0.05) differences from C, P and I.

The use of dietary parsley and inulin on the hematological parameters (Table 8) of laying hens showed no significant effect (p>0.05) across groups.

CONCLUSIONS

Antioxidants from plants can also be transferred to chicken eggs.
The use of parsley and inulin in the diet of laying hens increases the amount of zinc present in the egg yolk.
The use of natural additives in the feed of laying hens does not negatively affect the egg laying percentage and feed consumption.

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AVIAN TUBERCULOSIS AND COMORBIDITY OF DOMESTIC CHICKENS: POSTMORTEM EXAMINATION

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Abstract

Avian tuberculosis is actively studied by researchers all over the world. But, as a rule, its variants are described as the main disease, when the death of the organism is caused by tuberculosis-specific injuries. At the same time, the predisposition of tuberculosis to a chronic course, allows the emergence of the so-called natural model of its association with other diseases and / or certain pathological processes. This fact is not always taken into account by veterinarians. The method of pathological autopsy of domestic chicken carcasses and the method of analysis of obtained results were used. The diagnosis of "bird tuberculosis" was based on the results of complex studies. There were diagnosed comorbid pathology: tuberculosis/reproductive syndrome at infectious bronchitis of chickens; tuberculosis/fatty hepatosis of domestic chicken based on analysis of pathological investigation. It was found that pathomorphosis of avian tuberculosis had certain differences due to its associated course with other pathologies.

Key words: *avian tuberculosis, comorbidity, domestic chickens, fatty hepatosis, infectious bronchitis, pathomorphological analysis.*

INTRODUCTION

Scientists all over the world are studying various aspects of avian tuberculosis, including the properties of its causative agent – *Mycobacterium avium* (Sattar et al., 2021; Tsiouris, 2021; Liakhovych & Maslak, 2021; Iancu et al., 2017; Kindu & Getaneh, 2016; Kriz et al., 2013; Macovei et al., 2013; Mayahi et al., 2013; Ciobotaru et al., 2012; Klanicova et al., 2011; Kul et al., 2005). This disease is a problem for birds and at the same time, its causative agent, like other atypical mycobacteria, is dangerous to humans, animals and fish (Lu et al., 2021; Urdes & Loh, 2021; Crilly et al., 2021; Mochizuka et al., 2021; Izumi et al., 2019; Yeh et al., 2019; Procopie et al., 2018; Beck et al., 2015; Lorencova et al., 2013; Miranda et al., 2012). Given the data of Romanian researchers Sarbu et al. (2018), who found mycobacteria in extreme conditions - sulfur caves (Puturosu Mountain, Romania), the ubiquity of these microorganisms is impressive. And this fact once again emphasizes the danger of tuberculosis pathogens. It is also important that wild and synanthropic birds carry different species of mycobacteria over a long distances, contributing to their spread (Muzyka & Stehnii, 2012). People with immunodeficiency are especially sensitive to *Mycobacterium avium* and other atypical mycobacteria. This category of the population is a potential consumer of egg and meat products that can be infected with these pathogens. In Ukraine chicken from private mini-farms is traditionally in grate demand, where, according to labels, ecologically clean poultry is obtained (at natural slaughter).

At the same time, these products are not always safe, as they often fall out of the quality monitoring system. Objective information on the quality of the products of private poultry farms helps to prevent the infection of its consumers and producers with pathogens common to birds and humans, in particular *Mycobacterium avium.* In most cases, the bird's body is an environment for pathogens of various diseases (there is an associated course). The phenomenon of disease association in the modern nomenclature of terms is called comorbidity (Valderas et al., 2009). The issues of comorbidity of avian tuberculosis are not fully investigated according to literature data. Knowledge of the pathological picture in this case can help in understanding of pathogenesis

and appropriate tactics of prevention, treatment and diagnosis of avian pathologies by veterinarians.

MATERIALS AND METHODS

The subject of the study were macroscopic changes in the carcasses of adult domestic Rhode Island Red meat-and-egg chickens aged from two to three years (n = 8) from a tuberculosis-prone private mini-farm. There were detected signs of tuberculosis-specific liver damage in some individuals of chickens that were slaughtered for domestic purposes.

The owner of the farm was regularly consulted by veterinarians: after the slaughter of chickens, the carcasses were carefully checked for safety for human consumption. Chickens were keeped for egg and meat products. The territory of the mini-farm is a place of residence and transit of many birds species (synanthropic and wild, including migratory): sparrows, turtle doves, buzzards, pheasants, crows, magpies, jackdaws, cuckoos, white and black storks, partridges (*Perdix perdix*), woodpeckers (*Crex crex*), owls, small woodpeckers and others. During the warm season, chickens could go to the open space, where thay coud have a potential contact with representatives of synanthropic and wild fauna and the likelihood contamination of food with various pathogens. Naturopathy - (treatment by natural means) prevailed in keeping chickens. The owner of the farm is guided by the principle formulated by Hippocrates: "Our food must be medicine and our medicine must be edible". Moistened mixtures of cereal grains, sunflower seeds, watermelons, melons, and pumpkins; depends on the season - grapes, raspberries, cherries, plums; chopped pumpkins, zucchini, potatoes, beets, carrots, cabbage; milk, sour milk cheese, shredded tulle of the Black and Azov Seas (*Clupeonella delicatula*), onions, garlic; striped garden snails (*Cepaea hortensis)*, common earthworms (*Lumbricus terrestris*) were used to feed the chickens. Outbreaks of infectious bronchitis had occurred among chickens in the winter, and the owner has successfully used inhalations with pine essential oil (*Pinus sylvestris* L.), which has antiviral, immunostimulatory, antispasmodic, analgesic, anti-inflammatory, and expectorant proportiess, to treat respiratory symptoms (Hrodzynskyi et al., 1992). All individuals of chickens recovered, in this birds normalized appetite.

However, in laying hens, egg laying was disrupted and / or stopped. Some hens laid defective eggs: yolk-free, with watery protein and abnormal inclusions (Figure 1).

Figure 1. Type of egg of a chicken suffering from infectious bronchitis: lack of yolk, watery protein, abnormal brown-red inclusions

As a result metabolic processes slowed down: the usual amount of food led to an excess of calories that not consumed by the body and, accordingly, to - lipid excess (fat accumulation). The owner was forced to kill the hens due to the cessation of egg laying. There were made veterinary and sanitary examination of chicken carcasses, in accordance with generally accepted rules (Dobin & Cocurichev, 1963). The affected organs of chickens were additionally examined if tuberculosis was suspected. The diagnosis of Tuberculosis avium was established postmortaly in two of the eight chicken carcasses (based on the complex studies). The aim of this study was to identify and classify pathological changes in the carcasses of slaughtered chickens in the associated course of tuberculosis and infectious bronchitis; tuberculosis and general obesity. The work was done at the Department of Normal and Pathological Morphology of the State Biotechnology University. Methods of clinical observation, pathological autopsy of chicken carcasses and their analysis were used.

RESULTS AND DISCUSSIONS

We found changes that characterise of avian tuberculosis in two female chickens during sectional studies. Among them, in particular, there were classic for this disease granulomas with a typical localization in the intestinal wall. They were easily visualized in the jejunum, where they were visible on examination of the serous membrane. The tubercle nodules were light yellow, ranging in diameter from three to five millimeters (Figure 2).

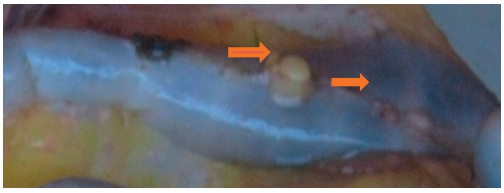

Figure 2. Fragment of the colon of a domestic adult chicken with comorbidity of tuberculosis / infectious bronchitis. Tuberculous granulomas on the serous membrane (arrows)

Within the duodenum, the tuberculous nodules were smaller than in the jeunum, were dense, some of tham had elongated (ellipsoidal) shape, and light pink-yellow in color, ranging in diameter from two to four millimeters (Figure 3).

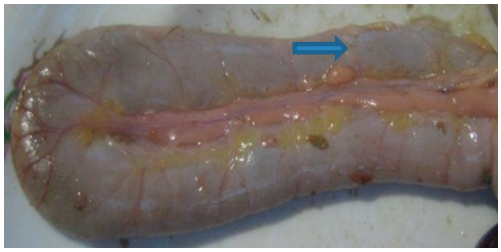

Figure 3. Fragment of the U-shaped duodenum and pancreas of a domestic adult chicken with comorbidity of tuberculosis /infectious bronchitis. Single tuberculous granulomas on the mesentery and serous membrane (arrow)

Tuberculous nodules localized in the mesentery was interesting finding. Such their placement is relatively rare in poultry. At the same time, these nodules were quite dense due to mineralization, had different diameters and different colors - from white to light pink-yellowish; placed along the vessels of the mesentery (Figure 4 A and B).

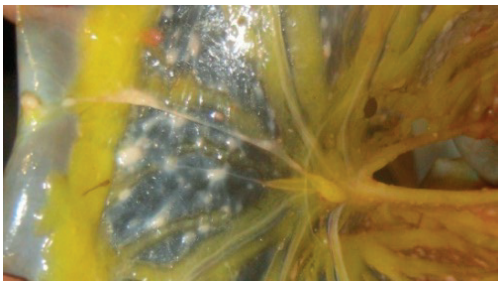

Figure 4 A. Small tuberculous nodules with localization at the root of the mesentery of an adult domestic chicken

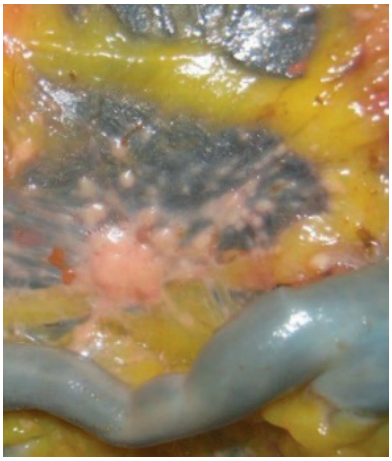

Figure 4 B. Draining mineralized tuberculous nodules with localization in the mesentery of an adult domestic chicken

For the study of tuberculous intestinal lesions in ornamental pheasants and peacocks never have these formations had such a density as in this case in domestic chickens (Lyakhovich et al., 2020; Liakhovych et al., 2018).

In peoples with tuberculosis, healing in areas of tuberculous changes, in particular, is associated with resorption and proliferation processes scarring, compaction, encapsulation, hyalinization, calcification) (Helberg et al., 2018). In birds, an important sign of tuberculosis, according to researchers (Dhama et al., 2011; Skoric et al., 2010), is granulomatous lesions without calcification. However, Özen et al. (2016) reported the phenomenon of mineralization of tuberculous granulomas, in particular, in the lungs of long-legged buzzard (buteo rufinus) - a bird of prey of the hawk family (*Accipitridae*). Trophic specialization of these birds differs significantly compared to domestic chickens.

After all, buzzards, like other members of the hawk family, eat, in particular, smaller birds, small mammals, fish. The trophic component is considered the leading source for the process of mineralization of tuberculous granulomas, as reported by Özen et al. (2016), and taking into account the information obtained on the fee diet in the studied chickens, which was rich in calcium. The formation of calcinates in the mesentery of the studied chickens, according to the existing classification of tuberculosis, should be classified as residual (post-tuberculosis). Their presence indicates a favorable variant of tuberculosis in the studied birds, especially taking into account the feeding rations and naturopathic direction, which is practiced by the owner of the mini-farm. In all specimens of the studied chicken carcasses in the thoracic cavity and internal organs contained significant accumulations of fat of a bright consistency of bright yellow color. Combination of specific liver lesions and steatohepatosis shoud be considered as favorable for tuberculosis process in another adult female chickens with generalized tuberculosis. On the background of steatohepatosis there were unfavorable conditions for the development of mycobacteria and changes for tuberculosis, in particular, the formation of specific granulomas (as evidenced by single tuberculous granulomas in the liver of the studied chicken). In this case, macroscopically, the chicken liver was deformed, sharply enlarged (had blunt edges, tight and in some areas - destroyed capsule), its surface was shiny, unevenly colored orange-beige-brown with pale yellow nodules (Figure 5), on the blade of the knife after the incision of the liver remained a fatty plaque; areas of complete disintegration (lysis) of the liver parenchyma with the formation of lipid cysts were detected. The consistency of the organ was flabby (the liver was easily torn during its examination). During the flatation test, liver fragments located on the water surface. In this model of liver pathology, tuberculous changes could not compete with lesions of steatosis relative to the common target (locus) of the lesion. Similar changes in tuberculosis of pheasants, the authors observed in the liver of those birds in the diet of which used rancid fats (Liakhovych et al., 2019). The authors also found steatohepatosis in generalized tuberculosis in an ornamental peacock (Liakhovych et al., 2021).

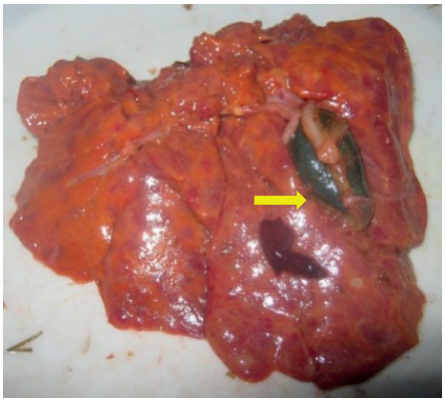

Figure 5. View of the liver and gallbladder of an adult chicken with steatohepatosis associated with tuberculosis (single tuberculous granulomas - arrow)

Researchers note that a high-energy diet to keep chickens in a cage provokes fatty liver syndrome (Rozenboim et al., 2016). The studied chickens in the winter were kept in crowded conditions without the possibility of active exercise. Infectious chicken coronavirus bronchitis is defined as a polysystemic disease with immune suppression (Shahnas et al., 2020). Herein is certain similarity of infectious bronchitis of chickens and tuberculosis. An important symptom of infectious bronchitis in chickens in adult females is the violation or even cessation of egg laying (Pereira et al., 2019). With the cessation of egg laying in the studied chickens due to the lack of folliculogenesis (it was possible the age reason or - due to exposure to infectious bronchitis), important chemical changes happens, in particular, the level of calcium in the body. It is also important that such a bird predisponsable to general obesity. Therefore, in the body of adult chickens with comorbidity of tuberculosis/infectious bronchitis a certain gradual (evolution) of changes can be expected: cessation of egg laying - steatohepatosis, according to that tuberculous liver damage will not be intense - general obesity - the appearance of tuberculous lesions, that localized in mesentery (moreover, with mineralization, because in the body of such birds there is an excess of minerals due to the

cessation of egg laying and - accordingly - the absence of egg shell formation). That is, minerals in such individuals are used to heal areas affected by tuberculosis.

CONCLUSIONS

Comorbidity was diagnosed on the basis of results of pathological examinations of domestic adult chickens carcasses: tuberculosis/infectious bronchitis; tuberculosis/steatohepatosis. The pathological picture of tuberculosis in domestic chickens due to comorbidity with infectious bronchitis and steatohepatosis had certain features. They were caused by processes that developed in the body of sick chickens. Due to the cessation of egg laying in the studied chickens, which recovered from infectious bronchitis, level of calcium increased, which is normally used by the body of an adult female to form an eggshell. Excess calcium was also facilitated by foods rich in it (in particular, cheese, fish, snails). In the liver parenchyma severe destructive changes with the formation of so-called lipid cysts have been developed as a result of steatohepatosis. The formation of intense classical tuberculous granulomas in such conditions is impossible. The comorbidity of infectious bronchitis of chickens and tuberculosis increases immunosuppression, which explains the natural impossibility of forming tuberculosis-specific nodules. After all, for tuberculous granulomas requires the appropriate phasing of immune cells. Therefore, the comorbidity model: steatohepatosis/hepatic tuberculosis is imposably. The predisposition of tuberculosis to a chronic course allows the emergence of a natural model of its association with other diseases and/or individual pathological processes. In such cases, the pathomorphosis of tuberculosis of domestic chickens has differences due to the associated course with other diseases.

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INFLUENCE OF THE ORGANOSELENIUM COMPOUND SELENOPYRAN ON THE ANTIOXIDANT SYSTEM OF LABORATORY ANIMALS WITH TOXICOSIS CAUSED BY CADMIUM COMPOUNDS

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Abstract

The article investigates the effect of an organoselenium compound on the antioxidant status of the body of laboratory animals when they are administered a cadmium compound. Cadmium compounds are widely known eco-pollutants, the toxic effect of which is due to their ability to stimulate free radical processes in the body of animals and humans. The purpose of this work was to identify aspects of cadmium toxicity due to its thiol specificity and features of the short-term adaptation of the antioxidant system to the administration of a toxicant in rats at the stages of ontogenesis. At the same time, the effectiveness of the organic selenium-containing compound was evaluated. In the tissues, the accumulation of lipid peroxidation products, as well as the content of selenium in the blood plasma, liver, and testicles of animals, were evaluated. In the course of the research, it was found that the prophylactic administration of selenopyran to experimental animals reduced the manifestation of toxicosis caused by cadmium. A similar effect of the drug is due to a decrease in the degree of damage to cell membranes and intracellular structures by free radical oxidation products.

Key words*: cadmium, diene conjugates, free radical oxidation, glutathione peroxidase, glutathione reductase, malondialdehyde, selenopyran, selenoprotein, superoxide dismutase.*

INTRODUCTION

In the formation of resistance to free radical damage, an important role belongs to the functioning of the enzymatic link of antioxidant protection. In turn, the structure of antioxidant enzymes provides for the presence of a large number of sulfhydryl groups, which determine both the spatial conformation of proteins and which are part of the active center.

Cadmium is a heavy metal capable of rapidly reacting with sulfhydryl groups in an aqueous medium, as well as replacing zinc in the active sites of enzymes. Cadmium compounds are widely known eco-pollutants, the toxic effect of which is due to their ability to stimulate free radical processes in the body of animals and humans (Voloshin et al., 2010; Gavryushina et al., 2021).

It is known that the microelement selenium largely mitigates the toxic effects of cadmium (Boryaev et al., 2015; Kravchenko et al., 2007).

The purpose of this work was to identify aspects of cadmium toxicity due to its thiol specificity and features of the short-term adaptation of the antioxidant system to the administration of a toxicant in rats at the stages of ontogenesis. At the same time, the effectiveness of the organic selenium-containing compound selenopyran (SP), 9-phenyl-symmetric octahydroselenoxanthene, was evaluated.

MATERIALS AND METHODS

The experiment was carried out on male rats of the Wistar breed. Three groups of animals were formed, receiving an isocaloric semi-synthetic diet. All groups of animals consisted of 2 subgroups: adults of sexual maturity (age over 90 days) and young adults in the period of puberty (age from 56 to 63 days).

The first group is the control. In the last 4 days of the experiment, the animals of the second group were intragastrically injected with an aqueous solution of $CdCl_2$ at a dose of 4 mg/kg of live weight.

Animals of the third group within 7 days intragastrically received an oily solution of selenopyran at a dose of 0.04 mg/kg of live weight, and $CdCl_2$ was administered similarly to the second group, against the background of the introduction of selenopyran.

In tissues (blood, liver, intestinal mucosa, myocardium, aorta), the accumulation of lipid peroxidation products was assessed by the content of diene conjugates (DC) and malondialdehyde (MDA), the activity of the main antioxidant defense enzymes (superoxide dismutase - SOD, glutathione peroxidase - GPO and glutathione reductase - GR) . The content of selenium in the blood plasma, liver and testicles of animals was studied.

Biometric processing of research results was carried out by the method of variation statistics for the reliability of the difference between the compared indicators using the Microsoft Office Excel software.

RESULTS AND DISCUSSIONS

The introduction of cadmium affected the function of the main life support systems of the body. Kidney damage was detected (in young and adult animals, the plasma creatinine content increased two times compared to the control group), and in the animals of the third group, this figure did not exceed the control value.

A significant increase in the activity of aminotransferases in the second group (by 30%) and bilirubin indicated liver damage, and in young animals to a greater extent than in adults. Selenopyran did not prevent an increase in alanine aminotransferase activity, however, aspartate aminotransferase activity and plasma bilirubin concentration remained at the control level.

In young animals, the accumulation of diene conjugates with the introduction of cadmium significantly increased in plasma (6.15±0.292 nmol/ml) and liver (72.2±0.86 nmol/g) compared with the control group (4.31±0.439 and 64.1±2.37, respectively).

In the second group, the administration of cadmium increased the content of malondialdehyde in the liver and aorta by 30 and 50%, respectively.

The introduction of selenopyran did not significantly reduce the degree of accumulation of diene conjugates in the blood and tissues.

In adult animals, the accumulation of diene conjugates in plasma, erythrocytes, liver, and intestines significantly decreased (compared to the control group) with cadmium administration, but tended to increase in the aorta and myocardium.

The accumulation of malondialdehyde in adult animals, both with the introduction of only cadmium, and against the background of selenopyran, significantly increased only in the myocardium (compared to the control).

The activity of antioxidant defense enzymes under cadmium load changed ambiguously.

Thus, the activity of glutathione reductase (Figure 1) in young animals remained at the control level in erythrocytes, myocardium and aorta, and significantly decreased in the liver and intestines, while in adults there was a significant decrease in the activity of the enzyme in the aorta (three times compared with the control), while no changes were observed in other organs.

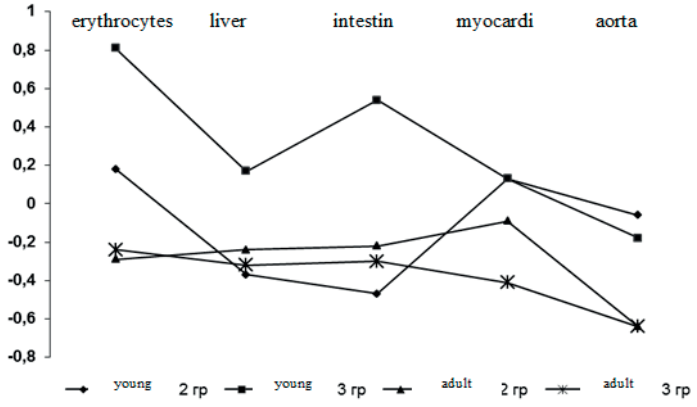

Figure 1. Changes in the activity of glutathione reductase in the organs of young and adult rats with experimental cadmium toxicosis (relative units to control values)

In young animals with toxicosis caused by cadmium, there was an increase in the activity of superoxide dismutase in the intestine, while in adults - in the intestine, liver and aorta, and in erythrocytes there was a significant decrease compared to the control.

The introduction of selenopyran against the background of cadmium loading led to an increase in the activity of glutathione reductase (Figure 1) in the liver and erythrocytes in young animals, and in adult rats in the myocardium and aorta. In adult animals, an increase in the activity of superoxide dismutase in erythrocytes and a decrease in this indicator in the intestine and myocardium were found compared with the second group.

The activity of glutathione peroxidase (Figure 2) in young animals of the second group in the liver and intestines decreased significantly in relation to the control, and increased in the myocardium.

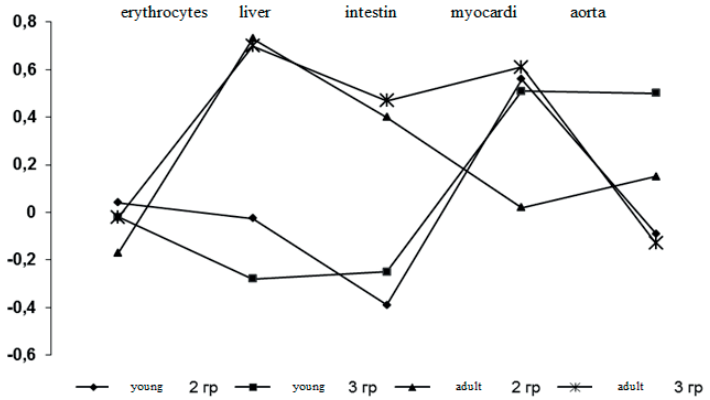

Figure 2. Changes in the activity of glutathione peroxidase in the organs of young and adult rats with experimental cadmium toxicosis (relative units to control values)

On the contrary, adult animals showed a significant increase in enzyme activity in the liver and intestines, and no changes in other tissues. The activity of this enzyme in adult and young animals correlated with changes in the content of selenium in the liver - a decrease in the concentration of selenium in the liver in young animals, and an increase in adults (Table 1).

Table 1. The content of selenium in the organs of young and adult rats with experimental cadmium toxicosis

Groups	Plasma, ng/ml		Liver, ng/mg		Testicles, ng/mg	
	young	adults	young	adults	young	adults
1	185±16.3	400±24.5	0.912±0.077	0.562±0.042	0.081±0.009	0.082±0.003
2	182±11.5	375±30.7	0.488*±0.068	0.635±0.065	0.061*±0.003	0.117*±0.006
3	287*±25.4	369±23.3	0.819*±0.118	0.698*±0.044	0.090*±0.007	0.147±0.036

* - differences with control are significant P<0.05

It is known that glutathione peroxidase is the main selenoprotein in the liver of mammals; therefore, the observed difference in enzyme activity is probably associated with its quantitative content in cells, and not with functional damage to macromolecules. The use of selenopyran against the background of a cadmium load increased the content of selenium in the liver in young individuals to the level of control animals, and in adults - by 24.2%, compared with the control, which did not correlate with changes in the activity of glutathione peroxidase. Apparently, in this case, slow metabolization of selenopyran in liver cells takes place, which corresponds to the short duration of the experiment, or its deposition, since the release of selenium from the molecule would lead to an increase in the synthesis of selenoproteins.

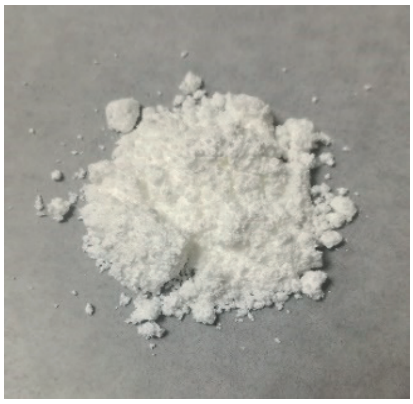

Figure 3. Cadmium hydroxid

It is known that the testes are the target organs of toxicosis caused by cadmium (Figure 3), and cadmium significantly affects the content of the trace element selenium in them (Table 1), the most important component of enzymatic antioxidant protection. At the same time, immature and adult individuals react differently. If the concentration of selenium in young individuals under a cadmium load in the testicles sharply decreased (by 25%), then in adults the opposite picture was observed (increase by more than 40%).

With the introduction of selenopyran, both in young and adult animals, the content of selenium in the testes increased, and this contributed to a decrease in the toxic effect of cadmium.

CONCLUSIONS

Thus, the prophylactic administration of selenopyran to experimental animals reduced the manifestation of toxicosis caused by cadmium.

A similar effect of the drug is apparently due to a decrease in the degree of damage to cell membranes and intracellular structures by free radical oxidation products.

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THE INFLUENCE OF THERMAL VARIATIONS ON THE INCIDENCE OF RABIES IN ANIMAL BIODIVERSITY

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Abstract

This paper presents the results of the study of the epidemiological situation of rabies in the last 10 years on the territory of the Republic of Moldova. It has been found that the most susceptible animal species to rabies virus are cattle (358 cases), dogs (304 cases), foxes (186 cases) and cats (150 cases), which constitute 91.14% of the total number of cases recorded in animals in the last 10 years. The fox is the rabies-reservoir species and the main vector of its spread in wild animal populations. In the livestock sector, cattle are the most affected and constitute 32.69%. Data obtained from the study show that rabies is developing sporadically. In the population of wild animals other than foxes, cases of the disease are not dependent on the existence of infected foxes in that area. It has also been found that there is no significant correlation of rabies in fox and dogs, fox and cattle, or dogs and cattle. At the same time, there has been found a correlation between the number of fox units and rabies cases and a cyclicity of rabies cases every 4 years (2012-2015 and 2016-2019). The influence of thermal variations on the incidence of rabies in living biodiversity has specific oscillating effects according to the years of study, with multiple divergences, which require a well-founded argument, based on the existing importance of the problem approached through high-performance scientific research.

Key words*: animal biodiversity, rabies, temperature.*

INTRODUCTION

Rabies remains a global complex problem, being one of the oldest viral zoonoses known to man. Rabies (lat. *rabies*, derived from rabhas - Old Sanskrit: to be violent) or madness has been recorded, over time, with a variable frequency, on all continents. Some countries, favored by their geographical position or as a result of drastic control measures, have managed to become and/or remain free from rabies (Australia, Iceland, Japan, New Zealand, Hawaii, England, Ireland, Spain, Portugal, Norway, Sweden, etc.). In general, the incidence of rabies cases has varied over time and varies widely across countries and from year to year. About 60,000 deaths are reported in humans each year in the world, but their actual number is of course much higher (OIE, 2018; OIE, 2021; Shankar et al., 2012; Бешенство, 2020). The global economic burden of rabies is estimated at 8.6 billion USD a year. Children under the age of 15 make up 40% of people who are bitten by animals suspected of rabies (FAO, 2018). Rabies is currently considered one of the most neglected diseases in the world, but it is a real burden for the developing countries (Jackson & Wunner, 2007). In the vast majority of human rabies deaths, dogs are the source of infection, accounting for up to 99% of all human rabies transmission, contributing up to 99% of all rabies transmissions to humans (Sudhi, 2014).

Rabies incidence in different countries. The incidence of rabies in different countries varies somewhat, influenced by the population dynamics of the rabies-reservoir species, by their state of immunity, the geographical peculiarities of the areas involved. In Europe, according to the World Organization for Animal Health (OIE), about two-thirds of rabies cases are reported in wild animals, and almost one-third of cases in domestic animals (OIE, 2021; Moga Mânzat, 2000).

All species of homeothermic animals are susceptible to rabies virus infection, but only a few of them are an important reservoir of the disease depending on the geographical region. Globally, the reservoirs differ depending on the continent, namely: in North America the most important reservoir are raccoons (*Procyon lotor*), skunks (*Spilogale gracilis* and *Mephitis*

mephitis) and bats, in South America, bats (*Desmodus rotundus*), in South Africa, jackals (*Canis adustus* and *Canis mesomelas*) and mongooses (*Suricata suricata*). According to the World Health Organization (WHO), in about 95% of human rabies cases reported in Asia and Africa, the dog is the main reservoir (Macpherson et al., 2013). In Europe, the fox (*Vulpes vulpes*) is the natural reservoir of rabies and the main vector of its spread in wild and domestic animal populations. In this context, since 1989, rabies vaccination of foxes has been the main tool in eliminating rabies in many European countries. In this way, it has been possible to reduce the number of cases of the disease or even to obtain the status of "rabies-free" countries (OIE, 2018, 2021; Müller et al., 2015; Najar & Streinu-Cercel, 2012; Shankar, 2012).

WHO, OIE and Food and Agriculture Organization of the United Nations (FAO) proposed to jointly eliminate as soon as possible the risks posed by rabies to both animal (either domestic or wild), and human health, and as a basic goal they pointed out that by 2030 there should be recorded zero human deaths due to rabies (Блохин et al., 2019; FAO, 2021; OIE, 2018; Robardet et al., 2019; WHO, 2021).

In order to reduce the number of rabies cases in the national territory, the Republic of Moldova has joined the WHO, OIE and FAO initiative to combat rabies and thus in 2019 approved the Measure Plan for the surveillance, control and eradication of rabies in the foxes of the Republic of Moldova for the years 2019-2023, to obtain the status of a rabies-free country.

According to the Measure Plan for the surveillance, control and eradication of rabies in the foxes of the Republic of Moldova for the years 2019-2023, to ensure the progressive reduction of rabies in the Republic of Moldova, until obtaining the status of a "rabies-free" country, it is expected to implement concrete action measures, one of which is the rabies vaccination of fox populations with vaccine baits (HG, 2019). For the successful implementation of the above-mentioned Plan, it is necessary to study the influence of environmental factors (especially temperature) on the incidence of rabies in animals.

MATERIALS AND METHODS

We performed a descriptive study evaluating the trend of rabies disease in the Republic of Moldova, both in wild animals and in humans between the years 2012-2021. We also performed an analysis of the literature and gathered epidemiological data from the WHO Rabies Bulletin Europe database, from the European Center for Disease Prevention and Control (ECDC) Epidemiological Reports and from the Annual Reports of the National Agency for Food Safety of the Republic of Moldova.

The materials analyzed for this paper were presented by the National Agency for Food Safety, the I.P. Republican Center for Veterinary Diagnosis, Institute of Zoology and the State Hydrometeorological Service. The statistical processing of the obtained cipher material was performed by the established methods for the biological field.

RESULTS AND DISCUSSIONS

The Republic of Moldova is one of the countries with the highest number of rabies cases in Europe, surpassed only by Ukraine, where about 1400 cases of rabies in animals and approximately 12 fatal cases in humans are registered annually (OIE, 2018, 2021).

Rabies is registered throughout the national territory, but most frequently it is registered in the districts of Edinet, Ungheni, Anenii Noi, Causeni, Falesti, Straseni, Nisporeni, Soroca, Cahul and Chisinau (Figure 1).

Figure 1. Distribution of rabies cases on the national territory for the years 2012-2021

According to the data presented by the I.P. Republican Center for Veterinary Diagnosis during the 2012-2021 study period, it has been found that most cases of rabies were reported in cattle, dogs, foxes and cats (Table 1). If we refer to dogs then most positive cases were recorded in rural areas. This is explained by the fact that the dogs from rural areas have been in more frequent contact with wildlife and thus increase the cases of disease transmission from wild animals to the domestic ones (wild animals-dogs-domestic animals).

Table 1. Number of rabies cases by animal species for the years 2012-2021 (10 years)

Year	Foxes		Dogs		Cats		Cattle		Other animals	
	cases	%	cases	%	cases	%	cases	%	cases	%
2021	3	13.0	8	34.8	3	13.0	8	34.8	1	4.3
2020	15	16.9	22	24.7	12	13.5	37	41.6	3	3.4
2019	17	18.3	22	23.7	14	15.1	34	36.6	6	6.5
2018	16	18.0	24	27.0	15	16.8	29	32.6	5	5.6
2017	13	22.0	13	22.0	7	11.9	22	37.3	4	6.8
2016	12	12.5	34	35.4	17	17.7	25	26.0	8	8.3
2015	30	15.0	51	25.6	25	12.5	74	37.0	20	10.0
2014	33	21.0	57	36.3	18	11.5	26	16.6	23	14.6
2013	25	19.7	37	29.1	19	15.0	42	33.0	4	3.1
2012	22	13.6	36	22.2	20	12.3	61	37.7	23	14.2
Total (10 years)	186	16.98	304	27.76	150	13.69	358	32.69	97	8.85

The data in Table 1 show that the fox is the main reservoir of rabies in wildlife. The presence of rabies in wildlife is a major risk for both animals and humans, thus being considered the most important anthropozoonosis with serious consequences (100% lethality) for animals and humans.

This eminent danger for domestic animals and humans has been accentuated lately as the foxes started nesting more and more often even in the localities (Table 2). This state of affairs increases the risk of rabies in both animals and humans.

Table 2. Distribution of fox breeding units in favorable ecosystems for 2021

Species	Agrocoenoses (2540 thousand ha)	Human settlements (314 thousand ha)	Forest Fund (335 thousand ha)
Foxes (*Vulpes vulpes*)	14.2 thousand	6 thousand	3.8 thousand

According to the Institute of Zoology, the density of foxes in autumn in the Republic of Moldova in the last 10 years is about 36,000 animals (Table 3). From a numerical point of view, in recent years, the population of foxes in the Republic of Moldova has remained constant, which means that their density is 10-12 specimens per 1000 ha. This amount is ten times higher than the optimal number of this species (1-2 foxes per 1000 ha).

Table 3. The (estimated) number of foxes in the Republic of Moldova for the years 2012-2021

Year	Herd of foxes (specimens) (thousand)		Effective (growth thousand)
	Effective spring	Effective autumn	
2021	24000	35000	11000
2020	25000	37000	12000
2019	29000	36000	7000
2018	28000	35000	7000
2017	28000	34000	6000
2016	27000	33000	6000
2015	26000	29000	3000
2014	28000	38000	10000
2013	30000	41000	11000
2012	32000	42000	10000
10 year average	27700	36000	8300

Data showing the total number of rabies cases in animals according to the reference years are distributed as follows in Figure 2.

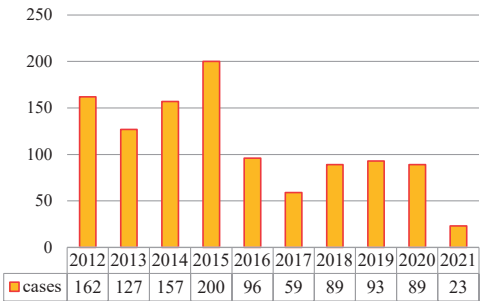

Figure 2. The total number of cases of rabies among animals for the years 2012-2021 (heads)

The data in Figure 2 show that the number of animal rabies cases is higher in the years 2012-2015 with a maximum of 200 cases in 2015 and an average of 161 cases in this period (4 years). In subsequent years, there has been a sharp decline in rabies in animals as compared to the previous period. The "relatively low" level is maintained during the years 2016-2020 with a maximum of 96 cases registered in 2016 and an average of 84 cases in these 4 years. The explanation of the established phenomenon is mainly predetermined by the reduction of the average number of foxes in the autumn unit in their natural habitat from 37,500 thousand in 2012-2015 to 34,500 thousand in 2016-2019.

At the same time, we mention that in 2021, from the data available at the time of writing this article, there were only 23 cases of rabies. The small number of rabies cases can be explained by the fact of application of prophylaxis measures by starting the vaccination of wildlife by terrestrial methods of distribution of vaccine baits directly in front of the mapped burrows throughout the country. Concomitantly, the baits were distributed by air via four special aircraft, automatically with the help of a "thrower" with a frequency of 25 baits per km^2 and a distance between the flight lines of 500 meters. Therefore, the applied intervention significantly reduced the number of cases of rabies, demonstrating the effectiveness of the measures taken and the post-vaccination resistance of the animal unit to the rabies virus produced by enhancing the body's immune properties and increasing the biological response of animals to this morbidity.

The research continued by studying the influence of ambient temperature on the incidence of rabies cases in wild and domestic animals. The temperature on the territory of the Republic of Moldova was recorded in three meteorological stations in the north in Briceni district, in the center in Chisinau municipality and in the south in Cahul. Based on the recorded data, the average temperature was calculated throughout the country during the years 2012-2021. The results of the study are presented in Table 4.

Table 4. Average monthly temperature for the years 2012-2021 in the Republic of Moldova (ºC)

Month	Year									
	2021	2020	2019	2018	2017	2016	2015	2014	2013	2012
January	0.21	0.22	-2.66	-0.80	-4.86	-3.16	-0.73	-2.03	-2.56	-2.70
February	0.13	3.94	2.26	-1.43	-0.81	4.61	0.53	-1.01	0.86	-8.42
March	3.51	7.62	7.20	0.93	7.60	6.13	5.03	7.81	1.92	4.33
April	8.32	11.13	10.33	14.91	9.53	13.13	10.13	11.10	12.21	12.93
May	15.32	14.21	16.46	18.86	16.10	14.63	17.10	16.33	18.70	18.33
June	19.91	21.22	22.60	21.23	20.93	21.01	20.13	19.62	20.62	22.41
July	23.61	22.70	21.56	21.66	21.76	22.96	23.53	22.00	21.16	25.36
August	21.31	23.42	22.93	23.63	22.83	22.22	23.93	22.13	21.81	22.73
September	15.31	19.71	17.86	17.52	18.01	18.52	19.31	17.66	14.31	18.66
October	9.70	14.23	11.86	12.76	10.73	7.93	9.56	9.73	10.76	12.43
November	6.70	4.81	7.90	2.56	5.36	3.44	6.56	3.72	8.16	6.03
December	0.53	2.10	2.80	-1.03	2.93	0.72	2.63	-0.31	0.06	-3.33
Annual average	10.38	12.14	11.75	10.89	10.84	11.00	11.47	10.55	10.66	10.72

The data in Table 4 show that the average annual temperature in the Republic of Moldova during the years of study is in the range of 10.38°C to 12.14°C. The minimum average temperature was 10.38°C in 2021, and the maximum average temperature was 12.14°C in 2020 (difference 1.76°C).

It is known that the rabies virus is quickly destroyed by sunlight and heat, but it is also well preserved in low temperatures and frost for long periods (Turcitu, 2007; Шевченко, 2013). In this context (Литусов, 2018) states that at 0ºC, the virus is stored for several weeks. He also mentions that in animal carcasses at low temperatures the virus can be

stored for up to 4 months. Based on this statement, it can be concluded that in the cooler years the rabies virus is preserved longer in the environment, causing the infection of more animals and increasing the number of rabies cases.

Table 5. Average annual temperature and number of rabies cases in the years 2012-2021 in the Republic of Moldova

Year	Cases of rabies (heads)	Temperature (°C)
2021	23	10.38
2020	89	12.14
2019	93	11.75
2018	89	10.89
2017	59	10.84
2016	96	11.00
2015	200	11.47
2014	157	10.55
2013	127	10.66
2012	162	10.72

However, the data in Table 5 show that the influence of thermal variations on the incidence of rabies cases in animal biodiversity has specific oscillating effects according to the years of study, with multiple divergences and the frequency of rabies cases is not subject to the above statement.

CONCLUSIONS

Although the number of animals detected with rabies has clearly decreased in recent times, the disease continues to cause material damage and obvious risks to the biodiversity of the wildlife, the national economy and civil society. The frequency constancy is defined by the occurrence of repeated cases of rabies in animals in the same localities, becoming outbreaks of major epidemiological significance. It should be noted that the large number and diversity of wild and domestic animals involved in the epidemiological process increases the potential for rabies cases in the animal and human population.

The data obtained from the study show that rabies is evolving sporadically. In the wildlife population other than foxes, cases of the disease are not dependent on the existence of infected foxes in the respective area. It has also been found that there is no significant correlation of rabies in fox and dogs, fox and cattle, or dogs and cattle. At the same time, there has been found a correlation between the number of fox units and rabies cases and a cyclicity of rabies cases every 4 years (2012-2015 and 2016-2019). The influence of thermal variations on the incidence of rabies in living biodiversity has specific oscillating effects according to the years of study, with multiple divergences, which require a well-founded argument, based on the existing importance of the problem approached through high-performance scientific research.

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A PRELIMINARY STUDY ON LIBIDO AND SEMINAL ATTRIBUTES OF DAJAL BREEDING BULLS

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Abstract

This study aims to deduce a baseline data regarding seminal attributes (fresh and post thaw semen) and libido of indigenous Dajal and Cholistani breeding bulls. Data were collected on weekly basis for three months and a total of 138 ejaculates were attained for analyses. Among fresh semen attributes, mass motility was significantly higher for Dajal bulls as compared to Cholistani bulls being 2.7±0.2 and 2.1±0.1, respectively. Dajal proved to bear the brunt of cryopreservation in a better way in terms of percentage of live spermatozoa (14.7±0.2% damage). The values of libido index score, reaction time and Time Lapsed between Two Ejaculates were also significantly higher for Dajal breeding bulls. This preliminary study is the first of its kind, which furnishes baseline data regarding various reproductive attributes of Dajal breeding bulls. It envisions future studies on Dajal bulls with a larger sample size related to effect of age, season, feeding regimen, various types of extenders on semen quality, and assessment of fertility rate in order to attain an enhanced productivity.

Key words*: Breeding bulls; Dajal cattle breed; libido estimation; semen analysis*

INTRODUCTION

The uninterrupted crossing of local/indigenous livestock with exotic bulls/semen has led to loss of indigenous genetic resources, extinction of native varieties and disturbance of eco-system (Mathias & Mundy, 2005; Tempelman and Cardellino, 2007). The ultimate consequence has resulted in replacement of indigenous local breeds with exotic ones (Köhler-Rollefson et al., 2009).

A terrifying loss of one indigenous livestock breed in two weeks has been reported by the FAO (Rischkowsky & Pilling, 2007; Köhler-Rollefson et al., 2009). The South-Asian countries have lately realized the dire need to conserve the indigenous genetic resources as a landmark to sustainable rural/sub rural livelihoods (Ruto et al., 2008).

Consequently, extensive research work has started pouring in, regarding the productive and reproductive aspects of indigenous livestock from Pakistan in order to conserve and propagate these breeds.

Pakistan is endowed with 15 indigenous cattle breeds which constitute 43% of total cattle population (Khan et al., 2008). Extensive research work has been conducted on Sahiwal and Red Sindhi breeds of cattle which have acclaimed them both at national and international level for being thermo-tolerant, hardy and tick-resistant breeds. Last decade has witnessed substantial work conducted and reported on productive and reproductive attributes of the formerly neglected Cholistani breed of indigenous cattle being reared by the nomads of Cholistan desert (Farooq et al., 2010; Farooq et al., 2012; Farooq et al., 2013a; Farooq et al., 2013b; Farooq et al., 2015). Resultantly, national conservation and propagation programs for this breed are under way.

Dajal is a locally-famed indigenous cattle breed of Pakistan mostly being used for draught purposes. It's utility and hence, its number has declined over the years owing to mechanization in agriculture and livestock sectors. It is a native breed of a small town, Dajal, located in District Dera Ghazi Khan (DGK), Punjab,

Pakistan (Figure 1). It has a white or greyish body color with distinct deep grey areas over the neck, shoulder and hump in mature males. It has long legs, a sturdy torso, compact body and well-built structure which give it a peculiar look of 'half bull and half horse'. Head is almost medium sized with short strong neck, black muzzle and eye lashes. Small dewlap, stumpy horns, small pointed ears, moderately developed hump, straight back, tight sheath and black tuft of tail are prominent points of Dajal animals (Figures 2 and 3) (Ward, 2003).

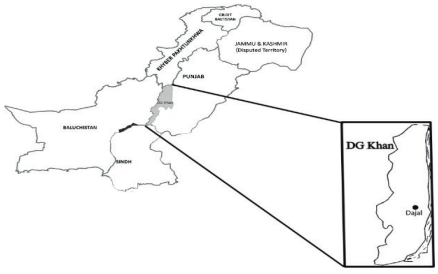

Figure 1. Geographical location of Dajal area, District Dera Ghazi Khan (DGK), Punjab, Pakistan

Figure 2. An elite specimen of Dajal breeding bull being incorporated in the present study reared at the Semen Production Unit, Karaniwala, Cholistan, Punjab, Pakistan

Figure 3. An elite specimen of Dajal Cow being reared at a private farm of Fazilpur, Dera Ghazi Khan, Punjab, Pakistan

Average body weight of Dajal breed is 550 kg to 650 kg of weight in mature bulls and 350 to 450 kg in cows. Cows are low yielder of milk and 1121±92 liters of milk per lactation have been reported (Aslam et al., 2002; Kenyanjui et al., 2009). A rough estimate of 72 thousand heads of Dajal has been reported (Khan et al., 2008).

It is not yet an established breed as it has not been depicted in the Livestock Census of Pakistan of 2006. There are unconfirmed reports attained from the native inhabitants and old-timer veterinarians of the Dajal area (native to Dajal breed of cattle) that an Assistant Commissioner, Francis, back in 1940s had initiated a Dajal Cattle Breeding Scheme as per an Inspection Note emanated from his office. However, the scheme waned off after independence of Pakistan in 1947 (Saeed, 2020). Later in 1972, the government of Pakistan established a Dajal Cattle Breeding Farm at state land of Fazilpur village of Dajal. Dajal cattle breed was reared here for two years and again in 1974, it was replaced by the Sahiwal cattle as per the provincial government's orders. The farm was designated for managing purebred Sahiwal cattle only and consequently Dajal vanished from the government attention till late 2016.

Scanty work on paternal lineage (Anwar et al., 2014) and diseases (Sohail et al., 2019) in Dajal have been reported from Pakistan. However, no work has yet been conducted on reproductive attributes of this neglected breed. Lately, few Dajal breeding bulls, selected on the basis of phenotype, have been brought to the Semen Production Unit (SPU), Karaniwala, Bahawalpur (Punjab, Cholistan, Pakistan) and are being utilized for semen collection in an attempt of its conservation and propagation. The demand of its frozen semen is confined to all the districts of DGK Division. Furthermore, the Livestock and Dairy Development Department, Punjab, Pakistan has initiated highlighting this breed at various local/national livestock shows known as 'Kissan Mailas' in local language. The present study is the first of its kind, designed with a general objective to highlight the indigenous Dajal breed of cattle from Pakistan. In specific, it aims to deduce a preliminary baseline data regarding libido and seminal attributes (fresh and post thaw semen)

of Dajal breeding bulls being reared at SPU Karaniwala, Punjab, Pakistan in comparison to Cholistani bulls.

MATERIALS AND METHODS

The present study was conducted for three months (April to June, 2019) at a state SPU located in the Cholistan Desert, Punjab, Pakistan. Geographical location of this area has been described earlier (Farooq et al., 2012). This is an arid tropical area with May and June (dry summer) being the hottest months of the year with the maximum temperature exceeding 45ºC. Negligible rains of monsoon are witnessed in July and August (wet summer). Winters are seen in December and January with temperature of 17-20ºC. Adult (5-6 years of age) breeding Dajal (n = 03) and Cholistani bulls (n = 03) having clinically healthy reproductive tract and donating semen of acceptable quality were selected for the study. The age of the study animals was attained from the records of the SPU, Karaniwala and their profile is presented in Table 1.

Table 1. Profile of Dajal and Cholistani breeding bulls incorporated in the study, being harbored at Semen Production Unit, Karaniwala, Punjab, Pakistan*

Bull #	Date of Birth	Age at Start of Research	Date of Entering SPU
DAJAL			
KWC-56	May, 2013	05 Years, 11 Months	03-07-2014
KWC-73	October, 2013	05 Years. 06 Months	02-08-2015
KWC-86	March, 2014	05 Year, 01 Month	17-06-2017
CHOLISTANI			
KWD-1	October, 2013	05 Years. 06 Months	17-06-2016
KWD-2	December, 2013	05 Years. 04 Months	17-06-2016
KWD-3	March, 2013	05 Years. 01 Months	17-06-2016

*Data Retrieved from the Records of SPU, Karaniwala

The body weight was taken fortnightly during the study period. They were kept under naturally prevailing climatic conditions, fed good quality seasonal fodder at the rate of 10% of body weight per bull and 2-3 kg of concentrate per bull per day. Vaccination against Hemorrhagic Septicemia and Foot and Mouth disease was carried out as per schedule. Preventive measures against worm infestation were undertaken twice in a year or whenever felt necessary. The details regarding feeding and management of these animals have been described elsewhere (Farooq et al., 2013).

At the time of semen collection, libido of each donating bull was estimated in terms of reaction time, libido index and time-lapsed-between-two-ejaculates (TLTE). A dummy bull (mostly Sahiwal bull and occasionally Cholistani bull) was used for bulls to mount on. All bulls were given sexual preparation before entry into the test area. The time between introduction of bull into the test area and first mount was defined as 'reaction time' (Singh and Pangawkar, 1989). A libido index, ranging from 0 to 6 was calculated for each bull depending upon his sexual interest in the teaser (Crichton & Lishman, 1985).

Semen from each experimental bull was collected at weekly intervals, using an artificial vagina (AV). The final temperature of AV before semen collection was in the range of 41-44°C. Two ejaculates were collected from each bull on each collection and a total of 12 collections were carried out. Infrequently, some bulls did not give the second ejaculate and hence, a total of 138 ejaculates with 23 ejaculates per bull were attained and processed further. After collection, each semen sample was processed for physical seminal attributes such as ejaculatory volume, color, pH, mass motility (score 0-5), individual sperm motility (40X, phase contrast microscope; Olympus BH-2, Tokyo, Japan) and sperm count (Photometrically at 560 nm wavelength using Bovine Photometer n° 1119, IMV, France), percentages of live and morphologically normal sperm and those with intact acrosome (Eosin-Nigrosin Staining) as prescribed (Jainudeen et al., 1982). Details of initial semen evaluation and relevant data have been presented elsewhere (Farooq et al., 2013).

The collected ejaculates were pooled and One-step slow method of dilution was adopted for each semen sample using Tris-fructose-egg-yolk glycerol extender, being used in the SPUs of Pakistan. Final sperm concentration of 30×10^6spermatozoa per insemination dose of 0.5 mL straws was resultantly, attained. Details of semen extension and freezing have been given elsewhere (Farooq et al., 2015). Frozen straws were stored in liquid Nitrogen until used for post thaw analyses.

The frozen straws were thawed at 37°C for 30 sec and subjected to post thaw seminal attributes *viz*. individual sperm motility (40X, phase contrast microscope; Olympus BH-2, Tokyo, Japan), percentages of live, morphologically normal sperms and those with normally intact acrosomes as per recommended protocols (Rasul et al., 2001). Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS V. 21 for Windows, Chicago, IL, USA). Data were presented as mean (± SE) values for libido, fresh seminal and post thaw seminal attributes. Independent sample t-test was implied for deducing differences between these attributes in Dajal and Cholistani bulls at the significance level of P≤0.05.

RESULTS AND DISCUSSIONS

The results regarding age, body weight and libido estimation parameters for Dajal and Cholistani breeding bulls are presented in Table 2.

Results indicated that though the age of bulls of each breed was almost same, yet there was significant (P≤0.05) difference in body weight being higher for Dajal (595.0±11.7 kg) as compared to Cholistani bulls (486.6±11.4 kg) (Table 2).

Table 2. Comparative overall mean values (± SE) for age, body weight and libido estimation parameters of Dajal (n = 03) and Cholistani (n = 03) breeding bulls

Parameters	Dajal	Cholistani
Age (months)	62.6±0.6	65.0±1.2
Body weight (kg)	595.0±11.7	486.6±11.4*
Libido index (Score 1-6)	4.3±0.2	3.9±0.1*
Reaction Time (min)	0.3±0.07	0.8±0.1*
Time elapsed between two ejaculates (min)	2.0±0.1	2.7±0.2*

*Significant within rows (P≤0.05)

In Pakistan, Dhanni, Dajal and Lohani are considered as draught indigenous cattle breeds whereas Cholistani and Red Sindhi are considered as milch breeds. While assessing birth weight as affected by various factors in a study, it has been reported that the birth weight, weaning weight and yearling weight in calves of Dajal cattle is higher as compared to other indigenous livestock breeds of Pakistan (Moaeen-ud-Din & Bilal, 2017). This study also endorsed the need of improving Dajal as a beef breed in the wake of the fact that Pakistan does not have a beef breed as yet. Genetic predisposition, environment and feeding regimen mainly maintain the body weight of farm animals as elucidated earlier.

The mean (± SE) value of libido index score for Dajal breeding bulls in present study was significantly (P≤0.05) higher being 4.3±0.2 as compared to 3.9±0.1 for Cholistani bulls (Table 2). Libido is considered as the most vital reproductive attribute for a breeding bull. It is directly related with the serum testosterone level and hence fertility rate. The sexual behavior, normally, is weaker in *Bos indicus* bulls as compared to *Bos taurus* bulls (Higdon et al., 2000). However, the score attained by the Dajal bulls in present study seems beyond satisfactory. Comparing the results with other studies conducted on Zebu cattle breed, slightly higher libido score of 4.8±0.07 has been reported from indigenous cattle bulls of Ethiopia (Abera et al., 2006) whereas a lower score of 3.2±0.17 has been reported for Sahiwal breeding bulls (Ahmad and Asmat, 2005). Keeping in view the same season and feeding regimes in this study, better libido score in Dajal breeding bulls may be attributed as an inherited character of this breed.

Regarding reaction time and TLTE, relevant mean values (± SE) for Dajal breeding bulls were 0.3±0.07 mins and 2.0±0.1 mins, respectively being significantly (P≤0.05) different and better as compared to those for Cholistani bulls (0.8±0.1 and 2.7±0.2 mins). The values for reaction time for both breeds are lower than those reported earlier for Cholistani bulls (4.5±1.1 mins) (Mahmood et al., 2013) and Sahiwal bulls (3.2±1.0 mins) (Ahmad & Asmat, 2005). Even higher value of 5.0±1.0 mins has been reported as a reaction time for Horroro indigenous bulls of Ethiopia (Abera et al., 2006). Similarly, the values for TLTE in both breeds of present study were lower than 9.2±1.5 mins reported for Sahiwal breeding bulls. Lower values for reaction time and TLTE for both breeds under study as compared to previous literature on Zebu bulls may depict effect of appropriate management skills, season or feeding regimen which influenced enhance

sexual activity in them resulting in a lower reaction time.

The results for physical attributes of fresh semen in Dajal and Cholistani breeding bulls of present study are given in Table 3.

Table 3. Comparative overall mean values (± SE) for fresh seminal attributes of Dajal (n = 03) and Cholistani (n = 03) breeding bulls

Parameters	Dajal	Cholistani
No. of ejaculates per bull	1.96±0.01	1.97±0.01
Ejaculatory volume (mL)	5.1±0.4	5.7±0.2
pH	6.1±0.09	6.0±0.04
Mass motility (Score 1-5)	2.7±0.2	2.1±0.1*
Individual sperm motility (%)	58.7±2.0	62.0±2.4
Sperm Concentration (million/mL)	739.1±17.2	892.0±21.0*
No. of dozes frozen per bull	155.2±3.0	187.5±4.3*
Live sperm (%)	67.2±2.0	79.9±1.5*
Morphologically normal sperm (%)	75.0±2.0	83.1±2.1*
Acrosome integrity (%)	75.6±1.6	82.0±1.8
No. of proximal cytoplasmic droplets	1.5±0.2	1.2±0.1
No. of distal cytoplasmic droplets	0.3±0.1	0.2±0.07

*Significant within rows (P≤0.05)

Mass motility was the only parameter significantly (P≤0.05) higher for Dajal bulls as compared to Cholistani bulls being 2.7±0.2 and 2.1±0.1, respectively. Other studies have also reported lower values of mass motility in Cholistani (Farooq et al., 2013a; Farooq et al., 2013b) and Sahiwal breeding bulls (Ahmad et al., 2003; Sarder, 2003). Similarly, Holstein-Friesian bulls when reared in subtropical environment of Pakistan also gave a lower mass motility score of 1.7±0.2 (Fiaz et al., 2010), than those recorded in our study. It has been well elaborated that the indigenous zebu (humped) breeds of cattle are tick-resistant, disease-resistant, thermo-tolerant and well-adapted to harsh environments (Farooq et al., 2010). Higher score in present study could be an inherent adaptability pattern of the breeds under study. As the mass motility score has been proven to be positively correlated to the libido index, hence better results of mass motility in Dajal bulls may be attributed to a higher libido score as seen in the present study.

The mean values (± SE) for remaining fresh seminal attributes *i.e.*, sperm concentration, number of dozes frozen per bull, and live and morphologically normal spermatozoa were significantly (P≤0.05) lower in Dajal bulls as compared to Cholistani (Table 3). Sperm concentration of 739±17.2 million/mL in Dajal bulls for present study is lower than the value of 918.0±65.7 million/mL reported earlier for Cholistani bulls (Farooq et al., 2013). Similarly, even higher concentration of 2541.9±1699.0 million/mL for Indian zebu bulls (Siddiqui et al., 2008) and 1471.0±37.0 million/mL for Sahiwal bulls (Sarder, 2003) has been reported. Difference in breed, geo-location and age could be a plausible justification for these differences. The live and morphologically normal spermatozoa in Dajal bulls (67.2±2.0 and 75.0±2.0%) were though significantly (P≤0.05) lower than those for Cholistani bulls (79.9±1.5 and 83.1±2.1%) (Table 3), yet the values were in the range reported elsewhere for various Zebu bulls (Sarder, 2003; Ahmad & Asmat, 2005; Farooq et al., 2013).

Results regarding mean (± SE) values of post thaw seminal attributes for Dajal and Cholistani breeding bulls are given in Table 4.

The individual sperm motility, morphologically normal sperm and those with intact acrosomes were significantly (P≤0.05) lower for Dajal breeding bulls as compared to Cholistani. Live spermatozoa, number of proximal droplets and distal droplets were however, non-significantly (P≥0.05) different between two study breeds. Values of all the studied post thaw attributes were at par with those reported for Cholistani (Farooq et al., 2013) and Sahiwal (Ahmad et al., 2003; Jain et al., 2008) breeding bulls elsewhere.

Table 4. Comparative overall mean values (± SE) for post thaw seminal attributes of Dajal (n = 03) and Cholistani (n = 03) breeding bulls

Parameters	Dajal	Cholistani
Individual sperm motility (%)	37.0±1.0	54.1±1.2*
Live sperm (%)	57.3±2.3	66.8±2.6
Morphologically normal sperm (%)	52.4±2.1	67.3±2.2*
Acrosome integrity (%)	54.3±1.5	68.0±2.2*
No. of proximal cytoplasmic droplets	1.0±0.3	0.6±0.2
No. of distal cytoplasmic droplets	0.5±0.1	0.4±0.1

*Significant within rows (P≤0.05)

Extension, freezing, cooling and thawing are few components of cryopreservation for bovine

semen which renders extensive physiological and morphological stress on spermatozoa. The present study also caters an objective of assessing the degree of cryodamage caused to the spermatozoa of Dajal and Cholistani breeding bulls during the intricate cryopreservation process. The effect of freezing on various post thaw attributes in terms of increase or decrease (%) is presented in Table 5.

Table 5. Comparative effect of freezing on post thaw seminal attributes of Dajal (n = 03) and Cholistani (n = 03) breeding bulls

Parameters	Dajal			Cholistani		
	Fresh Semen	Post thaw semen	Decrease / Increase (%)	Fresh Semen	Post thaw semen	Decrease / Increase (%)
Individual sperm motility (%)	58.7±2.0	37.0±1.0	36.9±2.5*	62.0±2.4	54.1±1.2	12.7±0.2*
Live sperm (%)	67.2±2.0	57.3±2.3	14.7±0.2	79.9±1.5	66.8±2.6	16.3±0.3
Morphologically normal sperm (%)	75.0±2.0	52.4±2.1	30.1±2.0*	83.1±2.1	67.3±2.2	19.0±0.3*
Acrosome integrity (%)	75.6±1.6	54.3±1.5	28.7±1.2*	82.0±1.8	68.0±2.2	17.0±0.2*
No. of proximal cytoplasmic droplets	1.5±0.2	1.0±0.3	33.3±1.9*	1.2±0.1	0.6±0.2	50.0±1.2*
No. of distal cytoplasmic droplets	0.3±0.1	0.5±0.1	40.0±1.2*	0.2±0.07	0.4±0.1	50.0±1.1*

*Significant within rows for Cholistani and Dajal breeding bulls (P≤0.05)

All the studied attributes, except live spermatozoa, were significantly (P≤0.05) decreased in post thaw semen, both for Dajal and Cholistani breeding bulls. The mean (± SE) values for damage/decrease in live spermatozoa were 14.7±0.2% for Dajal and 16.3±0.3% for Cholistani bulls. Hence, Dajal proved to bear the brunt of cryopreservation in a better way in terms of percentage of live spermatozoa. Remaining values were within the range presented for other Zebu bulls (Ahmad et al., 2003; Sarder, 2003; Jain et al., 2008; Farooq et al., 2013; Farooq et al., 2015).

The present study is a preliminary one which furnishes baseline data regarding various reproductive attributes of Dajal breeding bulls. It envisions future studies on these bulls with a larger sample size related to effect of age, season, feeding regimen, and various types of extenders on semen quality, and assessment of fertility rate in order to attain an enhanced productivity. We recommend that a directional approach and positive strategies may be devised by all the stakeholders to conserve and propagate this dying breed considering it an indigenous asset. In addition, this breed, owing to its resemblance to Bhag Nari breed of cattle, may be propagated through genetic selection as a beef breed for Pakistan. It will not only fulfill the ever-increasing beef demand of the country but can also become an export item in future.

CONCLUSIONS

The present study, in a nutshell, revealed that Dajal showed promising results regarding libido index, reaction time, TLTE and fresh seminal mass motility as compared to Cholistani.

Furthermore, the degree of damage incurred upon the spermatozoa of Dajal breeding bulls during the cryopreservation process is substantially less.

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INFLUENCE OF POLYPHENOLIC COMPOUNDS OF GREEN WALNUT EXTRACT ON SPERMOGRAM INDICES OF BREEDING RABBITS

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Abstract

Lately, the research and study of oxidative stress on the functioning of the male reproductive system has intensified. Free radical oxidation processes play a very important role in the functioning of each living cell, on the one hand, this is a necessary stage of various metabolic processes, but on the other hand, the processes of free radical formation intensify, which can cause various pathological changes in the cells and tissues of living organisms. In living systems there are mechanisms for generating active forms of oxygen, as well as biological systems for protecting intact cells from the influence of active forms of oxygen (ROS). In the norm between these systems there is an equilibrium, which provides a normal functioning of subcellular structures and organs as a whole, but this balance very often can be disturbed in the direction of non-compensation of ROS generation and the appearance of oxidative stress. This is a state of the living cell, in which the discoordination of ROS formation processes and the functioning of the antioxidant protection system takes place. At the same time, under normal conditions, the modified macromolecules undergo regeneration or are destroyed. In the processes of oxidative stress the recovery regimes are insufficient, and as a result damaged molecules accumulate in the body (Повarова et al., 2015). It is currently considered that the balance between the ROS generation system and the antioxidant protection system after their elimination have a decisive significance in regulating the functioning of cells. A significant imbalance of the antioxidant-prooxidant system can cause inhibition of the fertility properties of ejaculate. According to the results of the literature, at present the following main interdependent causes of dysregulation of male fertile function in the process of development of oxidative stress are highlighted. First of all, a decrease in the mobility of male reproductive cells is observed, which occurs due to a decrease in the elasticity of the membrane of reproductive cells, and therefore a decrease in the mobility of the flagellum (Галимова et al., 2016). The involvement of oxidative stress in the pathogenesis of male infertility has predetermined the study and research of the effectiveness of various antioxidants in regulating and proceeding metabolic processes in the process of spermatogenesis.

Key words*: mobility, reproductive sex cells, ROS, stress.*

INTRODUCTION

ROS are formed as necessary by-products during normal inter- and intracellular signaling enzymatic reactions. Mammalian spermatozoa represent a growing list that exhibit the ability to generate ROS when incubated under aerobic conditions, such as hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$) and hypochlorite (ClO^-). Due to their highly reactive nature, ROS can easily combine with other molecules, directly causing oxidation that can lead to structural and functional changes and lead to cell damage (Guérin et al., 2001).

If previously for a very long time the oxidative stress was considered the result of ROS overproduction, currently it qualifies as a result of redox - deregulation. Reversible oxidation of thiols in cysteine remnants of key proteins acts as an on/off switch, which controls the function of male reproductive cells. But if dysregulation occurs, these remnants are irreversibly oxidized, which leads to incorrect functioning and finally to the death of male reproductive cells (Micic et al., 2019).

In low concentrations ROS have a positive effect: they influence on prostoglandin metabolism, endothelial function, participate in gene regulation and cell growth, in intracellular signaling processes and in other types of signaling transduction, as well as play an important role in the regulation of antimicrobial protection (Ефремов et al., 2017).

Although a significant negative correlation has been found between ROS and IVF fertilization rate, however, controlled ROS generation has

been shown to be essential for capacity development and hyperactivation (Lamirande et al., 1993), these being the two spermatozoa processes that are necessary to ensure fertilization. *In vivo* physiological concentrations of ROS are involved in ensuring membrane fluidity, maintaining fertilization capacity and acrosomal reaction of spermatozoa (Bucak et al., 2010). Maintaining an adequate level of ROS is therefore essential for proper sperm functionality. ROS causes adverse effects on the plasma membrane of spermatozoa, DNA and physiological processes, thereby affecting the quality of spermatozoa.

The main source of ROS in physiological conditions is mitochondrial respiration. Mitochondrial dysfunction can deepen electron loss and thus increase ROS levels to toxic levels, deregulating redox homeostasis. It is assumed that ROS in fairly high concentrations are capable of causing destructive peroxidation by the formation of reactive aldehydes.

The axosome and associated dense fibers of the middle part of the spermatozoa are covered by mitochondria, which generate energy from intracellular ATP depletion deposits. Excess ROS affects motility and the ability to fertilize. The mechanisms of spermatozoa damage (Villaverde et al., 2019) can also be caused by many chemicals, such as polymer catalysts, phthalates, etc. Many of these substances intensify oxidative stress and therefore deregulate spermatogenesis (Menezo et al., 2014).

Under unfavorable conditions, the reproductive sex cells use their own apoptotic pathway, which involves the formation of mitochondrial ROS, loss of mitochondrial membrane potential, activation of capsase, influence of phosphatidylserine. High levels of ROS in spermatozoa cause oxidation of lipids, proteins and DNA, which lead to lipid peroxidation, oxidation of protein base structures and enzymes, and can cause mutations as a result of DNA oxidation (Micic et al., 2019). The presence of a high level of ROS in the seminal fluid can lead to negative changes in ejaculate parameters and a decrease in the indices of pregnancy, both by natural means and by artificial insemination methods (Ефремов et al., 2017).

DNA damage as a result of oxidative stress directly influences the quality of embryos (Micic et al., 2019).

As a result of the high level of oxidative stress, the amount of damaged DNA in male reproductive cells becomes higher, which can cause male infertility, miscarriages can occur and periodic pregnancy loss.

Spermatozoa are sensitive to oxidative stress due to insufficient level of antioxidant protection, small volume and limitation of cytoplasmic space, which does not allow to hold a set of protective enzymes (Ефремов et al., 2017). In addition, there is a lack of a single mechanism for detecting and restoring DNA damage (Bisht et al., 2017).

It should be noted that the lipids of spermatozoa membranes are very sensitive to oxidative stress, because they contain a significant amount of polyunsaturated fatty acids, phospholipids and stearins (Aitken et al., 2016). Given that the lipid components of the spermatozoa membranes participate in the regulation of their maturation, in the process of spermatogenesis, capacitation, acrosomal reaction and finally merging with the egg, it is clearly visible that lipid peroxidation can disrupt the functions listed above (Ефремов et al., 2017).

The vulnerability of spermatozoa to oxidative damage is further deepened, because they actively generate ROS, to stimulate the increase of tyrosine phosphorylation, which is related with their mobility (Aitken et al., 2014).

The excessive increase in the concentration of lipid peroxidation products is accompanied by loss of sperm mobility, which may limit the survival period in the female genital pathways (Bisht et al., 2017; Nowicka-Bauer et al., 2020). At the same time, the life span of spermatozoa (the moment of complete loss of motility) is closely correlates with their level of superoxide dismutase activity (Nowicka-Bauer et al., 2020). At the same time, it is necessary to take into account the fact that a significantly increased amount of ROS is generated by the leukocytes in the semen.

Increased ROS along with decreased antioxidant defenses lead to redox imbalance, reduced sperm mobility and damage to sperm DNA. Spermatozoa are very sensitive to the harmful effects of ROS due to the large

amounts of unsaturated fatty acids that are found in their cell membranes. Reactive oxygen species promote peroxidation. The sequence of events involves lipid peroxidation, loss of membrane integrity with increased permeability, reduced mobility of spermatozoa, structural DNA damage, and apoptosis (Sanocka-Maciejewska et al., 2005). There were several intrinsic and extrinsic factors associated with increased oxidative stress in the male reproductive system.

At the physiological level, ROS are associated with the development of spermatozoa fertilization properties, favoring chromatin compaction in maturing spermatozoa, mobility, chemotaxis, spermatozoa fertilization capacity, acrosomal reaction and oocyte interaction (Du Plessis et al., 2015). Excessive ROS production is a major cause of sperm injury. Indeed, due to the high amount of membrane unsaturated fatty acids and the lack of cytoplasmic antioxidant enzymes, spermatozoa are very sensitive to oxidation (Agarwal et al., 2017), with consequent negative effects on the quality/functioning of spermatozoa (Aitken et al., 2016).

In addition to the physiological role of ROS, excessive ROS generation and oxidative stress appear to be associated with harmful effects on spermatozoa, leading to changes in morphological and dynamic cellular properties and, ultimately, to a lower fertilization capacity.

Spermatozoa are particularly sensitive to ROS-induced oxidation due to the presence in their plasma membrane of high levels of polyunsaturated fatty acids such as docosahexaenoic acid which contains six double bonds per molecule (Aitken et al., 2016). Indeed, ROS mediate the extraction of hydrogen from the hydrocarbon side chain of a fatty acid, yielding to a carbon-centered lipid radical (L$^{\bullet}$) whose interaction with oxygen produces a lipid peroxyl radical (LOO$^{\bullet}$), capable of reacting with an adjacent radical. As a result of internal molecular rearrangements, conjugated dienes and hydroperoxides are generated (Yoshida et al., 2015).

LPO products can also react with proteins, DNA and phospholipids, generating end products involved in cell dysfunction. In particular, the interaction of LPO products with amino residues can lead to protein oxidation, affecting the structural and functional characteristics of the protein (Niki, 2014). In this context, it has been observed that LPO products like 4-hydroxy-2-nonenal (4-HNE) are able to propagate ROS generation by interacting with proteins of the mitochondrial electron transport chain of spermatozoa (Aitken et al., 2016).

Lipid peroxidation is strictly associated with alteration of membrane fluidity and permeability, inhibition of membrane-bound enzymes and receptors, and activation of the apoptotic cascade, supporting the involvement of oxidative stress in abnormalities of spermatozoa mobility and morphology. Among LPO products, 4HNE appears to be highly responsible for the cytotoxic effects on the cell membrane of spermatozoa, inducing loss of membrane integrity, changes in mobility, and compromising sperm-oocyte interactions. It has been observed that the effects mediated by 4HNE depend on several factors: the state of cellular differentiation, the amount of substrates for 4HNE attack and antioxidant defense systems (Nowicka-Bauer et al., 2020).

It is traditionally accepted that nucleic acids are another crucial target of oxidative stress. Both nuclear and mitochondrial DNA are vulnerable to the attack of hydroxyl radicals ($^{\bullet}$OH), which leads to the formation of several biomarkers of oxidative stress. ($^{\bullet}$OH) can react with guanine to produce 8-hydroxy-2'-deoxyguanosine (8-OH-G), an important marker of DNA oxidative damage, detectable in several biological samples (Burton et al., 2011).

The lack of adequate antioxidant systems makes spermatozoa highly susceptible to DNA oxidation. The oxidation of sperm DNA is also due to the lack of complete strategies for DNA repair in spermatozoa. Indeed, if 8-oxoguanine glycosylase (OGG1) is able to remove the 8-OHdG residue from DNA by producing an abasic site, the spermatozoa do not possess any base excision repair system for insertion of a new base (Aitken et al., 2014).

Several studies have indicated that the generation of ROS is associated with DNA fragmentation and chromatin deficient packaging, promoting apoptosis with relevant consequences on spermatozoa count (Aitken et al., 2014).

MATERIALS AND METHODS

The study was conducted on 20 breeding rabbits, which were administered "per os" in a dose of 1 ml of green walnut hydroalcoholic extract with a total polyphenol content of 548.37 mg/100 g gallic acid equivalent (GAE). The total content of polyphenolic compounds was determined by the Folin-Ciocalteu method. The method consists in determining the content of total polyphenols from plant sources by measuring the optical density of an extract, which by complexation with Folin-Ciocalteu reagent determines the absorption at wavelength 750 nm, using UV-Vis spectrophotometer. For the study was used the spectrophotometer "ПЭ-5400 УФ", with a spectral range from 190 to 1000 nm.

The animals were divided into two groups: the experimental group and the control group, with 10 animals in each group. The extract was administered for two cycles of spermatogenesis. Spermogram indices, the ability to fertilize of reproductive material and biochemical indices of blood serum were studied.

RESULTS AND DISCUSSIONS

In Table 1 are presented some physiological indices of the spermogram of breeding rabbits.

Table 1. Physiological indices of spermogram of breeding rabbits

Group	Volume, ml	Concentration, mlrd/ml	Mobility, points	Survival, hours
Control	0.66±0.13	0.18±0.02	7.8±0.43	13.9±0.41
Experimental	1.2±0.16	0.23±0.08	8.5±0.37	21.3±0.29

As follows, from the results obtained and presented in Table 1 is observed a sharp difference in the studied indices. The volume of reproductive material obtained from breeding rabbits in the experimental group increases almost twice, the concentration of reproductive cells increases by 0.05 billion/ml, mobility increases by 0.7 points and survival at room temperature by 7.4 hours.

In the following study was investigated the fertility rate of female rabbits. In this experiment were included 60 female rabbits, which were divided into two groups, of 30 animals in each group. The experimental group was artificially inseminated with reproductive material received from the rabbits which were administered the green walnut extract, and the control group was also inseminated with reproductive material obtained from the rabbits which did not receive a supplement. The research results are presented in Table 2.

Table 2. Fertility rate of artificially inseminated rabbits

Group	Number of parturients, who gave birth	Number of offspring received, in average from a female rabbit	Duration of gestation, days
Control	22	6.5±0.5	28-32
Experimental	29	8.6±0.4	28-30

Thus, as can be seen, the rabbits from the control group were fertilized in an amount of 73.3%, and the rabbits from the experimental group were fertilized in an amount of 96.6%. It is also observed the increase in the number of offspring and the shortening of the gestation period in the rabbits from the experimental group.

Another investigation included the study of some biochemical indices in the blood serum of breeding rabbits. The research results are presented in Table 3.

Table 3. Biochemical indices in the blood serum of breeding rabbits

Group	GST, nM/L	Phosphorus, mM/L	Mg, mM/L	Total protein, g/L
Control	16.53±0.15	1.47±0.14	0.760±0.11	71.2±1.21
Experimental	22.1±0.32	1.7±0.18	0.840±0.18	74.7±1.46

Thus, as we can see, the polyphenols in green walnuts have an essential influence on the quantitative changes of glutamate-S-transferase (GST), which proves its positive action on the fecundity of male reproductive cells of breeding rabbits and the essential protective effect in oxidative stress.

Phosphorus is an essential trace element that is one of the main constituents of the body's cells. Phosphorus is a participant in many metabolic processes. Phosphorus compounds play an important role in energy metabolism (ATP and creatine phosphate are energy accumulators that provide many processes in the body with energy). This trace element is also part of nucleic acids.

Magnesium is also an element that participates in the processes of energy synthesis, is an active participant in the synthesis of proteins, the secretion of parathyroid hormone, forms complexes with nucleic acids, phospholipids of cell membranes, stabilizing their fluidity and permeability. By regulating the cholesterol content in the blood, it improves blood circulation in the body's parenchymal tissues, including the testes. Magnesium is the second most concentrated intracellular cation, it is part of 300 enzymes.

Proteins, in turn, serve as a building material for all cells and tissues of the body. It is from proteins that enzymes, many hormones and antibodies are built. In addition, they perform the function of carriers of hormones, vitamins, minerals, fat-like substances and other components of metabolism in the blood, and also provide their transport to cells. Proteins are also responsible for maintaining the correct acid-base balance (pH). Finally, it is a source of energy. Proteins having these various functions and actively participating in the metabolic processes in the body have an essential influence on the functioning of the male reproductive system and on the quality of the reproductive material and finally influencing the quantity and quality of the offspring obtained.

CONCLUSIONS

It is necessary to evaluate the oxidative stress and to use antioxidants to regulate the activity of the reproductive system.

It is very important to determine the dose and duration of antioxidants for blocking ROS, to determine which antioxidants could be used to regulate the activity of the reproductive system and prevent male infertility.

It is also necessary to establish at what level of functioning of the reproductive system it is necessary to administer antioxidants.

Simultaneously with advanced technologies for artificial insemination, the need arises to combine different antioxidants to preserve male reproductive material at hypothermic temperatures, which optimally maintain the functioning of male reproductive cells in order to achieve a higher fecundity.

Early administration of antioxidants to farm animals increases the quantitative and qualitative indices of male reproductive material and therefore increases the efficiency of ova fecundity.

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INFLUENCE OF GREEN WALNUT EXTRACT ON THE ANTIOXIDANT STATUS OF THE ORGANISM OF BREEDING ROOSTERS

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Abstract

In recent times, the use of medicinal plants has significantly increased in correcting the disturbances in the organs and systems of living organisms, which can be caused by climate change, environmental pollution with various wastes, which contain heavy metals and other toxic substances released into the atmosphere as a result of processing of different raw materials et al. Researchers in this field have noticed that a number of plants have antioxidant, detoxifying properties, blocking properties of heavy metals from the internal circuit of living organisms and at the same time prevent the development of many disorders, which can cause different morbidity states of the body. In this paper will be analyzed and processed bibliographic scientific sources of researchers, which deal with the study of the physiological, biochemical, antioxidant, detoxifying, antiradical properties and the influence of biologically active components from Juglans regia L.. There will be presented some results obtained in the research conducted in our laboratory, on the influence of hydroalcoholic extract from green walnuts (Juglans regia L.), where will be taken into account the antioxidant influence and blocking properties of heavy metals, antiradical activity et al. The purpose of this paper was to determine the influence of biochemical complexes, which are contained in Juglans regia L. on antioxidant activity. From the results obtained after processing the specialized literature we notice that Juglans regia L. possesses high antioxidant properties, contain a series of biochemical compounds, such as vitamin C, flavonoids, quercetin et al. Due to the effect of Juglans regia L. on the amelioration of complications of various functional disorders in biological objects, it is necessary to further conduct complex physiological and biochemical studies on the use of Juglans regia L. components in various disorders in organs and systems of the human and animal organism.

Key words*: active forms of oxygen, antioxidants, antioxidant activity, extract, detoxification.*

INTRODUCTION

The walnut (*Juglans regia* L.) is a tree of the family Juglandaceae and contains many biologically active compounds with a particularly pronounced influence on the systems and organs of biological objects (Carvalho et al., 2010; Cosmulescu et al., 2010). Different parts of walnut are used as natural remedies in folk and classical medicine to balance some processes in living organisms possessing antidiarrheal, anthelmintic, antiseptic and astringent properties. Numerous studies have demonstrated the antioxidant potential of walnut products, i.e. hazelnuts (green or dried) and mesocarp of green walnuts (Ghasemi et al., 2011; Oliveira et al., 2008; Rahimipanah et al., 2008), leaves (Pereira et al., 2007), bark (Noumi et al., 2011) or flowers (Nabavi et al., 2011). According to studies conducted by (Rahimipanah et al., 2010), green walnut mesocarp is a valuable source of phenolic compounds and can be used as an alternative natural antioxidant in the pharmaceutical and food industries. Several studies suggest that regular consumption of nuts can have beneficial effects against disorders mediated by oxidative stress (Shimoda et al., 2009). Walnut fruits are impressively rich in polyphenolic compounds. It has been identified by Stampar et al. (2006) thirteen phenolic compounds in the green walnut mesocarp: chlorogens, caffeic, ferulic, synapic, gallic, ellagic, protocatechuic, syringic, vanylic acids, catechin, epicatechin, myricetin and juglone. The content of phenolic compounds depends on environmental conditions, genotype and stage of development of nuts (Solar et al., 2006), as well as geographical location or climatic conditions (Amaral et al., 2008). Regarding the seasonal variation in the content of phenolic compounds,

the highest content was determined in May and July (Amaral et al., 2008). Oliveira et al. (2008) have shown that the green walnut mesocarp is an important source for obtaining compounds with protective action for health, having high antimicrobial potential. It is proven, that walnuts have a higher total polyphenol content than most foods (Verardo et al., 2009).

Phenolic compounds have a major contribution to antioxidant activity because they are effective hydrogen donors (Banerjee et al., 2005). Phenolic acids, flavonoids and naphthoquinones are the main phenolic compounds of walnuts and walnut leaves. A special, unique component of walnuts is juglone (5-hydroxy-1,4-naptoquinone), a chemical compound released by walnut that can be toxic at various levels to many plant species. Juglone is present in considerable quantities in all green parts of the walnut (Cosmulescu et al., 2011) and in the walnut mesocarp, while the content of juglone in the endocarp is very low or absent (Jakopic et al., 2008). It is already proven (Colaric et al., 2005) that green walnuts are rich in phenolic compounds, and juglone is a well-known component of mesocarp, its content being significantly higher than the content of other phenolic compounds (Solar et al., 2006).

Juglone (5-hydroxyl-1,4-naphthoquinone), due to the antioxidant capabilities of phenolic compounds, juglone can serve to combat oxidative stress, thus protecting against the development of various diseases and aging processes. However, being a quinone molecule, juglone could also act as a redox cycle agent and produce reactive oxygen species. Juglon contains an intramolecular hydrogen bond between the hydroxyl and keto groups and is active in hydrogen atom donation (Jin et al., 2010). Juglon can have either pro- or antioxidant characteristics, depending on the concentrations (Chobot et al., 2009). Thus, some studies have reported the generation of ROS by juglone, while others describe its antioxidant properties (De Castro et al., 2004).

Juglone also activates mitogen-activated protein kinases that could promote cell survival, thus protecting against conditions such as heart damage.

By producing reactive oxygen species (ROS), biomolecules undergo oxidative stress. Antioxidants reduce ROS, and the balance between ROS and antioxidants defines oxidative stress. Phenolic compounds can inhibit these reactions by directly quenching ROS, inhibiting ROS-producing enzymes, chelating transition metal ions, transferring hydrogen atoms, and regenerating vitamin E (Tejero et al., 2007). Intramolecular hydrogen bonds play an important role in free radical stability (Nenadis et al., 2008).

Male reproductive cells are particularly sensitive to ROS-induced oxidation due to the presence in their plasma membrane of high levels of polyunsaturated fatty acids such as docosahexaenoic acid, which contains six double bonds per molecule (Aitken et al., 2014). Indeed, ROS mediates hydrogen by extracting from the hydrocarbon side chain a fatty acid, yielding to a carbon-centered lipid radical (L•) whose interaction with oxygen produces a lipid peroxyl radical (LOO•), capable of reacting with an adjacent fatty acid that propagates the process. As a result of internal molecular rearrangements, conjugated dienes and hydroperoxides are generated (Phaniendra et al., 2015). LPO products can also react with proteins, DNA and phospholipids, generating end products involved in cell dysfunction. The interaction of LPO products with amino residues can lead to protein oxidation, affecting the structural and functional characteristics of the protein (Niki et al., 2014). Similar 4-hydroxy-2-nonenal (4HNE) products are able to propagate ROS generation by interacting with proteins in the mitochondrial electron transport chain of spermatozoa (Aitken et al., 2014). Lipid peroxidation is strictly associated with changes in membrane fluidity and permeability, inhibition of membrane-bound enzymes and receptors, and activation of the apoptotic cascade, supporting the involvement of oxidative stress in spermatozoa mobility and morphology abnormalities (Nowicka-Bauer et al., 2020). Among LPO products, 4HNE appears to be highly responsible for cytotoxic effects on cells, sperm membrane, which induces loss of membrane integrity, changes in motility and compromises sperm-oocyte interactions (Baker et al., 2015; Walters et al., 2018; Nowicka-Bauer et al., 2020). It has been observed that the effects mediated by 4HNE

depend on several factors: the state of cellular differentiation, the amount of substrates for 4HNE attack and antioxidant defense systems (Walters et al., 2018).

The importance of oxidative stress in the etiology of disruption of male reproductive cell function was recognized because early studies by Thaddeus Mann and colleagues at the University of Cambridge demonstrated that mammalian spermatozoa are vulnerable to a process of lipid peroxidation that attacks unsaturated fatty acids in these cells, destroying the plasma membrane and compromising their functional competence. Induction of such stress may involve increased generation of reactive oxygen species (ROS) by these cells and/or a deficiency in the levels of antioxidant protection it provide. The net impact of oxidative stress includes a loss of motility, a decrease in sperm's ability to undergo acrosomal reaction, an impaired ability to fuse with the vitreous membrane of the oocyte, and DNA damage.

In addition to the negative influence, ROS also possesses regulatory functions of several intracellular processes, modifying the activation of various transcription factors (Burton et al., 2011) involved in intracellular signaling cascades for sperm physiology. In addition, ROS can improve the ability of sperm to bind to the pellucid area, inducing sperm-oocyte fusion (Wagner et al., 2018). By the way, antioxidant molecules can change the maturation of spermatozoa. In particular, catalase or superoxide dismutase (SOD) has been shown to inhibit sperm capacity or acrosomal reaction, supporting evidence of central involvement of ROS in sperm functioning (Wagner et al., 2018).

One of the major sources of superoxide anion in spermatozoa are mitochondria (Koppers et al., 2011). These organelles generate ROS as a normal byproduct of aerobic metabolism due to the leakage of electrons from the mitochondrial electron transport chain, which are then swept away by the universal electron acceptor, oxygen, to generate the superoxide anion. Mitochondrial ROS are also produced as part of the intrinsic apoptotic cascade that becomes activated whenever the phosphoinositide signaling pathway is compromised (Koppers et al., 2008).

Mitochondrial ROS generation and apoptosis may also be important in the mechanisms underlying sperm senescence. All mammalian spermatozoa have a finite lifespan and after a few days (depending on the species) will become senescent *in vivo* and *in vitro*, losing their viability, mobility, tyrosine phosphorylation state and DNA integrity with the passage of time (Matsuura et al., 2010; Yoshida et al., 2015). Oxidative stress appears to be a component of the senescence process, judging by the fact that sperm mobility and DNA integrity can be significantly improved in vitro if oxygen stresses are reduced and/or antioxidants are incorporated into the medium (Aitken et al., 2012).

MATERIALS AND METHODS

To achieve this purpose, hydroalcoholic extracts were obtained from green walnuts. The extract was administered to breeding roosters at a dose of one milliliter per head of the animal. To exclude irritation of the mucosa of the buco-esophageal tract the extract was diluted with distilled water. The experiments included two groups of five roosters in each - the experimental group and the control group.

Roosters from the experimental group were administered *per os* 1 ml of hydro-alcoholic extract from green walnuts, with a total polyphenol content of 548,37 mg/100g gallic acid equivalent (GAE). The extract was administered for two cycles of spermatogenesis, with the automatic device for the administration of drugs to animals. The total content of the polyphenolic compounds was determined by the Folin-Ciocalteu method. The method consists in determining the content of total polyphenols from plant sources by measuring the optical density of an extract which by complexation with Folin-Ciocalteu reagent absorbs in the VIS domain at wavelength $\lambda = 750$ nm. For the study was used the spectrophotometer "ПЭ-5400 УФ", with a spectral range from 190 to 1000 nm.

The determination of antioxidant capacity (AOC) was performed by the CUPRAC (CUPric Reducing Antioxidant Capacity) method according to the process described by Apak et al. (2005). The principle of the method is based on the property of antioxidants in the

research sample to reduce bivalent copper to monovalent copper in the presence of the chelating agent - neocuproine. This chelator forms stable colored complexes with the monovalent copper ion which has a maximum absorption at 450-490 nm. The intensity of the staining is directly proportional to the AOC (Apak et al., 2005).

The determination of ceruloplasmin was performed according to the process described by Колб et al. (1982), the principle of the method is based on the fact that this component of blood plasma possessing oxidative properties, catalyzes the oxidation reaction of some polyamines, including p-phenylenediamine. As a result of the reaction are formed compounds of blue - violet color, the intensity of which correlates with the activity of the enzyme and is estimated spectrophotometrically.

For the determination of biochemical indices were used biochemical analyzers "Sinergi" and "BioTECH", including sets corresponding to each index.

RESULTS AND DISCUSSIONS

The results of research of the fermentative antioxidant status in the blood serum of roosters given green walnut extract are presented in Table 1.

Tabel 1. Fermentative antioxidant status in the blood serum of roosters who received green walnut extract

Group	SOD, u/c (min/L)	G-GTP, u/L	Catalase, μM/L	G-S-T, nM/sL
Control	127.0±4.30	7.21±0.88	30.1±1.22	23.81±3.2
Experimental	156.0±3.71	10.4±1.09	35.1±1.1.1	34.2±4.18

As can be seen from the obtained data, the fermentative antioxidant status is very active in the control group. Superoxiddismutase is an enzyme that is part of the body's antioxidant defense system. It catalyzes the process of dismutation (disproportion) of the superoxide radical into hydrogen peroxide and molecular oxygen. Hydrogen peroxide is further converted into water and molecular oxygen under the action of catalase and glutathione peroxidase. The superoxide radical is produced in the process of oxidative energy reactions and is a product of the reduction of molecular oxygen by one electron. It is produced in almost all oxygen-consuming cells, can affect all cell components and the intercellular substance, and is also a precursor to the more toxic hydroxyl radical.

Tabel 2. Cationic and anionic antioxidant status in the blood serum of roosters

Group	Phosphorus, mM/L	Magnesium, mM/L	Zinc, μM/L	Iron, μM/L
Control	1.59±0.06	0.86±0.34	28.5±1.35	13.53±0.87
Experimental	1.69±0.06	0.87±0.5	34.7±1.03	15.28±0.77

It is known that about 80% of the total amount of phosphorus (700 g in the adult human) is deposited in the skeleton in the form of hydroxyapatite. The rest is found in extracellular fluid and soft tissues. Most phosphorus is combined with lipids, proteins and carbohydrates, participating in the formation of phospholipids, nucleotides and macroergic compounds. Phosphates are also one of the body's buffer systems.

Magnesium is involved in a wide range of biochemical reactions, having a direct activating impact on enzymes, such as phosphofructokinase, creatinine kinase and adenylate cyclase.

It intervenes in the synthesis, transport and use of macroergic compounds - ATP; ensures in the mitochondria the coupling of oxidation with phosphorylation.

It participates in the synthesis of nucleic acids and proteins, intervening in the activation of amino acids. Magnesium intervenes in ion transfer (K+) and modulates the activity of calcium channels. It has the mission of stabilizing cell membranes, as well as ribosomes and lysosomes.

Tabel 3. Protein antioxidant status, total and chemical antioxidant capacity in the blood serum of roosters in experiment no. 1

Group	CUPRAC,mM/L	CP, mg/L	AAT with ABTS, μM/L	SH-thiol groups of proteins, μM/g	Total protein, g/L
Control	1.3±0.32	108.9±0.35	116.1±0.71	4.69±0.35	53.4±0.51
Experimental	2.71+0.20	123.53±1.54	119.0±0.81	5.78±0.28	53.6±0.71

Ceruloplasmin being a protein of the blood, which depends on the copper content, performs its functions, contributing to the metabolism of other anions and cations, which promotes transmembrane metabolism and detoxification of cells and the organism as a whole. Copper, as usual, provides fermentative functions, including ceruloplasmin, which in turn distributes zinc through the intercellular spaces and also ensures the homeostatic status of the cell. It is necessary to note that ceruloplasmin oxidizes iron to Fe^{3+} after it is transported by transferrin to form hemoglobin. As a result of the research it is observed that polyphenols quantitatively influence the ceruloplasmin content, which in turn through fermentative reactions influences other indices of the antioxidant system. CUPRAC (CUPric Reducing Antioxidant Capacity) is increased in the experimental group compared to the control group, at the same time there are changes in AAT with ABTS, SH-thiol groups of proteins and in the total protein content.

CONCLUSIONS

Together with the evaluation of oxidative stress, the monitoring of redox status in the blood and ROS levels represent a new potential and a less invasive practice in the field of biomedicine to evaluate the functioning of the reproductive system and the quality of male reproductive material with the ability to fertilize.

Redox parameters can therefore be considered useful for the development of new strategies based on antioxidant supplementation to reduce systemic oxidative stress in males, improving the quality of reproductive material.

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INFLUENCE OF HYDROALCOHOLIC EXTRACT FROM GREEN WALNUT ON CERULOPLASMINE CHANGES IN BLOOD SERUM OF BREEDING ROOSTERS

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Abstract

Ceruloplasmin is a ferment with a high copper content, manifesting an increased ferroxidase activity, which is detected as a soluble isoform in plasma or as a membrane-associated isoform in different cell types. The ceruloplasmin-ferroportin system is the main route of cellular iron exit in vertebrates and is responsible for the physiological regulation of cellular iron levels. Ceruloplasmin is a copper-containing ferroxydase and plays an important role in the ionic state regulation of iron oxidation - Fe^{2+} to Fe^{3+}. As a result, iron is incorporated into transferrin without the formation of toxic iron products. Maintaining the normal transport and metabolism of iron is a function of ceruloplasmin to maintain the vitality of tissues and organs. This review focuses on the structural and functional characteristics of the two proteins, with special emphasis on their coordinated regulation at the transcriptional and post-transcriptional level. Ceruloplasmin (CP) is a glycoprotein that plays an essential role in iron homeostasis. According to the accepted theory, the bivalent iron transported from the cell by ferritin, it is necessary to oxidize certainly by ceruloplasmin in order to slightly facilitate the activity of transferrin. Therefore, the ceruloplasmin-feroportin system is the main pathway of cellular iron metabolism and is responsible for regulating iron levels in the cell. Oxygen is a paradox for cells in that it is both an essential nutrient needed for survival and a precursor for toxic, potentially deadly byproducts. Ceruloplasmin represents a protein with specific domains capable of both facilitating the production of cellular energy and preventing the formation of oxygen radicals. This ability to perform dual tasks lies in the complex shape and structure of the protein and involves strategically placed copper ions, which can help both give and take up electrons from substrates, including iron, oxygen, and iron-binding proteins. Copper is the essential element for the wide range of ceruloplasmin activities that maximizes iron metabolism. A defect or mutation in the ceruloplasmin gene that denies copper incorporation into ceruloplasmin disrupts iron metabolism. Ceruloplasmin is also involved in many redox reactions. Its effect as a pro-oxidant or antioxidant is due to the presence of other factors. In the presence of superoxide (for example, in the inflamed vascular endothelium), it will act as an oxidation catalyst for low-density lipoproteins. About 95% of all copper in the body is found in connection with apoceruloplasmin, therefore, determining the amount of ceruloplasmin is one of the main methods of assessing copper exchange.

Key words*: ceruloplasmin, copper, iron, ferritin, oxidation.*

INTRODUCTION

The production of ceruloplasmin occurs in the liver. Hepatocytes largely synthesize ceruloplasmin. In the liver, P-type ATPase enzymes are required to incorporate copper into apoceruloplasmin (Maio et al., 2010). It is mainly synthesized in liver parenchymal cells, with small amounts coming from macrophages and lymphocytes (Vlasova et al., 2019). After the peptide chain is synthesized, copper is added from an intracellular ATPase (Das et al., 2018). Copper is essential for the normal folding of ceruloplasmin, as well as for the normal attachment of oligosaccharides. A large part of apoceruloplasmin, which does not contain copper or ATPase, undergoes intracellular degradation, although a small part will reach in the circulation, but has a short half-life of 4 to 5 days. Copper plays an important role in the body's redox processes due to its ability to change valence and thus to be a donor and acceptor of electrons (Harvey et al., 2008). In addition, copper is of great importance: for antioxidant protection (superoxide dismutase, ceruloplasmin, metallothionein), connective tissue formation (lysyl oxidase), electron transport (cytochrome C oxidase), blood coagulation, deamination of primary amines (amin oxidase) et al. (Филатов,

2010). Copper is also an integral part of enzymes, participating in the metabolism of vitamins, hormones, proteins, carbohydrates, as well as some immune processes. About 90% of the copper in the blood plasma is part of ceruloplasmin (Cu-alpha-2-globulin complex), the rest is free (Камышников, 2009). A change in the concentration of copper indicates possible pathological processes in the body, for the control of which it is necessary to screen the content of copper and/or ceruloplasmin.

Ceruloplasmin is then released into the bloodstream to be transported to the distal sites and to perform its functions in other metabolic processes, namely iron metabolism.

A form of ceruloplasmin anchored to GPI was also initially identified in Sertoli cells (Fortna et al., 1999).

Sertoli cells are the somatic component of the seminiferous tubule and are believed to provide physical and biochemical support to the process of spermatogenesis. Sertoli cells are closely associated with developing germ cells and are known to synthesize and secrete a number of proteins considered to be essential in maintaining and controlling spermatogenesis (Griswold, 1988).

Known proteins would be secreted by Sertoli cells in rat testes, which include transferrin, ceruloplasmin, sulfated glycoprotein-1 (SGP-1), sulfated glycoprotein-2 (SGP-2) and androgen binding protein (ABP). Transferrin and ceruloplasmin are transport proteins of metals, which bind iron and copper, respectively. SGP-1 has been identified as a precursor for the sulfatide/GMi activating protein, which is a necessary component in the degradation of glycosphingolipids (Collard et al., 1988). SGP-2 can be detected by immunofluorescence on mature sperm (Sylvester et al., 1984) and has recently been shown to have identity with SP-40,40, a protein that can inhibit complement-mediated cytolysis (Collard et al., 1987; Kirszbaum et al., 1989). ABP binds androgens and effectively increases the concentration of androgens in the testicular fluid (French et al., 1973). Changes in the levels of these proteins may be useful measures for the hormonal and environmental regulation of Sertoli cell function.

Transferrin is an important marker for the function of Sertoli cells, as its synthesis and secretion can directly affect germ cells. Transferrin was first demonstrated as a product of Sertoli cell secretion by Skinner and Griswold (Skinner et al., 1980). Tight intracellular junctions between adjacent Sertoli cells prevent access of serum transferrin in the adluminal compartment, thus testicular transferrin has the proposed function of delivering iron to germ cells. A model has been proposed (Huggenvik et al., 1985) in which iron, which was delivered to the basal part of the Sertoli cell via serum transferrin, is bound to testicular transferrin for subsequent delivery to the adluminal compartment. Many important elements of this model have been verified experimentally (1 and references in it). Iron delivery is essential for the process of spermatogenesis and therefore the means by which transferrin is regulated is of great interest. The adjustment of transferin synthesis in Sertoli cells has been studied by RIA hybridization and nucleic acid. Sentoli cells respond to FSH, insulin, retinol and testosterone with increased mRNA production of transferin protein both in vivo and in vitro (Huggenvik et al., 1985). In situ hybridization confirmed that transferrin is specifically produced by Sertoli cells in the testis and in addition this synthesis varies according to the stages of the seminiferous epithelium cycle. (Morales et al., 1987).

Regulators of Sertoli cell function may ultimately influence the maintenance and control of germ cell development. Germ cells (Galdieri et al., 1984; Ireland et al., 1987; Le Magueresse et al., 1988), including a number of factors such as vitamins (Hugly et al., 1987) and hormones (Fakunding et al., 1976), have been shown to regulate the function of Sertoli cells. Thus, it has been described that germ cells stimulate the phosphorylation of Sertoli cell-specific proteins in a Ca^{2+} dependent and cyclic adenosine monophosphate (cAMP) independent manner in culture studies (Ireland et al., 1987). Also, germ cells provoke an increase in the secretion of ABP with a concomitant decrease in the production of estradiol (Galdieri et al., 1984; Le Magueresse et al., 1988). It has also been shown that transferrin secretion is increased when Sertoli cells are cultured in the presence of germ cells or the conditioned germ cell environment

(Djakiew et al., 1988; Le Magueresse et al., 1988). It is now clear that germ cells can influence neighboring Sertoli cells to increase protein secretion. However, the mechanism by which germ cells mediate their effect has not been characterized.

Regulation of gene expression in mammalian testis is probably the result of complex interactions between several types of cells and their respective functional states.

In general, among the various functions of ceruloplasmin, the following main ones can currently be highlighted (Вавилова et al., 2005; Санина et al., 1986): 1) transport and regulation of copper turnover in the blood and organs; 2) ferrooxidase action and immobilization of serum iron; 3) antioxidant action; 4) participates in acute phase reactions; 5) increases the stability of all cell membranes. The properties of ceruloplasmin deserve great attention, on the basis of which it was classified as an acute phase protein of inflammation. It has been established that the level of ceruloplasmin in the blood serum changes significantly in different infectious diseases, processes accompanied by destructive and necrotic changes of tissues et al. (Вавилова et al., 2005).

Ceruloplasmin has a pronounced oxidase activity, in plasma it also limits the release of iron reserves, activates the oxidation of ascorbic acid, noradrenaline, serotonin and sulfhydryl compounds, also inactivates reactive oxygen species, preventing lipid peroxidation.

The insufficiency of copper ions in the blood, as a result of the deficit of ceruloplasmin, causes the increase of their absorption in the intestine, which favors even more their accumulation in the body with the subsequent influence on a series of important vital processes in the body (Камышников, 2009).

Ceruloplasmin (also called ferroxidase) catalyzes the oxidation of ferrous ions to a high level:

$$CP–Cu^{2+} + 4Fe^{2+} \rightarrow CP–Cu^{+} + 4Fe^{3+}$$
$$CP–Cu^{+} + O_2 + 4H^{+} \rightarrow CP–Cu^{2+} + 2H_2O$$

The metal ions with variable valence are able to effectively catalyze the prooxidant products, in particular, the hydroxyl radical ($^{\bullet}OH$). By promoting the incorporation of oxidized Fe^{3+} into ferritin, ceruloplasmin inhibits superoxide and ferritin-dependent lipid peroxidation. The properties of ceruloplasmin described above served as a basis for explaining its anti-inflammatory activity, which together with a rapid increase in its concentration (2-3 times) in the blood, already at the beginning of the inflammatory reaction, allows it to be classified as an "acute phase"protein.

Transferrin is also a glycoprotein in blood plasma, which binds firmly but reversibly to iron cations and provides the transport of these ions into the body of mammals. The polypeptide chain of the transferrin molecule consists of two Fe^{3+} binding sites. Transferrin is synthesized mainly in the liver. The level of expression of this Fe^{3+} binding protein is determined by the iron content in the body's biological media - it increases in iron deficiency states and decreases with an excess of this metal with variable valence. Saturated Fe^{3+} transferrin can penetrate through endocytosis only into those cells on the cytoplasmic membrane of which the specific transferrin receptor (TFR-1) is expressed.

Together with ceruloplasmin, it improves the binding of iron ions with transferrin, and in the case of their high concentration in the blood plasma with ferritin. It is believed that the blood plasma proteins ceruloplasmin and transferrin, together with tissue ferritin, form a ferrocinetic system, the main antioxidant system that controls the processes of lipid peroxidation induced by ferrous ions. Acting as a ferroxidase, ceruloplasmin plays an important role in the regulating of the ionic state of iron - the oxidation of Fe^{2+} to Fe^{3+}. This makes it possible for iron to be incorporated into transferrin without the formation of toxic iron products.

Chelate compounds, which have the ability to bind metal ions with variable valence (ferritin, hemosiderin, transferrin, ceruloplasmin, lactic and uric acid) are the most important component of the body's antioxidant system, as they neutralize the main catalysts of oxidation of free radicals in the body.

The iron released by transferrin is bound to a specific protein, ferritin, which supplies iron to the mitochondria, it is incorporated into the heme with the participation of ferrohelatase. The storage of iron in oxidized form prevents

its involvement in oxidative processes (Карбышев et al., 2018).
Under physiological conditions, transferrin is saturated with iron at a rate of about 30%. Transferrin carries out the exchange of iron, transporting it between the erythroid elements of the bone marrow and macrophages. Together with pathological changes, the transaction of iron from macrophages to transferrin is interrupted. This can occur during inflammation due to a decrease in the iron content in erythrocytes and an increase in its deposition in cells. Transferrin also regulates the transport of iron in hepatocytes.
The displacement of iron atoms in the cell occurs due to the interaction of the iron-transferrin complex with specific receptors on the plasma membrane. The iron-transferrin complex enters the cytosol of the cell, where an iron atom is released, and the transferrin is removed from the cell, remaining capable of repeated and multiple binding of iron ions. Reticulocytes have the highest density of transferrin receptors on the plasma membrane. The iron in these cells binds to protoporphyrin to form heme, which combines with globin to form hemoglobin or myoglobin.

MATERIALS AND METHODS

To carry out these researches in the experiments were used 10 roosters, which were divided into two groups of five animals in each group - control group and experimental group. To roosters from the experimental group were administered "per os" in a dose of 1 ml of green walnut hydroalcoholic extract with a total polyphenol content of 548.37 mg/100 g gallic acid equivalent (GAE). The extract was diluted in a ratio of 1/4 with distilled water and administered with the automatic device for the administration of medicinal products to animals.
The determination of ceruloplasmin was performed according to the process described by (Колб et al., 1982), the principle of the method is based on the fact that this component of blood plasma possessing oxidative properties, catalyzes the oxidation reaction of some polyamines, including p-phenylenediamine. As a result of the reaction are formed compounds of blue - violet color, the intensity of which correlates with the activity of the enzyme and is estimated spectrophotometrically.
The determination of antioxidant capacity (AOC) was performed by the CUPRAC (CUPric Reducing Antioxidant Capacity) method according to the process described by (Apak et al., 2005). The principle of the method is based on the property of antioxidants in the research sample to reduce bivalent copper to monovalent copper in the presence of the chelating agent - neocuproine. This chelator forms stable colored complexes with the monovalent copper ion which has a maximum absorption at 450-490 nm. The intensity of the staining is directly proportional to the AOC (Apak et al., 2005).

RESULTS AND DISCUSSIONS

In these researches the antioxidant activity of polyphenols from hydroalcoholic extract of green walnuts was evaluated according to several indices of the antioxidant system of breeding roosters. The research results are presented in Table 1.

Table 1. Indices of the antioxidant system in the blood serum of breeding roosters

Group	CP, mg/L	CUPRAC, mM/L	SOD, u/c (min/L)	Zinc, µM/L	Phosphorus mM/L	Iron, µM/L	Mg, mM/l	SH-thiol groups of proteins, µM/g
Control	108.9±0.35	1.3±0.32	153.1±0.52	32.73±0.88	1.65±0.53	12.63±0.68	0.733±0.0085	4.69±0.35
Experimental	123.53±1.54	2.71+0.20	171.3±0.92	35.39±0.80	1.82±0.12	15.55±0.88	0.896±0.048	5.78±0.28

Ceruloplasmin (CP) is a blood protein that contains copper and performs multiple fermentative functions in several important vital processes. Copper provides the fermentative function of ceruplasmin, which in turn distributes copper among the intercellular

spaces of the organism. One of the main functions of ceruloplasmin is the oxidation of iron to Fe^{3+}, after it is transported by transferrin to form hemoglobin. Transferrin is the main protein for iron, it can bind only with the oxidized form of Fe^{3+}. From the intestine iron comes out in the form of Fe^{2+}, in such a form it is not available to transport, therefore it is oxidized by ceruloplasmin and is included in the metabolism. In addition, ceruloplasmin performs the role of transport of copper, part of which distributes it for the synthesis of other enzymes (cytochrome-C-oxidases). In addition to iron, ceruloplasmin activates the oxidation of ascorbic acid, noradrenaline, serotonin and other compounds.

As a result of the research and the experimental material obtained, it is observed that polyphenols quantitatively influence the ceruloplasmin content, which in turn through fermentative reactions influences other indices of the antioxidant system. CUPRAC (CUPric Reducing Antioxidant Capacity) is increased in the experimental group up to 123.53±1.54 compared to the control group with a value of 108.9±0.35 mM/L. At the same time, the content of superoxide dismutase (SOD), zinc, phosphorus, iron, magnesium and SH-thiol groups of proteins is increasing.

Along with the indices of the antioxidant system, the hematological indices were also evaluated. The research results are presented in Table 2.

Table 2. Hematological indices of breeding roosters included in experiments to study the action of polyphenols from hydroalcoholic extract of green walnuts on the body

Hematological indices	Group	The obtained results				
Erythrocytes, 10^12/L	pre-experimental	3.22±0.33	3.45±0.25	3.62±0.12	3.58±0.04	3.28±0.02
	experimental	3.64±0.32	3.67±0.08	3.95±0.08	3.93±0.17	3.4±0.11
Hemoglobin, g/L	pre-experimental	138.6±3.51	139.3±5.5	142.0±3.0	145.3±3.05	136±3.0
	experimental	151±2.0	143.3±2.08	151.6±2.51	157.6±3.2	142.3±10.1
Hematocrit, %	pre-experimental	44.9±1.6	46.3±2.25	46.13±0.8	48.1±1.4	44.6±0.55
	experimental	49.5±1.73	47.2±1.22	49.8±0.63	51.6±2.9	46.0±2.0
Mean corpuscular volume MCV, fl	pre-experimental	132.4±0.69	131.5±0.87	129.6±0.38	134.1±2.55	135.8±0.40
	experimental	130.0±1.12	128.4±1.58	126.0±1.81	131.3±1.7	135.0±1.81
Mean corpuscular hemoglobin MCH, pg	pre-experimental	38.9±0.65	39.4±0.57	39.6±0.41	41.1±0.85	41.5±0.47
	experimental	39.6±0.16	39.0±0.21	38.4±0.17	40.1±0.62	41.7±0.75
Mean corpuscular hemoglobin concentration, g/dL	pre-experimental	30.7±0.35	30.5±0.16	30.7±0.45	30.5±0.35	30.6±0.38
	experimental	30.5±0.30	30.3±0.45	30.5±0.30	30.6±1.15	30.9±0.79
Erythrocyte distribution - standard deviation RDW-SD, fl	pre-experimental	42.8±0.38	41.3±0.51	43.5±0.36	45.0±3.63	43.1±0.55
	experimental	42.1±1.17	39.6±1.77	42.3±2.53	42.0±0.60	41.2±1.2
Erythrocyte distribution - coefficient of variation RDW-CV, %	pre-experimental	9.1±0.39	9.0±0.40	9.6±0.22	9.7±0.75	8.8±0.21
	experimental	8.9±0.23	8.5±0.45	9.2±0.36	8.7±0.07	8.5±0.36
Reticulocyte RET, 10^6/L	pre-experimental	0.8±0.09	0.9±0.12	1.1±0.18	1.09±0.21	0.9±0.13
	experimental	1.1±0.12	1.3±0.26	1.2±0.11	1.1±0.35	1.1±0.33
Immature reticulocyte fraction IRF, %	pre-experimental	4.5±0.21	5.8±0.31	4.9±0.28	6.1±0.45	5.4±0.27
	experimental	5.9±0.34	6.9±0.45	6.1±0.37	7.2±0.38	6.8±0.31

From the obtained results we observe that ceruloplasmin by activating the antioxidant fermentative system, has a pronounced influence on the hematological indices. These changes are observed depending on the group and for each individual animal. For example, the animal with the number one in the control group (pre-experimental) has an erythrocyte content of 3.22±0.33 10^12/L and corresponding to the animal with the number one in the experimental group, an increase in erythrocytes up to 3.64±0.32 10^12/L is observed, as well as at the other animals individual changes of erythriocyte concenration

are observed. Anological changes are also observed in the other hematological indices of the breeding roosters.

At the same time, the volume and mobility of the reproductive cells in the ejaculate of the breeding roosters were studied. The research results are presented in Table 3.

Table 3. Physiological indices of the spermogram of breeding roosters, which were given hydroalcoholic extract of green walnuts

Spermogram indices	Interval, days							
	norm	I	10	20	30	40	50	60
Volume, ml	0.5	0.5±0.07	0.8±0.08	0.8±0.07	1.2±0.12	1.3±0.16	1.3±0.15	1.2±0.12
Total mobility		26.1±0.32	47.0±0.30	50.0±0.24	58.3±0.26	71.4±0.42	89.8±0.26	91.2±0.26

Therefore, significant changes in the volume and total mobility of the reproductive material of breeding roosters are observed. All these changes are observed from the first day of administration of the preparation and during about two cycles of spermatogenesis. So, as you can see, there is an increase and an improvement in these indices.

CONCLUSIONS

Ceruloplasmin has a beneficial influence on the functioning and stabilization of the fermentative antioxidant system, which is manifested by detoxification of the animal organism at the cellular level.

Ceruloplasmin influences the hematological indices through its apparent influence on copper and iron metabolism.

The administration of hydroalcoholic extract of green walnuts, which contains polyphenols, beneficially influences the quality of the reproductive material.

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RESEARCH ON THE DYNAMICS OF ERYTHROCYTIC SERIES IN RELATION TO AGE, IN CHICKENS

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Abstract

In this paper we would like to observe the dynamics of the red blood cell series of chickens, during their growth and development. Research has taken place on two groups made out of 10 chickens: one group (named group 1) containing an industrial hybrid (Cobb 500), and one group (named group 2) containing common breed chicken. The haematological determination has been made at the age of 14, 45 and 75 days. The results obtained showed that at 45 days, the mean corpuscular haemoglobin presented a significant difference ($p<0.05$), higher with 6.26% than the chicken in the group 2. Regarding the determination made at the age of 75 days, we have found a significant increase ($p<0.05$) between the two groups regarding: the red blood cells number (with 18.43% higher than the group 2), the haematocrit value (with 7.64% higher than the group 2), the haemoglobin concentration (with 16.6% in favour of the group 2). Regarding the mean corpuscular volume, it presented a significant increase (with 8.64% higher than the group 1). During the experiment, the concentration of mean corpuscular haemoglobin didn't present any significant changes ($p>0.05$), the results of this parameter falling within the physiological values.

Key words*: chicken, erythrocytes, haemoglobin, haematocrit.*

INTRODUCTION

Currently, the broiler is a remarkable practical accomplishment of the genetics and nutrition, this accomplishment that has been materialized thanks to the scientific and technological progress. The main goal is represented by the exploitation of the broilers, by discovering the methods or possibilities of achievement of a bigger and constant production in a short period of time, with a minimum of investment and expenditures. The consumption of chicken meat offers nutritional advantages that cannot be denied within healthy alimentation, such as: moderate caloric intake, protein content based on all the essential amino acids, a low proportionality of cholesterol and fats. (Gonciarov et al., 2015).

The nutritional value of chicken meat comes both from its protein richness and respectively from amino acids, but also from its high coefficient of digestibility of the nutritive components, in the case of protein reaching up to 96%-98%, as well as the maintenance of its physicochemical and microbiological characteristics, throughout their entire period of validity (Petcu, 2006; Petcu et al., 2015; Khawaja et al., 2016).

The chicken meat can be considered a dietary product, because it presents a high digestibility coefficient of nutritional components, has an optimal content of fat, a low quantity of connective tissue and a high quantity of muscle tissue (Savu & Petcu, 2002; Chikumba et al., 2013).

For the achievement of the desired products, the chickens need to benefit from all the necessary conditions, as well as a good overall health. In this regard, the research of erythrocytic series offers plenty of information, the erythrocytes being actively involved in the transportation and oxygen provision to the tissue level. Also, the investigation of the red series offers information about the installation of anemia, being known that this affection it's often encountered in the event that the feed it's not well balanced in amino acids and vitamins (Bălăceanu et al., 2017) or when the absorption processes on an intestinal level are affected (Pop et al., 2006).

The addressed topic has emerged following the finding that the specialized literature (Orawan et al., 2007; Kim et al., 2013; Bedánová et al.,

2016) is quite poor regarding the values of the parameters of the erythrocyte series of chickens. We mention that the papers examined by us (Alabi et al., 2015, Sugiharto et al., 2016) did not offer data regarding the erythrocyte parameters of chicken on age groups, which constituted an additional challenge for us.
The data obtained can be regarded as reference values, because we determined the erythrocyte parameters by age categories.

MATERIALS AND METHODS

This study aims to gather data regarding the erythrocyte parameter changes of chickens, during their growth and development.
The biological material had been represented by two batches of chickens as follows: the first group was made out of ten broilers (Cobb 500 hybrid) and the second group was made out of ten common chicken. The both groups benefited from the same maintenance conditions, the food being represented by concentrated feed (minced cereals) and green plants.
At the ages of 14, 45 and 75 days, in order to perform haematological determinations, blood has been collected on anticoagulant (initially from the crest by puncture or by sectioning), then from the axillary vein (by puncture) in order to achieve the haematological determinations.
The working methods used in the current study had been the following: the determination of red blood cells (RBC), the determination of the haematocrit (HCT), the determination of haemoglobin (Hb), the determination of mean corpuscular volume (MCV), the determination of mean corpuscular haemoglobin (MCH), the determination of mean corpuscular haemoglobin concentration (MCHC).
The determination of the erythrocyte number was realised by directly counting them on the microscope using the haemocytometer.
The haematocrit and haemoglobin were determined using the HemoSmart apparatus.
The determination of the mean erythrocyte volume, mean erythrocyte haemoglobin, and mean erythrocyte haemoglobin concentration were determined using the calculation formulas described in the specialized literature (Cotor et al., 2012).
Comparisons regarding the statistical relevance of the differences between the two experimental groups were made using the t test (Student).

RESULTS AND DISCUSSIONS

The results obtained will be presented below in the form of tables and figures, for each moment of our determinations.
Regarding the values of the erythrocyte parameters of the 14-day-old chickens, from the two experimental groups, the results are presented in Table 1.

Table 1. The average values of the erythrocyte parameters in the case of the two experimental groups, at the age of 14 days

Group number	Erythrocyte parameters					
	Direct erythrocyte constants			Indirect erythrocyte constants		
	RBC *(millions/ mm^3 of blood)*	HTC *(%)*	Hb *(g/dl)*	MCV *(fl)*	MCH *(pg Hb/E)*	MCHC *(g Hb/dl E)*
Group 1	2.38	38.16	9.13	170.75	35.87	24.62
Group 2	2.44	37.53	9.06	168.62	35.87	24.87

Regarding the average number of erythrocytes (RBC), the difference between the two experimental groups at the age of 14 days was 2.52% in favour of group 2, this difference not being statistically significant.
Regarding the mean haematocrit value (HTC), the difference between the two experimental groups was 1.68% in favor of group 1, this difference not being statistically significant.
Regarding the mean haemoglobin value (Hb), the difference between the two experimental groups was 0.77% in favour of group 1, this difference not being statistically significant.
Regarding the mean value of MCV, the difference between the two experimental groups was 1.19% in favour of group 1, this difference not being statistically significant.
Regarding the mean value of MCH, there is no difference, the average value being the same for both of the experimental groups.
Regarding the mean value of MCHC, the difference between the two experimental groups was 1.02% in favour of group 2, this difference not being statistically significant.
In Table 2 we present the average values of all haematological parameters determined in the

case of both experimental groups, at the age of 45 days.

Table 2. The average values of the erythrocyte parameters in the case of the two experimental groups, at the age of 45 days

Group number	Erythrocyte parameters					
	Direct erythrocyte constants			Indirect erythrocyte constants		
	RBC (millions/ mm^3 of blood)	HCT (%)	Hb (g/dl)	MCV (fl)	MCH (pg Hb/E)	MCHC (g Hb/ dl E)
Group 1	2.46	36.75	8.7	166.12	33.75	24.62
Group 2	2.62	38.58	9.21*	164.62	35.87*	23.87

*P<0.05

Regarding the average value of the total number of erythrocytes (RBC) in the case of the two experimental groups, at the age of 45 days, the difference was 6.5% in favour of group 2, this difference not being statistically significant. However, there is an increase in this difference compared to the value obtained after the first determination.

Regarding the average value of the haematocrit (HTC) in the case of the two experimental groups, at the age of 45 days, the difference was 4.98% in favour of group 2, this difference not being statistically significant.

Regarding the average value of the haemoglobin (Hb) in the case of the two experimental groups, at the age of 45 days, the difference was 5.86% in favor of group 2, this difference being statistically significant (P<0.05).

Regarding the mean value of MCV in the case of the two experimental groups, at the age of 45 days, the difference was 0.91% in favour of group 1, this difference not being statistically significant.

Regarding the average value of MCH in the case of the two experimental groups, at the age of 45 days, the difference was 6.28% in favour of group 2, this difference being statistically significant (P<0.05).

Regarding the average value of MCHC in the case of the two experimental groups, at the age of 45 days, the difference was 3.14% in favour of group 1, this difference not being statistically significant.

In Table 3 we present the average values of all the haematological parameters determined in the case of both experimental groups, at the age of 75 days.

Table 3. The average values of the erythrocyte parameters in the case of the two experimental groups, at the age of 75 days

Group number	Erythrocyte parameters					
	Direct erythrocyte constants			Indirect erythrocyte constants		
	RBC (millions/ mm^3 of blood)	HCT (%)	Hb (g/dl)	MCV (fl)	MCH (pg Hb/E)	MCHC (g Hb/ dl E)
Group 1	2.17	35.73	8.18	174.5*	33.16	22.5
Group 2	2.57*	38.46*	9.53*	160.62	34.75	24.5

*P<0.05

Regarding the average value of the total number of erythrocytes (RBC) in the case of the two experimental groups, at the age of 75 days, the difference was 18.43% in favor of group 2, this difference being statistically significant (P<0.05).

This difference can be attributed to the diet (Akinleye et al., 2008), because during the growing season the broilers preferred to feed almost exclusively on concentrated feed (ground cereals), that are poorer in vitamins, compared to the green plants the common breed chickens (which also ate green fodder), benefited from. The low value of the average number of red blood cells in the case of chickens from group 1 (very close to the physiological minimum), makes us to assert that these chickens suffered from anemia, most likely deficiency type (the main deficiency being the folic acid present in the leaves and probably, the iron deficiency). We mention that these results were also communicated by other authors (Ghiță et al., 2021).

Regarding the average value of the haematocrit (HCT) in the case of the two experimental groups, at the age of 75 days, the difference was 7.64% in favour of group 2, this difference being statistically significant (P<0.05). We mention that we expected to notice this difference, because the erythrocytes being in smaller numbers in the blood of the chicks from group 1, it was logical that the volume occupied by them to be smaller.

Regarding the average value of haemoglobin (Hb) in the case of the two experimental groups, at the age of 75 days, the difference was 16.5% in favor of group 2, this difference being statistically significant (P<0.05).

This finding, as well as the fact that in the case of group 1, the average value of haemoglobin is slightly lower than the physiological minimum, justifies us to say that the chickens in group 1 suffered from anemia.

The values obtained by us fall within the limits communicated by other authors (Orawan et al., 2007).

Regarding the average value of VEM in the case of the two experimental groups, at the age of 75 days, the difference was 8.64%, this time in favour of group 1, this difference being statistically significant ($P<0.05$).

This finding, as well as the fact that in the case of group 1, the average values of erythrocytes, haematocrit and haemoglobin are lower than in the case of group 2, justifies us to affirm that the chickens in group 1 suffered from macrocytic anemia (anemia with high MCV).

This type of anemia is characteristic of deficiency anemia, installed when the body is vitiated by nutrients that influence mitosis in the hematogenous bone marrow (folic acid, cobalt, vitamin B_{12}, vitamin E, etc.) (Zagrai et al., 2020).

Regarding the average value of MCH in the case of the two experimental groups, at the age of 75 days, the difference was 4.79% in favour of group 2, this difference being statistically insignificant. This observation is logical, which means that even if there are fewer erythrocytes, they contain haemoglobin in physiological concentrations, the anemia being eminently due to the low number of erythrocytes, so in this situation, we discuss a normochromic anemia (Cotor et al., 2021).

Regarding the average value of MCHC in the case of the two experimental groups, at the age of 75 days, the difference was 8.89%, in favour of group 2, this difference being statistically insignificant.

This observation is logical, which means that even if the erythrocytes are less, they contain haemoglobin in physiological concentrations, the anemia being eminently due to the low number of erythrocytes, so in this situation we discuss a normochromic anemia.

In Figures 1, 2, 3, 4, 5 and 6 we present in dynamics the changes of the erythrocyte parameters studied for both experimental groups in the 3 moments (the age of 14, 45 and 75 days).

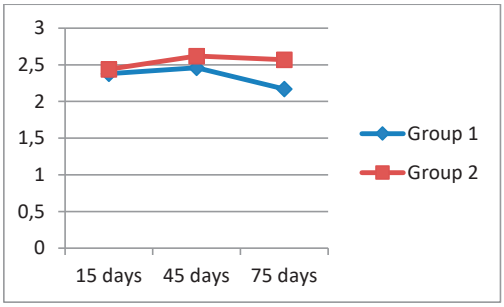

Figure 1. RBC dynamics in the case of the two experimental groups

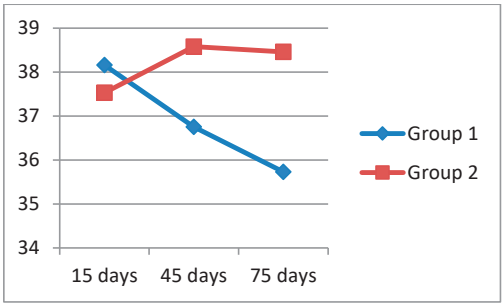

Figure 2. HCT dynamics in the case of the two experimental groups

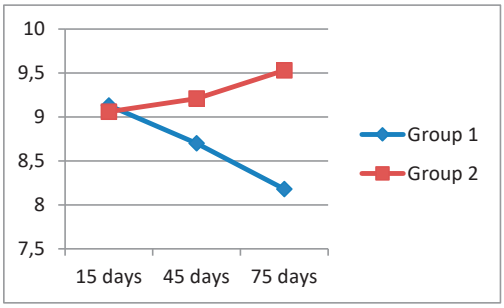

Figure 3. Hb dynamics in the case of the two experimental groups

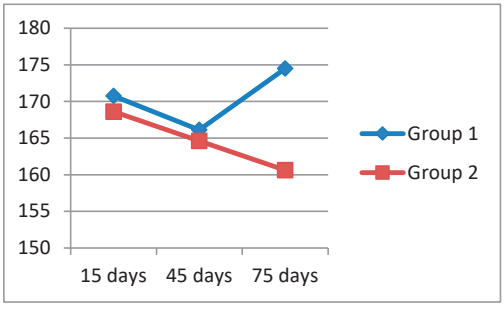

Figure 4. MCV dynamics in the case of the two experimental groups

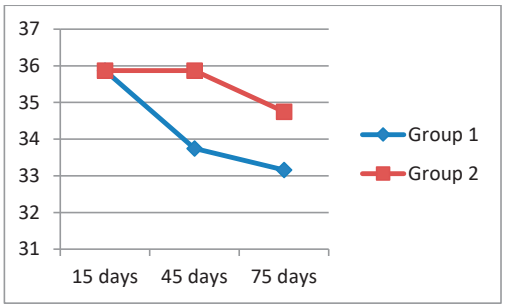

Figure 5. MCH dynamics in the case of the two experimental groups

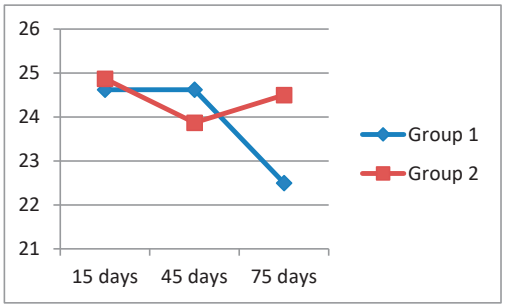

Figure 6. MCHC dynamics in the case of the two experimental groups

CONCLUSIONS

The number of erythrocytes of the chickens in the two experimental groups was within the physiological limits throughout the experiment except for the age of 75 days when a significantly higher difference of 18.43% was found in favour of group 2.

The value of haematocrit showed a significant difference only at the age of 75 days, the difference being 7.64% higher in favour of group 2.

Haemoglobin values showed significant differences both at the age of 45 days (higher by 5.86% in favour of group 2) and at the age of 75 days (higher by 16.6% in favour of group 2).

The mean corpuscular volume showed a significant difference only at the age of 75 days (being 8.64% higher in favour of group 1).

The mean corpuscular haemoglobin showed a significant difference only in the case of 45-day-old chicks (the difference being 6.26% higher in favour of the chicks in group 2).

The mean corpuscular haemoglobin concentration did not show significant differences throughout the experiment, the values of this parameter falling within the physiological limits.

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RESEARCH ON THE INFLUENCE OF AGE ON REPRODUCTIVE INDICES IN KARAKUL OF BOTOȘANI SHEEP

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Abstract

The research on the influence of age on the reproduction indices in Karakul of Botoșani sheep was carried out by processing the data found in the breeding and calving registers, the lamb register and the existing breeding registers within the S.C.D.C.O.C. Popăuți for the period 2017-2019. Following the research carried out on the influence of the age of Karakul of Botoșani sheep on the reproduction indices, the obtained results indicated a statistically significant link between the age batch of which the sheep belong and the reproductive indices analyzed. The data obtained indicated values of higher reproduction rates in the 52-56 months' age batch (107.8% fertility rate, 96.4% fecundity rate, 95.5% birth rate and 112.9% prolificacy rate) compared to the results obtained by sheep in the 16-21 months' age batch (94.6% fertility rate, 90.6% fecundity rate, 89.1% birth rate and 106.1% prolificacy rate).

Key words*: age, Karakul of Botoșani, pelts, reproduction indices.*

INTRODUCTION

The researches regarding the influence of age on the reproduction indices for the sheep from the Karakul of Botoșani breed were carried out in the creative unit of this breed of sheep, respectively "Research and Development Station in Sheep and Goat Breeding - Popăuți Botoșani". The study carried out aim at researching some aspects regarding the reproductive indices of the specialized sheep for the production of pelts. The proposed study started from the research hypothesis represented by the way in which the technological factors underlying the breeding works of the Karakul of Botoșani breed can intervene in increasing the reproduction indices and have a positive influence on the productive parameters of the sheeps from this breed.

Reproductive function can be considered as a physiological basis for animal husbandry, as it directly contributes to the numerical increase of livestock, and through the use of breeders of high zootechnical value there is not only an improvement of breeds but also an increase in production obtained from the new generations (Pascal, 2015).

In the sheep species, the sexual maturity of individuals usually appears before that of body weight and is influenced both by the precocity of the breed, individuality but also by specific technological conditions. In sheep, it is estimated that sexual maturity occurs at the age of 8-9 months in males and at 7-10 months in females, although sex cells are suitable for fertilization from the age of 5-6 months (Fecioru, 2008; Pascal et al. 1995).

Technological factors through the level of nutrition and body development of sheep have a high influence on reproductive activity. The calving season has an influence on the age at the first calving, so by obtaining early calvings that allow the intensification of the breeding activity by using the young sheep from the first year at calving.

Karakul of Botoșani breed have breeding characteristics which are characteristic to the parents that formed the basis of formation of this sheep breed. Separation of young sheep by sex should be done from the age of 4-5 months with the emergence of the genetic instinct. In this breed of sheep, sexual maturity is established at the age of 7-9 months for males and at 8-9 months for females, at which time the sexual cell is suitable for fertilization. A consequence of the lateness of the breed is the use for breeding at the age of 16-18 months, respectively in the autumn of the second year of life as a result of

staggered calvings and improper development until the breeding season of the first year of life. Karakul of Botoșani sheep have a sexual activity up to the age of 9-11 years with an average operating age of about 7.5 years. Due to the specific production of this breed, respectively the production of pelts, they are exploited as long as they can produce a lamb from which a pelt is obtained. Breeding systems used in sheep of this breed and how these systems increase breeding rates by obtaining a high number of products of high biological value through the rational use of breeders.

MATERIALS AND METHODS

The biological material studied for the assessment of the influence of age on the reproduction indices in Karakul of Botoșani sheep is represented by the Karakul of Botoșani sheep from the Research and Development Station in Breeding Sheep and Goats Popăuți Botoșani, creative unit of this sheep breed. The study batch was represented by the sheep from the unit from August 2017 to December 2020, composed at the beginning of the study of 920 sheep from the Karakul of Botoșani breed. The processing of data on the reproduction indices of Karakul sheep and the influence of age on them were assessed for the breeding seasons 2017, 2018, 2019.

Assessment methods and techniques used in recording, observing, assessing reproduction indices, investigating, simulating and reporting to standards. The qualitative variables studied are: category of sheep, sex, color varieties, age at the time of introduction to reproduction.

For the statistical processing of the study data obtained as a result of the appreciation of the reproduction indices, the Microsoft Excel program was used, which allowed the planning, collection, access, preparation, data management and presentation of results and version 26 of IBM SPSS (Statistical Product and Service Solutions). Continuous variables were analyzed for normality and then expressed by mean ± standard deviation, minimum and maximum (Popa, 2008). The Student's t test (when comparing 2 batches) and the ANOVA One-Way method (for multiple batches) were used to compare the averages of the parameters between batches. A value of statistical significance coefficient $p<0.05$ was considered significant.

In order to highlight the influence of the age of the Karakul of Botoşani sheep in the breed on the analyzed reproduction indices, they were distributed over the 3 years of study taken into account by age batches expressed in months at the time of use for breeding, 7 batches were prepared with sheep of the Karakul of Botoșani breed: 16-21 months, 29-33 months, 39-45 months, 52-56 months, 64-68 months, 75-79 months, over 89 months. The results obtained converge in a sense with other studies done on other breeds in Romania (Pascal, 2007).

RESULTS AND DISCUSSIONS

The influence of the age of the Karakul of Botoșani sheep at the time of their use in breeding was assessed for the following reproduction indices: fertility index, fecundity index, prolificacy index and birth rate.

The studied batch with sheep from Karakul of Botoșani breed was distributed by age categories in the breeding season specific to each reference year. The largest share of the sample was in sheep aged between 29 and 33 months (21.4%), followed by those aged between 39 and 45 months (20.8%) and those aged between 16 and 21 months (19.6%). Slightly lower shares were sheep aged between 52 and 56 months (16.2%) and those aged between 64 and 68 months (15.1%). The lowest shares in the total sample were sheep aged between 75 and 79 months (5%) and those over 89 months (2%) (Table 1).

Table 1. Karakul of Botoşani sheep by batches' age

Karakul of Botoşani sheep age at breeding						
			Year			Total
			2017	2018	2019	
Karakul of Botoşani sheep age at breeding	16-21 months	N	176	147	82	405
		%	20.1%	26.1%	13.1%	19.6%
	29-33 months	N	174	129	139	442
		%	19.9%	22.9%	22.1%	21.4%
	39-45 months	N	156	140	134	430
		%	17.8%	24.9%	21.3%	20.8%
	52-56 months	N	100	101	133	334
		%	11.4%	17.9%	21.2%	16.2%
	64-68 months	N	177	39	95	311
		%	20.2%	6.9%	15.1%	15.1%
	75-79 luni	N	63	5	35	103
		%	7.2%	0.9%	5.6%	5.0%
	>89 months	N	29	2	10	41
		%	3.3%	0.4%	1.6%	2.0%
Total		N	875	563	628	2066
		%	100.0%	100.0%	100.0%	100.0%

The evaluation of the fertility index is done by assessing the number of products obtained from the total number of sheep distributed during the breeding season. The standard of the Karakul of Botoșani breed regarding the fertility index according to the specialized literature is estimated at a minimum of 92.6%.

The fertility index obtained by the sheep in the studied batch for the reference period 2017-2019, compared to the entire sample is 1.018 lambs for each sheep distributed at the breeding or, as a percentage, 101.80%. Thus, it can be observed that out of a total of 2066 Karakul of Botoșani sheep heads distributed during the three years, 2104 products were obtained (Table 2).

Table 2. Karakul of Botoșani sheep fertility index

Fertility index	
Number of sheep distributed at breeding	2066
Fertility index	101.8%
Number of products obtained	2104

In order to highlight the influence of the age of the Karkaul de Botoșani sheep on the fertility index, we applied the ANOVA One-Way analysis method. A significantly lower fertility rate can be observed at 16 to 21 months of age and between 29 and 33 months compared to sheep aged between 39 and 68 months.

The highest value of the fertility index being registered in the age category 52-56 months with a number of 360 products obtained from 334 sheep distributed at the breeding, with a value of the fertility index of 107.8% (Table 3).

Table 3. Fertility index by age category

Fertility index				
Sheep age at the breeding	Number of sheep distributed at breding	Fertility index	Number of products obtained	P
16-21 months	405	94.6%	383	<0.001
29-33 months	442	97.7%	432	
39-45 months	430	106%	456	
52-56 months	334	107.8%	360	
64-68 months	311	105.5%	328	
75-79 months	103	100%	103	
> 89 months	41	102.4%	42	

The lowest value of the fertility index was registered in the 16-21 months' age batch with a number of 383 products obtained from 405 sheep distributed for reproduction, obtaining a value of 94.6% of the fertility index. The results of the statistical analysis also show that the differences are statistically significant between the different age categories of Karakul of Botoșani sheep in terms of fertility index ($p<0.001$) (Figure 1).

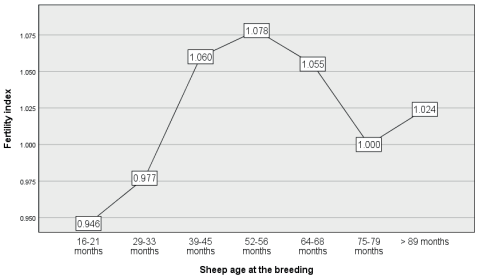

Figure 1. Karakul of Botoșani fertility index

Data on differences in the number of products obtained in relation to each age batch of Karakul of Botoșani sheep are presented in Table 4. It can be seen that among all the existing age batches in the studied batch, the differences in the fertility index of sheep are statistically significant with a value of $P<0.005$. Age batch 52-56 months obtaining the best values of the fertility index.

Table 4. Multiple comparisons obtained products

Multiple comparisons			
Dependent variable: Obtainded products			
Bonferroni			
(I) Sheep age at the breeding	(J) Sheep age at the breeding	Mean difference (I-J)	P
16-21 months	39-45 months	-.115*	.001
	52-56 months	-.132*	.000
	64-68 months	-.109*	.007
29-33 months	39-45 months	-.083*	.046
	52-56 months	-.100*	.012
39-45 months	16-21 months	.115*	.001
	29-33 months	.083*	.046
52-56 months	16-21 months	.132*	.000
	29-33 months	.100*	.012
64-68 months	16-21 months	.109*	.007
*. The difference between the averages is statistically significant at a threshold of 0.05.			

The fecundity rate is expressed by the ratio of the number of ewes that gave birth and aborted to the total number of sheep inoculated. The standard of the Karakul of Botoșani breed regarding the fecundity index according to the specialized literature is between 92.6% and 98.9%.

The appreciation of the fecundity index for the reference period 2017-2019, compared to the entire sample is 0.938 or, in percentage, 93.80%. So, from 2066 inoculated sheep, a number of 1938 products were obtained (Table 5).

In order to highlight the influence of the age of the Karakul of Botoșani sheep in the breeding on the fecundity index, we applied the analysis of Anova One-way variance.

By analyzing the results of the statistical analysis it can be seen that the best value in terms of fecundity index was obtained in sheep aged 52-56 months, by obtaining a fecundity rate of 96.4% with a number of 322 sheep that gave birth and aborted out of a number of 334 inseminated sheep (Table 6).

Table 5. Fecundity rate Karakul de Botoșani

Fecundity rate	
Total number of sheep inseminated	2066
Fecundity index	93.8%
Total number of sheep that gave birth and had an abortion	1938

Table 6. Fecundity index by age category

Fecundity index				
Sheep age at the breeding	Total number of sheep inseminated	Fecundity index	Total number of sheep that gave birth and had an abortion	P
16-21 months	405	90.6 %	367	0.009
29-33 months	442	93.0 %	411	
39-45 months	430	95.8 %	412	
52-56 months	334	96.4 %	322	
64-68 months	311	94.5 %	294	
75-79 months	103	90.3 %	93	
> 89 months	41	95.1 %	39	

The lowest value of the fertility index was recorded in sheep belonging to the age batch 75-79 months with an index of 90.3% with a number of 93 sheep that gave birth and aborted out of a number of 103 inseminated sheep (Figure 2).

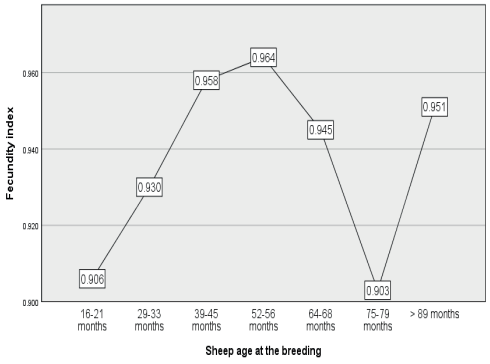

Figure 2. Karakul of Botoșani fecundity index

It can be seen that the differences between the age batches of the sheep in the study batch, in terms of the number of calvings and abortions are statistically significant ($p<0.05$).

The highest differences were achieved between the age category 16-21 months and the category 52-56 months. The age batch 52-56 months obtaining the highest values of the fecundity index (Table 7).

Table 7. Fecundity index by age category

Multiple comparisons			
Dependent variable: Number of births and abortions			
Bonferroni			
(I) Sheep age at the breeding	(J) Sheep age at the breeding	Mean difference (I-J)	p
16-21 months	39-45 months	-.052*	.038
	52-56 months	-.058*	.024
39-45 months	16-21 months	.052*	.038
52-56 months	16-21 months	.058*	.024
*. The difference between the averages is statistically significant at a threshold of 0.05.			

The evaluation of the birth rate is done by reporting the number of ewes that have calved to the total number of sheep distributed at breading during one breeding season. According to the specialized literature, the birth rate for sheep belonging to the Karakul of Botoșani breed is 101.5%. The assessment of the birth rate for the reference period 2017-2019, for the Karakul of Botoșani sheep compared to the entire sample is 0.928 or, in percent, 92.80%. ewes calved during the study period (Table 8).

Table 8. Karakul of Botoșani birth rate

Birth rate	
Total number of sheep distributed at breeding	2066
Birth rate	92.8%
Number of sheep that gave birth	1917

In order to highlight the influence of the age of Karakul of Botoșani sheep in the breeding campaign on the birth rate, we applied the analysis of variance Anova One way. We observe a significantly lower birth rate at the ages between 16 and 21 months where out of a number of 405 sheep distributed in the breeding only 361 heads gave birth, obtaining a birth rate of 89.1%, compared to sheep aged 39-45 months where out of a number of 430 sheep heads distributed during the breeding, 411 heads gave birth thus obtaining a birth rate of 95.6% also the category aged 52-56 months where out of a total number of 334 ewes distributed in the herd, 319 heads gave birth, obtaining a birth rate of 95.5% (Table 9).

Table 9. Birth rate by age category

Birth rate				
Sheep age at the breeding	Total number of sheep distributed at breeding	Birth rate	Number of sheep that gave birth	P
16-21 months	405	89.1%	361	0.002
29-33 months	442	91.9%	406	
39-45 months	430	95.6%	411	
52-56 months	334	95.5%	319	
64-68 months	311	93.6%	291	
75-79 months	103	88.3%	91	
> 89 months	41	92.7%	38	

Regarding the results of the statistical analysis, they also show that the differences are statistically significant between the different age categories in terms of birth rate (p = 0.002). Therefore, the age of the Karakul of Botoșani sheep at the time of use for reproduction influences the birth rate. Sheep in the age batch 39-45 months obtaining the highest value in terms of birth rate (95.6%) (Figure 3).

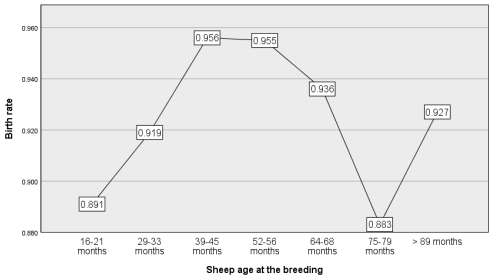

Figure 3. Karakul of Botoșani birth rate

The prolificacy index is expressed by relating the total number of products obtained to the number of ewes that gave birth. The standard of the Karakul of Botoșani breed regarding the fertility index according to the specialized literature is between 101.1% and 107.3%.

The assessment of the prolificacy index for the reference period 2017-2019, compared to the entire sample is 1,098 or, as a percentage, 109.80%. Thus, from 1917, ewes that gave birth were obtained a number of 2104 products (Table 10).

Table 10. Prolificay index Karakul of Botoșani

Prolificacy index	
Number of ewes that gave birth	1917
Prolificacy index	109.8%
Number of products obtained	2104

In order to study the influence of the age of the Karakul of Botoșani sheep in the breed on the prolificacy index, we used the analysis of variance Anova One way. A slightly lower prolificacy index can be observed in young sheep aged between 16 and 21 months, where out of a total of 361 ewes that gave birth, a number of 383 products were obtained, obtaining a value of the prolificacy index of 106.1% compared to sheep over 39 months of age. The highest value in terms of prolificacy index was recorded in the age batch 75-79 months where out of a total of 91 ewes that gave birth, a number of 103 products was obtained (Table 11).

Tabel 11. Prolificacy index by age category

Prolificacy index				
Sheep age at the breeding	Number of sheep that gave birth	Prolificacy index	Number of products obtained	p
16-21 months	361	106.1%	383	0.004
29-33 months	406	106.4%	432	
39-45 months	411	110.9%	456	
52-56 months	319	112.9%	360	
64-68 months	291	112.7%	328	
75-79 months	91	113.2%	103	
> 89 months	38	110.5%	42	

The results of the statistical analysis show that the differences are statistically significant between the different ages in terms of the prolificacy index (p = 0.004). In view of all the aspects presented, related to the influence of the age of the sheep on the prolificacy index, it is confirmed that the age of the Karakul of Botoșani sheep in the mountain influences the value of the prolificacy index (Figure 4).

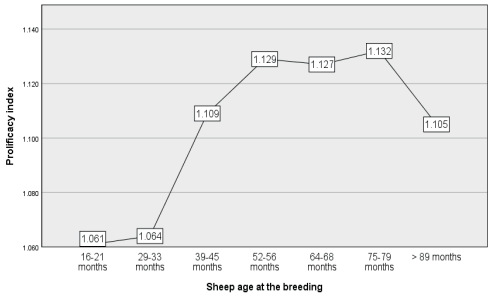

Figure 4. Karakul of Botoșani prolificacy index

CONCLUSIONS

As a result of the age distribution of the Karakul of Botoșani sheep herd during the 3 breeding seasons, it was found that the largest share of the sample had sheep between 29 and 33 months of

age (21.4%), followed by those aged between 39 and 45 months (20.8%) and those aged between 16 and 21 months (19.6%).

Statistical processing of data on the influence of Karakul of Botoșani sheep age on fertility index indicated that differences are statistically significant between different age categories of Karakul of Botoșani sheep in terms of fertility index ($p<0.001$).

Significantly lower fertility rates at 16 to 21 months of age and 29 to 33 months of age compared to sheep aged 39 to 68 months.

Regarding the influence of the age of sheep from the Karakul of Botoșani breed on the fecundity index, the statistical analysis of the data shows that there are statistically significant differences between different age categories in terms of fecundity index ($p = 0.009$).

Following the interpretation of the results of the statistical analysis regarding the influence of the age of sheep from the Karakul of Botoșani breed on the birth rate, they show that the differences are statistically significant between the age categories within the batch studied in terms of birth rate ($p = 0.002$).

Regarding the influence of the age of sheep from the Karakul of Botoșani breed on the prolificacy index, the results of the statistical analysis show that the differences are statistically significant between the different age categories within the study batch ($p = 0.004$).

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RESEARCH ON PRODUCTIVE PERFORMANCE IN MEAT COW BREEDS FROM NEAMT COUNTY ZOOTECHNICAL HOLDINGS

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Abstract

In this scientific paper, the productive performances of beef cattle belonging to the Aberdeen Angus and Charolaise breeds from 6 farms in Neamț County were analyzed, studying the following indicators: birth weight (kg), body weight (kg), and average daily gain (g) at the age of 200 days (kg), and also 365 days (kg). The data on average values and variability of meat production indices show that the highest performances were 42.33 kg at birth, 246.29 kg and the average daily increase of 1030.71 grams per 200 days, and 433.69 kg with the average daily increase of 1074.33 kg, at the age of 365 days. On the last place was farm 1, which registered an average weight at birth of 30.70 kg, at 200 days this indicator was 188.59 kg, and the average daily gain was 786.20 grams; at 365 days the average weight was 320.84 kg and the average weight was 793.11 kg. Farm 1 raises the Aberdeen Angus breed, and farm 5 raises the Charolaise breed, which has better results due to the constant care of the breeder to improve the genetic material.

Key words*: Angus, beef, Charolaise, performance, production.*

INTRODUCTION

Meat is a special food source, compared to which the preferences and tastes of consumers have been and are constantly growing. Beef is a complete food, rich in protein and special properties regarding the taste, smell, aspect, etc. It contains 34.9% dry matter, of which 18.7% protein, 15.3% fat and 0.9% mineral salts, with an energy value of 2270 kcal/kg. It also contains all the essential amino acids - lysine 1.78 g/100 g meat, leucine 1.68 g, arginine 1.32 g, valine 1.14 g, isoleucine 1.04 g, phenylalanine 0.80 g, threonine 0.80 g, histidine 0.58 g, methionine 0.46 g, and tryptophan 0.22 g/100 g meat (Stanciu, 1999).
Beef production in cattle is influenced by many factors, which requires systematization, thus: genetic, physiological, and also environmental factors (Acatincăi, 2010). Genetic and physiological factors can influence meat production by 30% up to 70% if we consider that for meat characters the genetic determinism is from intermediate to strong (Georgescu et. al., 1998).
Environmental factors influencing the phenotypic performance of beef cattle relate in particular to the technology of exploitation, feeding and reproduction having a decisive role.
The maintenance system can create comfort or discomfort with positive or negative effects on the growth and fattening process (Maciuc, 2012).
Food influences meat production through the level and type of feeding, the quality of feed, the form and method of their administration. The energy-protein and mineral-vitamin level must satisfy the requirements of the vital functions and allow obtaining a maximum growth gain (Dinescu et al., 1996).
The breeding management process has a decisive role in raising and exploitation beef cattle, which directly influences the increase of the herd, and thereby the increase of the total meat production.

MATERIALS AND METHODS

In this paper, we present the research results from 6 farms in Neamț County where beef cattle are bred and exploited: Aberdeen Angus (103 heads) and Charolaise (73 heads).
The research objectives were: birth weight (kg), body weight at the age of 200 days (kg) and 365 days (kg), the average daily gain at the age of 200 days (g) and 365 days (g).

Differential significance tests were also applied through the Fischer test (F) which is used to verify the equality of dispersions of two normally distributed independent variables and the Tukey test, the most widely used multiple comparison procedure also called the honest significant difference test.
The data came from direct observations and determinations in the farm as well as from the farm's primary database, but also from the Own Performance Control (CPP) records made by the Aberdeen Angus Association and the Romanian Meat Cattle Breeders Association. All data were statistically processed and summarized in tables and figures.
The six farms studied were coded from 1 to 6 and are shown in Table 1.

Table 1. Diary cattle farms encode, total number of animals in the herd and raised breeds

Farm encode	Name of the farm	Number of animals	Breed
1	S.C. Valdo Forest SRL	87	Aberdeen Angus (AA)
2	Bordianu Cristian	179	Aberdeen Angus (AA)
3	S.C. CGC Agria S.A.	189	Aberdeen Angus (AA)
4	Minuț Maria Simona	38	Charolaise (CH)
5	Arteni Mugurel	49	Charolaise (CH)
6	I.I. Dârlea Ofelia Nicoleta	65	Charolaise (CH)
TOTAL		607	

RESULTS AND DISCUSSIONS

From the data on the average values and variability of the meat production indices presented in table 2 at farm 1 it is found that an average birth weight of 30.70 kg was achieved for the 50 cases, the average weight at the age of 200 days was 188.59 placed between extremely wide limits, namely 146.10-238.00 kg and the average daily increase recorded was 786,200 grams with equally wide limits between 570.00-1050.00 grams.
From the analysis of the degree of dispersion for the weight achieved at the age of 200 days, the standard deviation having the maximum value of 51.981 kg, and the maximum coefficient of variation of 27.563%, it is found that in this farm there is a heterogeneous cattle population in this age category.
At the age of 365 days, an average body weight value of 320.842 kg was recorded between extremely wide limits, precisely 217.94 - 374.39 kg and an average daily gain of 793.11 grams with very wide limits of 509.59-947.95 g. From the analysis of the degree of dispersion for the weight achieved at the age of 365 days, the standard deviation having the maximum value of 133.671 kg, and the maximum coefficient of variation of 41.663%, shows the heterogeneity of cattle in this age category.

Table 2. Average values and variability of farm meat production in farm 1

Indicators	n	$\overline{X}$	$\pm s_{\bar{x}}$	s	V%	Minimum	Maximum
Weight at birth (kg)	50	30.70	0.628	4.441	14.467	24.00	40.00
Weight at 200 days (kg)	50	188.59	11.249	51.981	27.563	146.10	238.00
Average daily gain at 200 days (g)	50	786.200	26.965	123.571	15.718	570.00	1050.00
Weight at 365 days (kg)	50	320.842	38.841	133.671	41.663	217.94	374.39
Average daily gain 365 days (g)	50	793.11	45.974	172.017	23.024	509.59	947.95

From the data on the average values and variability of the meat production indices presented in Table 3 at farm 2 it is found that an average birth weight value of 30.29 kg was achieved for the 28 cases, the average weight at the age of 200 days was 189.05 kg placed between some fairly wide limits of 162.43-217.10 kg and the average daily gain recorded was 767.50 grams, with wide limits between 625.00- 900.00 grams.
From the analysis of the degree of dispersion for the weight achieved at the age of 200 days, the standard deviation having the value of 18,915 kg, and the coefficient of variation of 21.24%, it is found that in this farm there is a heterogeneous cattle population.
At the age of 365 days, an average body weight value of 321.54 kg was recorded, the limits being between 264.00-431.20 kg, and an average daily gain of 799.62 g with limits of 641.10 g, and 1098.63 g. From the analysis of the degree of dispersion for the weight achieved at the age of 365 days, the standard deviation having the value of 39.635 kg, and

the coefficient of variation of 12.327%, it is found that this age category has an average homogeneity.

Table 3. Average values and variability of farm meat production in farm 2

Indicators	n	$\overline{X}$	$\pm s_{\bar{x}}$	s	V%	Minimum	Maximum
Weight at birth (kg)	28	30.29	0.388	2.052	6.776	28.00	36.00
Weight at 200 days (kg)	28	189.05	7.149	18.915	21.241	162.43	217.10
Average daily gain at 200 days (g)	28	767.50	36.608	103.544	13.491	625.00	900.00
Weight at 365 days (kg)	28	321.54	11.442	39.635	12.327	264.00	431.20
Average daily gain 365 days (g)	28	799.62	31.296	108.411	13.558	641.10	1098.63

From the data on average values and variability of the meat production indices presented in table 4 at farm 3 it is found that an average birth weight value of 30.85 kg was achieved for the 25 cases; the average weight at the age of 200 days was 223.25 kg, placed between quite wide limits (168.35-314.61 kg), and the average daily gain recorded was 907.00 grams with very wide limits of 625.00-1350.00 grams.

Table 4. Average values and variability of farm meat production in farm 3

Indicators	n	$\overline{X}$	$\pm s_{\bar{x}}$	s	V%	Minimum	Maximum
Weight at birth (kg)	25	30.85	0.731	3.865	12.528	25.00	37.00
Weight at 200 days (kg)	25	223.25	8.113	40.563	18.169	168.35	314.61
Average daily gain at 200 days (g)	25	907.00	41.107	205.533	22.661	625.00	1350.00
Weight at 365 days (kg)	25	369.66	15.165	45.495	12.307	300.00	440.00
Average daily gain 365 days (g)	25	905.32	40.356	121.069	13.373	726.03	1090.41

From the analysis of the degree of dispersion for the weight achieved at the age of 200 days, the standard deviation having the value of 40.563 kg, and the coefficient of variation of 18.169%, it is found that in this farm there is a population of cattle with average homogeneity for this age category.

At the age of 365 days, an average body weight value of 369.66 kg was recorded, the limits being between 300.00-440.00 kg, and an average daily gain of 905.32 grams with limits of 726.03-1090.41 g. From the analysis of the degree of dispersion for the weight achieved at the age of 365 days, the standard deviation having the value of 45.495 kg, and the coefficient of variation of 12.307%, results that at this age category the cattle population is characterized by an average homogeneity.

From the data on the average values and variability of the meat production indices presented in table 5 at farm 4 it is found that an average birth weight value of 38.81 kg was achieved for the 27 cases, the average weight at the age of 200 days was 242.50 kg placed between quite wide limits of 180.00-311.00 kg and the average daily increase recorded was 1017.05 grams, with limits between 711.40- 1355.70 grams.

From the analysis of the degree of dispersion for the weight achieved at the age of 200 days, the standard deviation having the value of 31.32 kg, and the coefficient of variation of 12.94%, it is found that in this farm there is a population of cattle with average homogeneity for this age category.

At the age of 365 days, an average body weight value of 376.42 kg was recorded, the limits being between 310.00-430.00 kg and an average daily increase of 925.08 grams with limits of 753.00-1068.00 g. From the analysis of the degree of dispersion for the weight achieved at the age of 365 days, the standard deviation having the value of 40.871 kg, and the coefficient of variation of 10.858%, results that at this age category the cattle population is characterized by an average homogeneity.

Table 5. Average values and variability of farm meat production in farm 4

Indicators	n	$\overline{X}$	$\pm s_{\bar{x}}$	s	V%	Minimum	Maximum
Weight at birth (kg)	27	38.81	0.935	4.860	12.521	28.00	47.00
Weight at 200 days (kg)	27	242.50	6.144	31.329	12.944	180.00	311.00
Average daily gain at 200 days (g)	27	1017.05	29.967	152.806	15.024	711.40	1355.70
Weight at 365 days (kg)	27	376.42	11.798	40.871	10.858	310.00	430.00
Average daily gain 365 days (g)	27	925.08	31.275	108.342	11.712	753.00	1068.00

From the data on the average values and variability of the meat production indices presented in table 6 at farm 5, it was found that an average birth weight value of 42.33 kg was achieved for the 15 cases, the average weight at the age of 200 days was 246.29 kg, the limits being 205.00-342.00 kg, and the average daily gain recorded was 1030.71 grams with limits between 862.43-1502.51 grams.

From the analysis of the degree of dispersion for the weight achieved at the age of 200 days, the standard deviation having the value of 35.274 kg, and the coefficient of variation of 14.322%, it is found that in this farm there is a population of cattle with average homogeneity for this age category.

At the age of 365 days, an average body weight value of 433.69 kg was recorded, the limits being between 390.00-470.00 kg and an average daily gain of 1074.33 grams with limits between 958.90-1164.00 g. From the analysis of the degree of dispersion for the weight achieved at the age of 365 days, the standard deviation having the value of 25.911 kg, and the coefficient of variation of 5.974%, results that at this age category the cattle population is homogeneous.

Table 6. Average values and variability of farm meat production in farm 5

Indicators	n	$\overline{X}$	$\pm s_{\bar{x}}$	s	V%	Minimum	Maximum
Weight at birth (Kg)	15	42.33	1.780	6.894	16.284	30.00	55.00
Weight at 200 days (Kg)	15	246.29	9.427	35.274	14.322	205.00	342.00
Average daily gain at 200 days (g)	15	1030.71	44.701	167.255	16.227	862.43	1502.51
Weight at 365 days (kg)	15	433.69	7.186	25.911	5.974	390.00	470.00
Average daily gain 365 days (g)	15	1074.33	6.871	64.591	6.012	958.90	1164.00

From the data on average values and variability of meat production indices presented in table 7 at farm 6, it was found that for the 31 cases an average birth weight of 38.26 kg was achieved, the average weight at the age of 200 days was of 241.95 kg placed between quite wide limits of 196.00-284.00 kg and the average daily increase recorded was 1020.28 grams with limits between 876.29-1260.00 grams.

From the analysis of the degree of dispersion for the weight achieved at the age of 200 days, the standard deviation having the value of 22.93 kg, and the coefficient of variation of 9.47%, it is found that in this farm there is a homogeneous cattle population for this age category.

At the age of 365 days, an average body weight value of 401.63 kg was recorded, the limits being between 280.48-454.96 kg and an average daily gain of 994.28 grams, with wide limits of 641.10-1197.00g. From the analysis of the degree of dispersion for the weight achieved at the age of 365 days, the standard deviation having the value of 50.209 kg, and the coefficient of variation of 12.501%, results that at this age category the cattle population is characterized by an average homogeneity.

Table 7. Average values and variability of farm meat production in farm 6

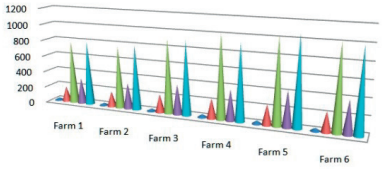
Indicators	n	$\overline{X}$	$\pm s_{\bar{x}}$	s	V%	Minimum	Maximum
Weight at birth (kg)	31	38.26	0.904	5.033	13.155	27.00	51.00
Weight at 200 days (kg)	31	241.95	5.261	22.933	9.478	196.00	284.00
Average daily gain at 200 days (g)	31	1020.28	22.853	99.616	9.763	876.29	1260.00
Weight at 365 days (kg)	31	401.63	13.419	50.209	12.501	280.48	454.96
Average daily gain 365 days (g)	31	994.28	40.239	150.561	15.143	641.10	1197.00

From the analysis of the average values and variability of the meat production indices to the descendants of the cattle in the six farms studied under presented in Tables 2, 3, 4, 5, 6 and 7 and from Figure 1 it is revealed that the best phenotypic performances were registered on the farm 5 and the weakest on the farm 1.

In the farm 5 where the best performances were recorded, the Charolaise breed is raised, which is a specialized breed for meat production, the adult cows can reach weights of 1000 kg, the intensively fattened youth achieve a high average daily gain of 1200-1400 g (Lupan et al., 1997).

However, if we compare the phenotypic performances obtained in farm 5 with the data from the literature, it is revealed that the average daily increase obtained is lower than the productive characteristics of the Charolaise breed. This situation indicates that there are

deficiencies to be remedied in this farm as well, especially of the environmental factors that influence the meat production.



	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6
Weight at birth (Kg)	30.7	30.29	30.85	38.81	42.33	38.26
Weight at 200 days (Kg)	188.59	189.05	223.25	242.5	246.29	241.95
Average daily gain at 200 days (g)	786.2	767.5	907	1017.05	1030.71	1020.28
Weight at 365 days (kg)	320.84	321.54	369.66	376.42	433.69	401.63
Average daily gain 365 days (g)	793.11	799.62	905.32	925.08	1074.33	994.28

Figure 1 Meat production performances in all the studied farms

The tests for the significance of differences in productive performance to the number of beef cattle studied on these farms show that there are significant differences between farm 1 and farm 6 of 7.56 kg in birth weight, as well as 7.97 kg between farm 2 and farm 6, 4.08 kg between farm 5 and farm 6, 11.63 kg between farm 1 and farm 5, 12.05 kg between farm 2 and farm 5, 8.11 kg between farm 1 and farm 4, 8.53 kg between farm 2 and 4, 11.21 kg between farm 1 and 3, of 11.62 kg between farm 2 and 3, for the highest significance threshold, $p < 0.01$. A significant difference was also found between farm 3 and 6 of 3.65 kg, as well as between farm 4 and 5, of 3.52 kg, where $p < 0.05$.

Significance tests of differences in productive performance in beef cattle studied on farms for weight at the age of 200 days show significant differences for the highest significance threshold $p < 0.01$ between farms 1 and 6 of 53.36 kg, between farms 2 and farm 6 of 52.90 kg, of 57.70 kg between farm 1 and farm 5, of 57.24 kg between farm 2 and farm 5, 53.91 kg between farm 1 and farm 4 and 53.45 kg between farm 2 and farm 4.

Significance tests of differences in productive performance in beef cattle studied on farms for weight at 365 days of age show significant differences for the highest significance threshold $p<0.01$ between farm 5 and 1 of 112.85 kg and between farm 5 and farm 2 of 112.15 kg.

CONCLUSIONS

The following conclusions can be drawn from the study:

The best phenotypic performances analyzed in terms of the five indicators, as follows: average birth weight (kg), average weight at 200 days (kg), average weight at 365 days (kg), average daily increase at 200 days (g) the average daily allowance at 365 days (g) were recorded on the farm 5.

Thus, at farm 5, an average weight of 42.33 kg, 246.29 kg at 200 days, and 433.69 kg at 365 days was recorded at birth and the average daily increase was 1030.71 grams at 200 days and 1074.33 grams at 365 of days. The Charolaise breed is raised on farm 5.

At farm 1, an average weight of 30.70 kg, 188.59 kg at 200 days and 320,842 kg at 365 days was recorded at birth, and the average daily gain at 200 days was 786.20 grams and 793.11 grams at 365 days. Farm 1 raises Aberdeen Angus breed.

An important aspect that determined the differences of the productive performances is the one related to the characteristics of the beef cattle breed that grows here, the Charolaise breed that is characterized by a hypermetric body development, where the height of cows is 135-140 cm and the height of bulls is 142-155 cm; regarding the body weight of cows the limits are 800-1000 kg and 1100-1300 kg in bulls. Also, the birth weight of calves is higher than those of the Aberdeen Angus breed, which directly leads to higher weight gains.

The higher performance achieved on the farm 5 is due to the farmer's special interest in improving the number of beef cattle he owns and also due to the fact that the farmer has higher education, the field of specialization: veterinary medicine. Thus, breeding on farm 5 is focused on the main sources of genetic progress (breeding bulls, selection).

The food that is administered to the cattle in farm 5 it is well balanced in energy and protein, this being another important factor that led to the best results in this husbandry operation.

That is why it is necessary to specialize the workforce, as to increase the level of knowledge and skills of those who work directly in production for the following activities:

• establishing diets according to body weight, age of animals, productive level for beef cattle according to the average daily gain;
• performing artificial insemination, thus reducing production costs by paying labor on one hand, and on the other hand the cow insemination can be done with tested and breeding bulls, which is the first and most important source of genetic progress in populations of beef cattle.

However, if a study is compared to the data from the literature with reference to the productive performance of the Charolaise breed, it is revealed that the average daily gain obtained on farm 5 is lower than these data.

This is partly due to the extensive farming system applied in this farm.

From this point of view, there is a need to concentrate, specialize, and optimize the size of beef cattle breeding units so as to introduce modern technologies (industrial type) in order to organize beef meat production, leading to increased profitability of this kind of farms.

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QUANTITATIVE AND QUALITATIVE VARIATION OF SAANEN GOAT MILK KEEPED IN EXTENDED LACTATION FOR TWO YEARS

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Abstract

Saanen goats, specialized in milk production, improved in terms of quantity and quality (protein and fat content), are normally milked for 270-300 days with an annual production of 650-1050 l of milk / lactation and an average protein content of 2.9% and fat content of 3.2%. This study was conducted in the first goat farm in Romania that initiated prolonged lactation of goats. The research was carried out on a batch of primiparous goats, maintained in an intensive exploitation system, which after the first calving were subdivided according to milk production measured for a week. Two batches of 128 goats with a production of 3-4 liters/day and 94 goats with a production of over 4 liters/day were established. By modulating the diet and the light regime, the goats were milked continuously for 690 to 742 days, monitoring the amount of milk, protein and lipid levels on a monthly basis. Along with controlled breeding programs, prolonged lactation contributes to ensuring on the domestic market a continuity in the supply of goat's milk, throughout the year.

Key words*: goats, milk quantity, prolonged lactation.*

INTRODUCTION

The increasing consumer interest in goat milk and milk products has led to the development of the goat milk system and of the goat milk processing industry, as well as the modulation of goat farming systems, seasonal breeding animals, in order to ensure a constant milk production all year round. The demand for goat's milk has also increased in our country, which has directed research on selection, improvement, reproduction and exploitation systems of goats in order to make the species profitable (Osman, 2019; Sava et al., 2016).

The main ways to maximize the productive potential adopted by goat breeders in our country were: import of specialized goat breeds for milk production and raised in purebred, crossbreeding between local goats, Carpathian or Alba de Banat with import males of specialized breeds for milk (Anghel et al., 2017, 2020), application of selection and improvement of local breeds programs by adhering to the breeding programs of racial genealogical herdbook and adopting the goat breeding and maintenance systems to allow the expression of genetic value.

Among the specialized breeds for milk production, the Saanen and French Alpine goats have been imported in large numbers into our country, so that 6 farms have over 350 goat heads, according to data held by the ANCC Caprirom Genealogical Register (Caprirom, 2020). These goat breeds record with specific breeding technology, milk production of over 700 litres in 280 days of lactation, respectively an average of 946 litres of milk in 306 days lactation to Saanen breed (Capgene, 2013a) and 886 litres of milk in 295 days lactation in the French Alpine breed, in the countries of origin. (Capgene, 2013b).

Regardless of the breed structure of the goat herd, an important role in the improvement of the goats' exploitation is played by the exploitation system, respectively: ensuring sufficient and balanced rations in relation to physiological needs related to gestation, rank and level of lactation, precocity and frequency of milking. (Assan, 2014) ensuring a constant feeding ration (Legarto et al., 2014; Lefrileux et al., 2009), ensuring an age-appropriate thermal and humidity regime, ensuring appropriate housing conditions and animal density so as to avoid the stress of overcrowding. These factors

also influence the quality of milk, the rhythm and frequency of milking, the type of feeding as a composition of rations but also as a mode of administration influence the percentage of fat and protein in milk (Murney et al., 2015).

Milk production is conditioned by the external factors mentioned but also by genetic determination and individual reactivity. There are operating systems in which milking is practiced 3 times a day, obtaining a production increase of up to 10% (Lacasse et al., 2011). This method is little used, being economically inefficient in relation to the personnel, water and electricity expenses that the additional milking entails.

In order to obtain a high milk production, in addition to the genetic factor that dictates the productive level, it is possible to resort to an increase of the milking period by the early or very early weaning of the kids, immediately after calving, thus gaining 45-60 days of lactation. (IDELE, 2015).

One method of obtaining a larger quantity of milk from a lactating goat is the application of protocols for lactation maintenance for a period longer than 310 days, respectively over 450-500 days, without the females being given to the reproduction. The category of long lactations does not include females who continue their lactation between 2 seasons but who gave birth and did not have a period of 2 months of breast rest (IDELE, 2013).

This study shows the way about rentability the goat milk production in the great farms without increase the number the goats.

MATERIALS AND METHODS

The study was conducted in a farm with a herd of 1800 genetically selected Saanen dairy goats, raised in an intensive system, in permanent free housing. The females were subdivided according to the rank of lactation, physiological state and milk production, all of them having a balanced and differentiated diet according to the three subdivision criteria. The farm used mechanical milking with an automated milking station, provided with Daily milk management which records the individual quantity of milk. The farm being correlated with the dairy factory, due to economic considerations, respectively the increased need for milk during the winter, as well as the insufficient spaces for sheltering the kids forced the adoption of the specific technology of prolonged lactation. Moreover, for Saanen goats specialized for milk production, a major problem encountered on the farm was the weaning of pregnant goats in the 3rd or 4th month of gestation, respectively at 270-310 days of lactation. For the production of prolonged lactation, only animals at the first lactation were chosen, with an average daily production of over 3 litres of milk. Milking was practiced twice a day, at 6 and 18 o'clock, the batches of goats in prolonged lactation being the first at the milking station.

Due to the increased need for milk on the farm during the winter, in previous years the counter season reproduction was practiced. Thus, the goats included in this study gave birth in December. The technology of raising the kids involves very-early weaning, so the kids are not breastfed by the goats, they receive the colostrum in the bottle. The goats are milked separately from 3 to maximum 10 days (colostrum milk) after which they are milked at the milking station, the milk being distributed to the dairy factory for human consumption.

In order to establish the productions and carry out the batches, we chose a to have a milk production control day every month.

Out of a total of 587 Saanen primiparous goats, we identified 257 goats, with a production of over 3 litres of milk/day, that entered the extended lactation program. Of these, 222 goats were the subject of the present study, completing lactation at the end of the control period. Thus, the quantity of milk in June was taken as a reference, before the beginning of the normal breeding season which could have influenced the quantity of milk. The selected goats were kept separately, in the youth stall that was not intended for breeding, in order not to come into physical and sensory contact with the males during the breeding. The two batches were set up: first batch of 128 goats with a daily milk production between 3 and 4 litres and the second batch of 94 goats with more than 4 litres of milk/ day. The goats were kept separately in stalls of 64 goats (batch 1) and 98 goats (batch 2) ensuring the stable surface as well as the optimal feeding area.

The rations consisted of concentrated granulated feed for lactating goats (produced in the own

concentrated feed factory based on a Dutch recipe) in the amount of 2.8 kg of feed/ head/day for goats with 3-4 litres of milk/day and 3.2 kg of feed/head/ day for goats with more than 4 litres of milk/day. In addition, 0.8 kg of alfalfa granules and oat straw at discretion were administered. This structure of the ration did not change throughout the prolonged lactation, only the quantities from one month to another being adjusted, in relation to the quantity of milk registered, respectively with the increase or decrease of the daily production.

Another aspect that was taken into account during this study was the maintenance of a constant photoperiod regime, so that the hormonal level of the hypothalamic-pituitary-ovarian axis and implicitly the prolactin level not to be epiphyseal influenced (Chemineau et al., 2007; Tong et al., 2018).

Following the analysis of the evolution of milk production in Saanen goats, by estimating the average production, the standard deviation (STDEV) from the average and establishing the minimum and maximum values by statistical analysis in Excel, we proceeded to establish the reference periods that can provide data on the effectiveness of the application of this technology in obtaining prolonged lactation. The four periods analysed were: the period January-June when the study batches were set up, the period of 300 days (January-October) as a reference for milk production in the conditions in which these goats were mounted, the period of 450 days starting November until the weaning of all the goats studied and the entire study period of 2 years.

During the quantitative monitoring, the monthly qualitative monitoring of the collection milk was also performed. The batches of goats kept in prolonged lactation are milked first on the milking station and after they have been milked, a sample of milk was collected from the cooling tank provided with a stirrer, before entering on the milking station and the other goats that are not the subject of the study. The protein and fat levels in the milk were determined with the LACTOSCAN milk analyser.

RESULTS AND DISCUSSIONS

The variation of the quantity of milk in batch 1 differed during the 2 years, respectively 106 weeks of study, the differences being also between the 2 followed batches.

Table 1. Daily evolution of milk production in Saanen goats with production of 3-4 kg milk/head/day in first lactating year (batch 1: n = 128).

Month	Refe-rence time	Average produc-tion	STDEV	Min.	Max.
Jan	CD*	3.491	0.271	3.020	3.982
Febr	CD	3.498	0.291	2.965	4.194
Mar	CD	3.539	0.289	3.018	4.251
Apr	CD	3.508	0.277	3.011	4.082
May	CD	3.495	0.293	2.918	4.214
June	CD	3.533	0.291	3.031	4.204
July	CD	3.524	0.295	3.019	4.280
Aug	CD	3.194	0.306	2.363	3.934
Sept	CD	3.071	0.346	2.376	3.919
Oct	CD	3.030	0.375	2.140	3.965

CD* - control day

During the first 10 months corresponding to physiological lactation for batch 1, recorded the milk production from the control day and the average daily batch production for the control months were calculated (Table 1). Thus, it is observed that the average daily production was between 3.030 +/- 0.375 kg/head/day (October of the first year) and 3.539+/- 0.289 kg/head/ day (March of the first year). The minimum production recorded during this period was 2.140 kg milk/ head/ day and the maximum was 4.280 kg milk/head/day given according to the characteristics of the breed in Saanen goats (Capgene, 2013a).

Table 2 shows the same parameters corresponding to the next 14 months of study. As shown in the data presented in the tables, the prolonged lactation in this case can be structured in two phases: the first 7 months with production close to the normal physiological lactation production followed by another 7 months in which the downward curve of lactation and weaning of goats is observed.

In the first 7 months of prolonged lactation in batch 1, respectively the interval from 300 days to 510 days, the average daily production of goats was between 2.060+/- 0.410 kg/head/ day and 2.968 +/- 0.436 kg/head/ day with extreme individual productions of 1.047 and 3.931 kg/ head/day.

In the next 7 months (510-742 days), respectively from June of the second year the milk production registered appreciable decreases, the average production being

comprised between 1.878 +/- 0.379 and 0.044 +/- 0.014 kg/head/day.

Table 2. Daily evolution of milk production in Saanen goats with production of 3-4 kg milk/head/day maintained in prolonged lactation (batch 1: n = 128)

Month	Refe-rence time.	Average production	STDEV	Min.	Max.
Nov	CD*	2.968	0.436	1.880	3.931
Dec	CD	2.881	0.407	1.925	3.811
Jan	CD	2.717	0.369	1.895	3.702
Febr	CD	2.563	0.378	1.652	3.495
Mar	CD	2.405	0.378	1.409	3.299
Apr	CD	2.223	0.467	1.073	3.126
May	CD	2.060	0.410	1.047	3.041
June	CD	1.878	0.379	0.967	2.957
July	CD	1.763	0.415	0.734	3.024
Aug	CD	1.454	0.468	0.263	2.524
Sept	CD	1.082	0.546	0	2.334
Oct	CD	0.699	0.528	0	1.806
Nov	CD	0.274	0.037	0	1.300
Dec	CD	0.044	0.014	0	0.777

CD* - control day

Starting with August, they weaned 4 goats, in September 26 goats and in October 80 goats, a total of 14 weanings in November.

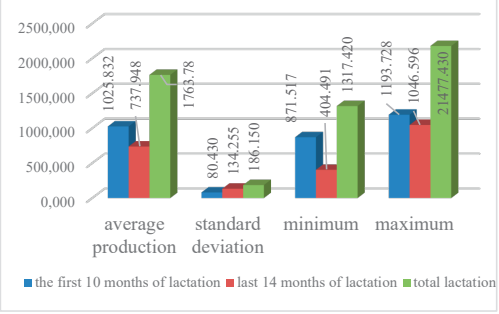

Figure 1. The ratio between the production during the normal lactation period and the additional lactation in goat batch 1

The analysis of the individual evolution of milk production during the 104 weeks (2 years) shows that the average production of the batch is stabilized for the first 7 months, after which a decrease of up to 200 g is observed from one month to another, until February-March in the second year of lactation. After week 83 of milking (approximately 600 days of lactation) there is a major decline in milk production.
The total production of each goat was calculated by multiplying the quantity on the control day by the number of days in that month.

Figure 1 shows the contribution brought by the additional milking period, respectively an average of 737.948 +/- 134.255 kg of additional milk/head/lactation (41.83%).
Table 3 shows the evolution of milk production in goats from batch 2, which in May and June recorded daily quantities of over 4 kg of milk/head/day. The initially selected batch was of 102 goats, of which the 94 we refer to in this study ended the prolonged lactation.

Table 3. Daily evolution of milk production in Saanen goats with a production of more than 4 kg milk/head/day, in first lactating year (Batch 2: n = 94)

Month	Refe-rence time	Average produc-tion	STDEV	Min.	Max.
Jan	CD*	4.794	0.762	4.017	9.600
Febr	CD	4.825	0.786	3.973	9.722
Mar	CD	4.857	0.795	3.943	8.906
Apr	CD	4.847	0.768	4.023	8.772
May	CD	4.818	0.727	3.930	8.292
June	CD	4.814	0.694	4.028	8.303
July	CD	4.756	0.673	4.016	8.199
Aug	CD	4.564	0.706	3.422	7.959
Sept	CD	4.367	0.723	3.129	7.705
Oct	CD	4.221	0.706	2.880	7.309

CD* - control day

Batch 2 of primipara goats, Saanen breed, during the period of 300 days of lactation recorded average daily values milk production between 4.221 +/- 0.706 (October) and 4.857 +/- 0.795 kg/head/day (Mars). The maximum value of daily production measured on the control day was 9.772 kg/head/day and the minimum recorded was 2.880 kg/head/day.

Table 4. Daily evolution of milk production in Saanen goats with a production of more than 4 kg milk/head/day, maintained in prolonged lactation (Batch 2: n = 94)

Month	Refe-rence time	Average produc-tion	STDEV	Min.	Max.
Nov	CD*	4.063	0.676	2.850	7.087
Dec	CD	3,892	0.712	2.476	6.783
Jan	CD	3.661	0.692	2.140	6.141
Febr	CD	3.496	0.672	2.028	5.430
Mar	CD	3.277	0.658	2.024	4.924
Apr	CD	3.067	0.705	1.240	5.049
May	CD	2.757	0.783	0	4.526
June	CD	2.522	0.805	0	4.308
July	CD	2.234	0.830	0	3.885
Aug	CD	1.934	0.889	0	3.788
Sept	CD	1.585	0.876	0	3.482
Oct	CD	1.192	0.817	0	2.879
Nov	CD	0.692	0.690	0	2.434
Dec	CD	0.297	0.447	0	1.857

CD* - control day

Table 4 shows the values of average daily and monthly milk production during the period of prolonged lactation.

As in the case of the first batch, a decrease in milk production is observed, remaining at values of over 2 kg/head/day for over 9 months, ranging from 4.063 +/-0.676 to 2.234 +/-0.830 kg/head/day. The maximum quantity of milk obtained during this period was 7.087 kg/head/day. In the last 5 months of lactation, the downward curve of milk production is obvious, the rate of decrease of production being progressive and higher than in the case of batch 1.

The end of lactation for the batch of goats with yield over 4 kg/day occurred earlier, the first three weaned goats being registered at 17-19 months of lactation. At 20 and 22 months of lactation (580-640 days) they weaned 10 more goats and at 670-710 days of lactation 47 goats. In the interval of 710-742 days, they weaned 31 goats.

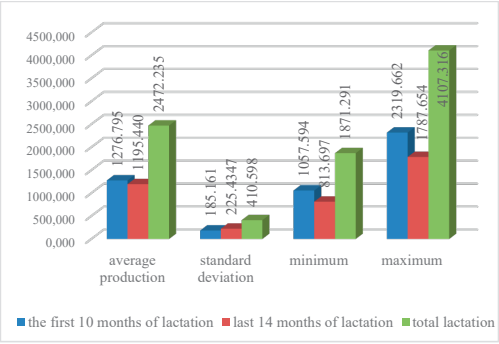

Figure 2. The ratio between production during the normal lactation period and additional lactation in goats batch 2

All the goats from batch 1 and 2 starting with July of the second year of lactation were reintroduced in the reproductive circuit, proceeding to detect the daily estrous. The goats that showed estrous, were mounted naturally starting with August. Goats that did not show estrous in the second year and had milk production were monitored until the final weaning, during which time the ration was adjusted in relation to the milk production of 1-2 kg milk/head/day, respectively he decreased at 1.4-1.6 kg granular concentrates/head/day.

By graphically representing the total milk production divided into periods, respectively the natural physiological lactation interval and the prolonged lactation interval by modulating the rations and the photoperiod, the productive contribution of the applied technology is observed.

Thus, from the average total production/goat of 2472.235 +/- 410.598 kg of milk obtained, 1276.795 +/- 185.161 kg represents the milk corresponding to the normal physiological lactation (51.65% of the total production/goat/lactation season) and 1195.440 +/- 225.437 kg (48.35%) is the extra milk obtained by keeping the goats on prolonged lactation (Figure 2).

During the quantitative monitoring of the milk, have been constantly checked the qualitative analysis bulletins provided by the laboratory of the dairy factory, respectively the protein and lipid level of the collection milk (common test for the 2 study batches) of all the goats kept in prolonged lactation.

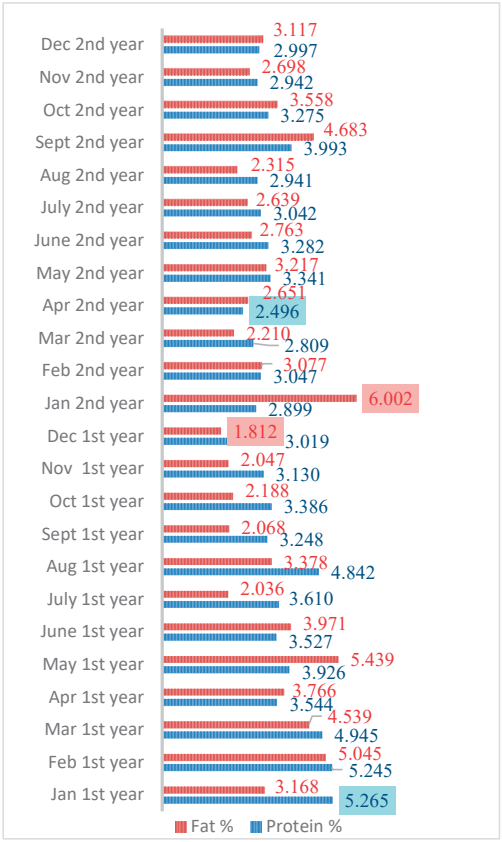

Figure 3: The percentage evolution of protein in milk collected during prolonged lactation

The percentage of protein during the whole lactation registered an average value of 3.531% +/- 0.785 with a minimum of 2.496 and a maximum of 5.265. The fat percentage ranged from 1.812% to 6.002%, with an average of 3.266% +/- 1.161 over the entire monitored period (Figure 3).

As can be seen from Figure 3, the critical periods were in January and July - December of the first year of control when due to the hyperprotein ration not properly correlated with the energy needs of goats, a slight metabolic acidosis appeared with clinical digestive manifestations.

In January of the second year, 80% of the study population showed digestive dysfunctions associated with nerve forms. Milk analysis and blood tests revealed the condition of metabolic alkalosis in the whole group. Both acidosis and metabolic alkalosis have been treated with conservative methods based on mineral supplements and phytotherapy.

CONCLUSIONS

The embrace of a specific technology for primipara Saanen goats to maintain lactation for as long as possible without breeding has resulted in obtaining a lactation of up to 23 months of which 17-18 months with an average production of over 2 kg/head/day. The quantitative intake during the additional lactation period was 41.83-48.35% extra milk.

The application of constant rations is one of the factors that have maintained lactation for over 600 days, but metabolic risks require detailed studies to make a correlation between the protein level administered and the energy required to ensure milk production.

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A STUDY OF THE INFLUENCE OF ENVIRONMENTAL FACTORS AND THE PREVALENCE OF PASTEURELLOSIS IN RABBITS

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Abstract

We studied the influence of environmental factors on the prevalence and clinical manifestations of pasteurellosis in rabbits of the California and New Zealand breeds over a period of one year. It was found that the minimum temperatures in winter and the maximum in summer were out of the optimal values: 4-7 and 16-18^{O}C, respectively. The relative humidity during most of the year was out of the recommended levels / 60-70% /, both at the average and at the maximum measured values. The concentration of ammonia exceeded the average values by about 16 mg / m³, and the maximum values by about 30 mg / m³. The dust content varied from 0.9 to 6 mg / m³ with average values of 3.6 mg / m³. The total number of isolated microflora in m³ air was 6.83 x 10³ (6.5-7.3 x 10³).Dominant in the biological material and washes / from cages, walls and inventory / were the representatives of Salmonella, Staphylococcus, Streptococcus, Pasteurella and Colibacteria; and from the molds Aspergillus flavus, A. fumigatus, Penicillium, Alternaria and Mucor. Antibodies against Pasteurella multocida were found in 60% of the population. Exacerbation of the disease and higher mortality was observed during pregnancy and during the suckling period. The main complications were - respiratory disorders (51%) followed by inflammation of the conjunctiva. About a quarter of the affected animals (26.4%) suffered from the mixed form of the disease. We found that Inflammation of the ears was extremely rare (0.2%). A relationship has been established between the number of environmental factors and the incidence of respiratory diseases. We can conclude that environmental factors are actively involved in the development, form and severity of pasteurellosis in rabbits.

Key words*: environmental factors, pasteurellosis, rabbit.*

INTRODUCTION

Rabbits are considered competitors to the other farm animals due to their rapid economic maturity, high fertility and meat of high dietary qualities (Zdanovich et al., 2020; Trubchaninova & Kapustin, 2014). The hygiene has a key role in the successful breeding of rabbits indoors, especially in the case of the intensive technologies.

According to Kumar et al. (2008) the rabbit buildings design is based on the behavioral features of the animals and their reactions to the environmental parameters. The relationships between the temperature, the air velocity and the relative humidity in the premises are the major microclimatic factors which have an influence on the rabbits. (Ogunjimi et al., 2008).

The air pollutants originate from the animals themselves as well as from their feces, feeds and bedding. The process of urine and feces degradation is accelerated at high temperatures (van Praag et al., 2010), and this permeates the air with NH3 and H2S.

Pathogenic bacteria, viruses and fungi are found in the air very often and this leads to health risks for both the animals and the staff (El-Raffa, 2004). The author proposes a hygiene program for breeding of rabbits to be elaborated and implemented, which could be an effective solution ensuring the protection of their health and productivity.

Another problem experienced in rabbit breeding is the prevention of the conditionally pathogenic infections. Their most common representative is *P. multicida* which exhibits a marked tropism towards the respiratory, nervous and reproductive systems (Kluger & Vaughn, 1978) and causes rhinitis, otitis, enzootic pneumonia, conjunctivitis, pyometra, orchitis, subcutaneous abscesses, septicaemias (Flatt, 1974). Data of Morisse (1981), Dincheva & Hristev (1983) show that the agent is isolated in the upper respiratory tract of 46-72% of the rabbits examined. According to Morisse (1981), the respiratory disorders are directly or indirectly responsible for 70% of the economic losses in a herd of meat rabbits. The bad

rearing conditions are the main reason for the contraction and the spread of the disease. The air features which play a key role are the temperature regime, the air humidity, airflow, lighting and the toxic gasses concentration. In general, the rabbits adapt well to the different factors of the barn environment and retain their normal physiological functions.

The aim of the present study is to examine the extent to which the barn environment conditions are responsible for the spread and the clinical manifestation of pasteurellosis in a rabbit farm inhabited by rabbits of the California and New Zealand breeds.

MATERIALS AND METHODS

Subject to the present study were two brick buildings (5 x 9 m) with 50 metal cages each, which were placed in two rows on two levels. The windows were installed on one of the side walls and had a total area of 8.4 m². The number of the animals controlled in the course of one year was 1150. The cages on the first level were at a height of 0.8 m above the floor. The feeding was with a ready-mixed standard feeds. The floor cleaning was dry, 1-2 times a week. The ventilation was natural. The natural light coefficient for the cages on level one was 3, and on level 2-4%.

The tests were carried out once a month around 10-11 o'clock on the level of the cages. Subject to controlling activities were the following: the air temperature- with a manual multi-functional Compact infrared thermometer 105518 with a scope from 50 to + 550ºC and a resolution of 0.1ºC, the relative humidity- with an aspiration psychrometer by Assmann, the air velocity (m/s)- with a catathermometer, the lighting level- with a lux meter PU 150 PRAHA, the ammonia- by means of the titrimetric method with sulfuric acid, and the carbon dioxide- with 'Higitest' test tubes. The air samples were collected following the Matusevich method, and the microflora was differentiated by means of different selective culture media. The dust was evaluated through the Koch's sedimentation method (Hristev, 2008). Apart from air samples, washes and biological material were also used for the bacteriological tests. Immunodiffusion reaction was used for the detection of antibodies in the blood serums. The reaction antigens were provided by NDRVMI Sofia. The blood samples were obtained from the ear vein. The results were processed variationally.

RESULTS AND DISCUSSIONS

Disturbances of the microclimate such as excess or lack of heat, excess humidity, increase of the harmful gases and microorganisms in the air are all reasons for reduction of the resistance which in turn leads to rapid spread of different infections.

According to Regulation 44 (2006), temperature values between 16 and 25°C and humidity between 65 and 70% are considered optimal with reference to rabbits. The average temperatures during our studies (Table 1) were within the accepted range. However, the minimum temperatures, mainly in winter, as well as the maximum ones during the summer exceeded these reference values by 4-7°C, on average. If we consider that the optimal temperature for rabbit breeding is 16-18°C (Pomytko, 1982; Morisse, 1981), then the difference between it and the maximum values reported increases up to 11-12°C. These differences increased mainly during the summer and, according to Dermendzhieva et al. (2017), Ogunjimi et al. (2008), the rabbits were under the conditions of temperature stress.

The heat stress during the summer affects negatively the development of the growing rabbits, their reproductive functions and reduces their resistance to diseases (Marai et al., 2002). The heat stress affects the fertility of the female rabbits as well as the embryonic development and the weight of the embryos. The milk secretion of the mothers is reduced while the mortality in newborns is increased. It is possible that a temporary coital and fertile impotence be observed in male rabbits (Kumar et al., 2008). The construction materials of the renovated conventional building in which the rabbits controlled by us were reared were, in all likelihood, with good heat technology qualities and, therefore, neither very low or below-zero centigrades during the winter, nor very hot ones during the summer were registered.

The relative humidity during most of the year was beyond the recommended references values- 60-70% both with reference to the average and the maximum values reported. If

we consider the reference values of 60-80% cited by Kumar et al. (2008) as the norm, then it could be concluded that the humidity was more often within the accepted parameters than not. The relative humidity exceeded the recommended reference values especially during the winter period and partly during the spring and the autumn. The lack of artificial ventilation impeded the air circulation during the cold days which led to trapping of the excess humidity and a lower temperature. The highest humidity and, at the same time, the lowest air velocity (0.17 m/s) were measured from the end of the working day until the morning of the following day.

Harmful and toxic gases are accumulated as a result of the life activity as well as the feces and urine decomposition processes. Similar conclusion was also reached by Calvet et al. (2011), Da Borso (2016), who also add the absent or misfunctioning ventilation system.

All these set the conditions for the development of intoxication of various degrees as well as decrease of the immunological protection of the rabbits.

During the early morning hours, the ammonia concentration usually exceeded the average values by 16 mg/m³ and the maximum ones- by 30 mg/m³. During our tests, no presence of hydrogen sulfide was ascertained.

The carbon dioxide emitted by the rabbits during breathing as well as a small fraction released during the urine and feces decomposition were within the reference value norms and did not exceed 0.25% even in the maximum values reported.

In older publications it is acceptable that the recommended air velocity in the rabbit buildings exceed the optimal one by 1-2 m/s. However, according to Regulation 44, it must not exceed 0.2 m/s, and the cooling quantity- 5-8 mcal/cm²/s. Kumar et al. (2008) recommend values of 0.3-0.4 m/s at air temperatures from 22 to 25^{O}C. According to Dermendzhieva et al. (2017), the dry cleaning and the high temperatures, especially during the summer, are the main reason for the rapid organic waste decomposition during which the ammonia, accompanied by the hydrogen sulfide, exceed the recommended hygiene norms. The dust content varies in quite wide ranges: from 0.9 to 6 mg/m³ at average values of 3.6 mg/m³. If we take the acceptable indicative dust content in the buildings for most types of animals (0.5-4 mg/m³) into account, then it was within the hygiene norms.

The total number of the microflora isolated in a m³ of air was 6.83 x 10³ (6.5-7.3 x 10³); 6-7 thousand of them were microorganisms and the rest were molds. The representatives of *Salmonella*, *Staphylococcus*, *Streptococcus*, *Pasteurella*, coliform were dominant in the biological material and the washes (from cages, walls and inventory), and the *Aspergillus* (*flavus*, *fumigatus*), *Penicillium*, *Alternaria* and *Mucor* in the molds. Presence of antibodies against *Pasteurella multocida* was detected in 60% of the population.

Table 1. Minimum, average and maximum values of the environmental factors examined

Environmental factors	Minimum	Average	Maximum
Temperature, °C	9.1	20.5±3.6	29.2
Relative humidity, %	62	78.3±8.5	91
Air velocity, m/s	0.17	0.56±0.3	1.8
Cooling value, mj/cm²/s	6.25	9.3±1.2	14.3
Ammonia, mg/m³	32	36.2±2.8	50
Carbon dioxide, vol. %	0.05	0.11±0.04	0.18
Microflora, thousand/m³	6.5 x 10³	6.83±0.5	7.3 x 10³
Dust, mg/m³	0.9	3.6±0.7	6

Having analyzed all data available, we consider that the barn environment factors on one hand, and the age, physiological condition and the overall resistance of the animals, on the other, determine the contraction and the form of display of the pasteurellosis (Table 2). Exacerbation of the disease and a higher mortality rate was observed during pregnancy and the suckling period. The respiratory disorders had the biggest share (51%) followed by inflammation of the conjunctiva. A quarter of the animals affected (26.4%) suffered from

the mixed form. The ear inflammation was the rarest condition (0.2%).

Table 2. Clinical forms of pasteurellosis manifestation

Form of manifestation	n	%
Respiratory disorders with clinical manifestation, number	586	51
Pneumonia	103	17.6
Conjunctivitis	224	38.2
inflammation of the ears	1	0.2
Subcutaneous abscesses	103	17.6
Mixed form	155	26.4

Note: n - Number of animals

The constructive and technological shortcomings of the reconstructed building were the reason for the dynamics in the temperature-humidity regime.

The random opening or closing of the door and the windows led to non-rhythmic circulation and velocity of the air (Figure 1).

The air flow was the most active from the side of the open windows and it gradually dwindled towards the opposite side of the building i.e. there were both 'turbulence' and 'dead' zones at hand.

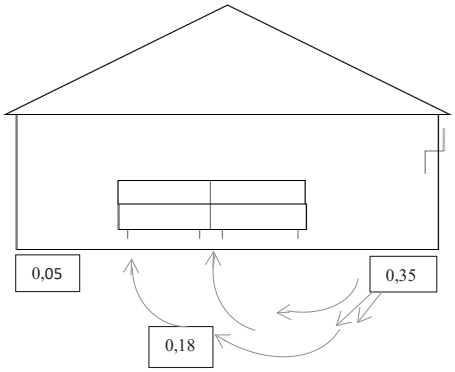

Figure 1. Cross-section of the air flow

This led to periods of increase of the toxic gases concentration. During an experiment exposing rabbits to ammonia, Morisse (1987) ascertains that the mucosa of their respiratory tracts becomes vulnerable and easily penetrable by the *Pasteurella* and other microorganisms on it. In another study (1981), the author proves that the air velocity is another environmental element involved in the contraction of the respiratory syndrome.

The more elements of the barn environment are beyond their acceptable optimum, the more often a respiratory pathology is registered (Table 3).

Table 3. Relative share of the rabbits which contracted pasteurellosis, depending on the involvement of major factors of the barn environment

Influencing factor	Relative share of sick animals, %
1, 2	20.7
2, 4	26.4
2, 3, 4	34.1
1, 2, 4	38.0
1, 2, 3, 4	51.0

Legend: 1 - ammonia; 2 - relative humidity of the air; 3 - air flow; 4 - temperature.

Taking all these into account, we can conclude that the contraction, form of manifestation and severity of the pasteurellosis in rabbits is largely dependent on the factors of the environment.

CONCLUSIONS

The minimum temperatures in the winter and the maximum ones during the summer in the buildings for rabbits studied by us were beyond the optimal values by 4-7 and 16-18^{O}C respectively. The relative humidity during most of the year exceeded the recommended hygiene norms (60-70%) both for the average and the maximum values reported. The concentration of ammonia exceeded the average values by around 16 mg/ m³ and the maximum ones- by 30 mg/m³. The content of dust varied from 0.9 to 6 mg/m³ at average values of 3.6 mg/m³. The total number of the microflora isolated in m³ of air was 6.83 x 10^3 (6.5-7.3 x 10^3). *Salmonella*, *Staphylococcus*, *Streptococcus*, *Pasteurella* and coliform as well as the molds *Aspergillus* (*flavus*, *fumigatus*), *Penicillium*, *Alternaria* and

Mucor were isolated in the biological material and the washes (from cages, walls and inventory). Antibodies against *Pasteurella multocida* were detected in 60% of the population. Exacerbation of the disease and higher mortality were observed during pregnancy and the suckling period. The respiratory disorders took up 51%, followed by inflammation of the conjunctiva. Slightly more than a quarter of the animals affected (26.4%) contracted the mixed form. Inflammation of the ears was extremely rare (0.2%). A relationship has been established between number of the non-optimal environmental factors and the incidence of pasteurellosis. A complex of ecological factors such as the concentration of ammonia, the air flow, humidity and temperature in the production premises played an active role in the contraction of the disease, the form of manifestation and its severity as well as in the pasteurellosis carriage in rabbits. The dust and the composition of the microflora in the air may be supplementary but not major etiological factors for the contraction of the disease.

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INFLUENCE OF CERTAIN ENVIRONMENTAL FACTORS ON BASIC PHYSIOLOGICAL, HEMATOLOGICAL AND BLOOD CELL PARAMETERS IN FREE-RANGE DAIRY COWS

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Abstract

We studied the influence of the temperature-humidity index (THI) on some basic physiological, hematological and blood cell parameters in free-range dairy cows from three cattle farms with different capacity and breeding techniques for a period of one year. Weak to significant variations in the hematological parameters and indices were found in connection with changes in THI. Despite the reported high THI during the light part of the summer days, the animals retain their immune protection: respiration increased by 10-13%, but the heart rate and body temperature remained within the upper reference range, the erythrocytes increased by 7-8%, the leukocytes by 6%, hemoglobin by 10-15% as well as the eosinophils and the monocytes. The values of the blood cell index (BCI), the lymphocyto-granulocyte index (LGI) and the neutrophil-lymphocyte index (NLI) also increased. Almost all studied physiological parameters, blood cell types and indices were dependent on the season and the type of farm - $P < 0.05$ - $P < 0.001$.

Key words: *basic physiological, hematological and blood cell parameters, dairy cows, temperature-humidity index.*

INTRODUCTION

The maintenance of temperature tolerance is of vital importance for the comfort of the cows (West et al., 2003). However, the high temperatures during the summer, even those during the transitional periods, disturb this temperature comfort as well as the well-being of the highly productive cows. The resulting disorders in the thermoregulation of the body lead to changes in the physiological processes, the behaviour and also to milk secretion disruptions (Kekana et al., 2018; Kim et al., 2018; Sejian et al., 2018; Mylostyvyi & Chernenko, 2019; Ouellet et al., 2021).

The heat exchange between the animal organism and the environment is a complex process aimed at maintaining the temperature homeostasis. The processes related to it depend on both a range of biological and many physical factors. Different physical and biological coefficients for their evaluation have been developed and introduced in an effort to minimize the complexity of the respective processes (Jendritzky et al., 2002). Thom (1959) index adapted for cattle is most often used for the assessment of the physical factors of the environment. Based on this index assessment of the environment, the effect i.e. the biological response of the animal is sought so as a conclusion to be made for the comfort, health, productivity and well-being (Hahn et al., 2009).

Physiological and biochemical indicators are most commonly used for the ascertainment of the degree and the nature of the biological response. The blood parameters are one of the most sensitive and fastest-reacting ones when it comes to changes in the cells and the organs in the event of a disease or a stress situation. These changes occur a lot earlier than the manifestation of the first clinical signs. Therefore, according to Mazzullo et al. (2014), Wood & Quiroz- Rocha (2010) their analysis is a suitable indicator for the organism response assessment.

Evtimov & Konstantinov (1968) also consider that the fastest response regarding the effect of the external factors on the animals may be obtained by taking the interior indicators. The publications of other authors (Mazzullo et al., 2014, Wood & Quiroz-Rocha, 2010) also fully support the above mentioned. According to Chulichkova (2017), some blood cell indexes can be used far less frequently for now.

Taking all these into account, we set ourselves the goal to monitor the reactions of dairy cows during winter, summer and transitional periods

by using some interior and blood cell parameters.

MATERIALS AND METHODS

The studies were carried out in the course of one year in three cattle breeding farms with a different capacity. The breeding technology in two of them was free in individual boxes, and in the third one- in groups on deep litter bedding.

The cows in the first farms were reared in open buildings with metal construction and a thermo-panel roof. Each animal was provided space of 9.4 m². The area of the individual boxes was 1.25/2.20 m. The floor was cement and in the boxes, it was covered with a solid rubber mat. The chest rails were made of planks. The open spaces providing natural light in each building had a total area of 170 m². The mechanical ventilation was regulated automatically with all devices turning on when the temperature was above 25ºC. The manure cleaning was performed via delta scraper device starting at an interval of 6 hours. The feeding was unlimited with a total mixed ration and a permanent access to water. The milking was performed twice a day in a 'herring bone' milking hall 2 x 12, equipped with herd management software product.

The animals in the second farm were reared in reinforced concrete buildings with concrete walls and roof panels. The side windows and the ridge vents were covered with polyethylene sheets during the winter period. Each cow was provided with space of 11.5 m². The area of the individual boxes was 1.10/2.10 m. The floor of the building was cement and in the boxes, it was covered with a soft rubber mat. The feeding was in the morning and in the evening through a mechanical mixer. The manure cleaning was performed via delta scraper device every three hours. Nipple drinkers were used for watering. The milking was performed twice a day in a 'herring bone' hall 2 x 8.

The cows in the third farm were reared on deep litter bedding in a semi-open building with brick walls without inner or outer screed. Each animal was provided space of 8.06 m². The open areas of the building ensured natural ventilation similar to the tunnel type. The additional mechanical ventilation was turned on gradually at temperatures over 18 and 25ºC. The feeding was unlimited with a total mixed ration and a permanent access to water. The manure cleaning was performed twice a year with a periodic addition of hay. The milking was done twice a day in 'DeLaval' hall 2 x 5.

All microclimatic parameters in the buildings as well as the physiological indicators of the animals were measured at 14.00 h in the course of three days every month. The temperature and the relative humidity in the controlled buildings were measured with an aspiration psychrometer by Assmann. Meanwhile, a weekly thermo-hygrograph reported the diurnal fluctuations of these factors. The temperature-humidity index (THI) was calculated following Kelly and Bond (1971):

$$THI = T - (0.55-0.0055 \times RH) \times (T-58),$$

where:

T is the temperature, ^0F

RH - relative humidity, %

Six animals at the same age and physiological condition were selected from each building. Their blood was sampled with Vacutainer K2E 5-4 mg, REF- 368856. The hemoglobin levels, the number of the red and white blood cells as well as the different classes of the white blood count were reported with an automatic hematology analyzer Dymind D7 CRP. The data regarding the different leukocyte types were used so as to calculate the leukocyte indexes (Chulichkova, 2017).

The body temperature was measured with a Kerbl digital thermometer, model 2130, and the respiratory and pulse frequency - with a chronometer in accordance with the approved propaedeutic methods.

The results were statistically processed via SPSS-21. We used a linear model of the following type:

$$Yijk = \mu+Si+Fj+eijk,$$

where:

Yijk- observation vector, μ- total mean, S and F are fixed effects of respectively i-th season, j-th- farm; eijk- residuals (dispersion).

RESULTS AND DISCUSSIONS

The appropriate temperature and humidity ensuring convenience, comfort, good health condition and productivity of the dairy cows in

accordance with Regulation 44 are at values of respectively 10 and 15°C and 65-75%. Igono et al. (1992) consider temperatures from 24 to 27°C to be upper threshold ones, while Ozhan et al. (2001) reckon that heat stress might be expected when the temperature exceeds 18-20°C. However, the breakpoint might vary depending on the degree of acclimatization, productivity, physiological condition, the air flow and the relative humidity (Mader et al., 2006).

The temperature-humidity index is used for studying the animals tolerance towards the high air temperatures and it also reflects the overall comfort of the dairy cows (Mahdy et al., 2014). 72 is considered to be the limit value of the index; it corresponds to temperature of 25°C and humidity of 50% (Igono et al., 1992; Ravagnolo et al., 2000).

Referring to the data above, the temperature and relative humidity means regarding the buildings studied by us and displayed in Table 1 show that there were longer or shorter periods with unwanted temperature-humidity regime, especially during the summer and the transitional periods. The temperature-humidity index representing the combined effect of the temperature and the humidity of the air followed the same trend. This index reached 85 during the summer period. Generally, it was within its reference values (70-71) during the transitional periods, while in the winter it fell below 50. Rao et al. (2014) comment that even when the temperature and the relative humidity are within the reference values (27°C and 80%), the THI is higher than that considered to be the limit (72). In this sense, we are inclined to agree with the conclusion reached by Vitali et al. (2009) that the upper minimum value of the coefficient should be 77 and the upper maximum - 87.

Table 1. Means of the microclimatic factors in the controlled buildings

	Transitional period			Summer			Winter		
Parameters	Farm 1	Farm 2	Farm 3	Farm 1	Farm 2	Farm 3	Farm 1	Farm 2	Farm 3
Temperature, °C	21.8	22	22.5	27.8	28.2	27.6	5.8	7.1	6.9
Relative humidity, %	68	73	70	79.0	64.8	75	73	85	76
Temperature-humidity index /THI/	69.8	71	71.4	78.3	82.4	85	43.9	46.3	48.6

According to Raushenbah & Erohin (1975) the barn environment and the rearing technology affect the interior parameters of the animals. The data of our studies show that at THI values between 72 and 78, the body temperature was within the maximum reference values (Table 2), while the respiration accelerated by 10-13%. Sabuncuoglu (2004) states that it is considered one of the most sensitive physiological indicators which follow the change of the temperature and the other physical factors of the environment. Thanks to the sensitive respiratory mechanism, the organism homeostasis is retained. The respiratory movements accelerate and so does the blood circulation in the blood vessels. In addition, the pulse is also quickened but remains within the its reference values. More blood is redirected towards the peripheral parts of the body and the lungs, which leads to quick release of the excess heat.

The hemopoiesis accelerated during the summer period, and as a result there was an increase in the erythrocytes number of the animals of the three farms by 7-8%, while in the transitional and the winter periods, their number experienced no significant change. The same was observed with reference to the hemoglobin (10-15%) and the leukocyte number (more than 6%).

Table 2. Physiological and hematological parameters

Parameters	Physiological limits	Farm 1	Farm 2	Farm 3
Body temperature, °C	37.5-38.5	38.42 ± 0.03	38.33 ± 0.04	38.45 ± 0.06
Breathing, n/min	10-30	32.7 ± 0.02	33.8 ± 0.6	33 ± 0.2
Pulse, n/min	32-80	77 ± 2.1	79.3 ± 0.8	76.7 ± 1.3
Erythrocytes x 10^{12}/L	5.85±0.9	7.4±0.2	6.8±0.4	7.3±0.6
Leukocytes x 10^{9}/L	6.15±1.6	9.4±2.1	11.1±2.1	10.6±2.7
Hemoglobin, g/L	89.6±12.8	121±2.1	112±1.9	125±2.1

Unlike the season, the <<farm>> factor influenced the hematological status of the cows to a much lesser extent. Omran et al. (2011) also report increased erythrocytes and hemoglobin in animals subject to heat stress. Habeeb (1987), however, ascertain decrease of the red blood cells considering that this is a result of a thyromolymphatic involution. Apart from a decrease in the erythrocytes, Al-Haidary (2004) reports increase in their volume. According to the author, this is an adaptation mechanism for ensuring the water, necessary for the organism evaporative cooling. Srikandakumar & Johnson (2004) even assume that the erythrocytes decrease may be caused by the free radicals attack against them as well as the insufficient nutrient intake due to the heat stress. The erythrocytes increase probably stems from the accelerated metabolic processes in the organism. Other authors presume that the increase of the blood elements and the hemoglobin is a result of the developing dehydration (Mirzadeh et al., 2010).

The leukocytes, which according to Nenashev & Bikteev (2008) vary widely, provide abundant information about the blood. The leukocyte increase ascertained by us was relatively high (more than 53%) when compared to that indicated by Abdel-Samee (1987) - 21-26%. Similar to our trend was reported by Lallawmkimi (2009) with reference to pregnant and suckler buffalo cows. The increase observed during the summer period, however, was not related to a change in the leukocyte groups. The fluctuations registered by us in most of the leukocyte groups were within the reference values (Table 3). This makes us consider that despite the high THI during the summer period, the animals immunological protection was retained. This is also proved by the increasing percentage of the leukocytes, neutrophils and eosinophils taking part in the blood cell parameter formation (BCC).

The eosinophils have various functions but resembling those of the other leukocytes. They are a source of many cytokines and growth factors related to immunomodulation functions of the organism. The slight increase (around 14% on average) of the eosinophils during the hot period ascertained by us is also confirmed by Ciaramella et al. (2005) in buffaloes aged over ten years. According to Mayengbam (2008), the changes in the eosinophil and basophil count caused by thermal exposition (40 and 45°C) are also negligible.

The highest values of the blood cell coefficient (BCC) were reported during the summer period (from 0.98 to 1.08), followed by the winter one (0.83-0.93). It remained the lowest during the transitional period (0.68-0.84). Regardless of the slight fluctuations, the coefficient demonstrated a certain dependence on the season and the type of farm ($p<0.001$). Most probably, this was a result of not only the high and the low temperatures of the barn environment which stimulated the cell immunity, but also of the antigen influence of the growing fetus (Chulichkova, 2017).

Table 3. Means of the blood cell indexes examined in the different farms during the different seasons

№	Parameters	Season 1			Season 2			Season 3			SD
		F1	F2	F3	F1	F2	F3	F1	F2	F3	
1	BCC*, conditional units	0.81	0.68	0.84	1.08	0.89	0.98	0.92	0.83	0.93	0.11
2	RNR*, conditional units	1.1	0.38	1.7	0.58	0.50	0.53	0.49	0.49	0.50	0.41
3	LGI*, conditional units	10.5	13.2	9.7	8.1	10.3	9.4	9.5	10.8	9.6	1.3
4	I neutr/lymph, conditional units	0.94	0.73	1	1.2	0.94	1	1	0.89	1	0.12
5	I lymph/eosin, conditional units	67.1	31.3	88.4	23.3	26.8	25.9	24.3	28.4	25.7	22.2
6	I neutr/eosin, conditional units	63.1	22.8	90	27.9	25.2	26.6	24.7	25.4	25.7	22.4
7	Leukocytes, 10^9/l	7.95	7.2	8.2	11.4	10.9	11.7	9.5	8.2	8.6	1.9
8	Erythrocytes, 10^{12}/l	5.93	6.03	6.07	7.38	7.45	6.93	6.05	5.69	5.53	0.76
9	Hemoglobin, g/l	109	116	108	125	122	121	108	107	109	7.32

10	Neutrophils, %	44.2	38.7	45	50.2	45.3	47.8	44.5	43.2	46.2	5.4
11	Eosinophils, %	0.67	1.67	0.50	1.83	1.83	1.83	1.83	1.67	1.83	0.9
12	Lymphocytes, %	47	53	44	42	48	47	44	48	46	5
13	Monocytes, %	8.2	6.5	10	6	4.5	3.7	6.3	6	5.5	2.1

*Note: *THI - temperature and humidity index; BCC - blood cell count; RNR - reactive neutrophil response; LGI - lymphocyte-granulocyte index; NLI – neutrophil – lymphocyte index; LEI – lymphocyte-eosinophil index; NEI- neutrophil- eosinophil index*

The antimicrobial neutrophil factors might provisionally be subdivided into two groups: components of the mature neutrophil whose quantity depend not on the degree of cell stimulation but on substances which are synthesized in the granulopoiesis process (lysozyme, lactoferrin et al), and substances which are formed or sharply activated upon neutrophil stimulation i.e. their quantity depends on the extent of the cell reaction. Our results showed that the coefficient characterizing the reactive neutrophil response (RNR) had the highest values during the transitional period when the temperature-humidity regime in the barn environment was within relatively acceptable values. Only the cows from building 2 made an exception by indicating lower coefficient values throughout the whole year. Despite that, the season and farm factors affected its values ($p<0.001$).

The animals immunological status maintenance is a result of not only the neutrophil activity but also that of the lymphocytes. In our case, the fact that the lymphocyte count was retained within the physiological norms during the different seasons means that normal phagocyte processes were maintained. This was also confirmed by the two-phase orientation of the changes in the lymphocyte-granulocyte index values (LGI) and the correlation between neutrophils and lymphocytes (NLI). Both indexes once again show a high-grade dependence on the season and the type of farm- $P<0.001$ (Table 4).

Table 4. Analysis of variance with reference to the influence of the season and the farm on the hematological parameters and the blood cell indexes

Hematological parameters and leukocyte Indexes	Model	
	Season F and significance	Farm F and significance
BCC, conditional units	267.013***	135.487***
RNR, conditional units	25.860***	13.495***
LGI	53.918***	78.777***
NLI	46.309***	72.065***
LEI	54.382***	9.725***
NEI	35.310***	13.260***
Leukocytes, 10^9/l	43.926***	2.865*
Neutrophils, %	5.309**	4.000*
Eosinophils, %	6.963**	0.896
Lymphocytes, %	1.504	7.819**
Monocytes, %	23.749***	2.610*
Erythrocytes, 10^{12}/l	82.052***	2.725*
Hemoglobin, g/l	89.135***	1.804

Note: ***P<0.001; **P<0.01; *P<0.05

The lymphocyte/eosinophil (LEI) and the neutrophil/eosinophil (NEI) indexes exhibited similar dependence on the season and the farm. In addition, the numerical expression of these indexes was almost equal both in the hot and in the cold winter period. The same indexes were 2-3 times higher during the spring and the autumn but only with reference to the animals bred in the open buildings. The more frequent and sharper fluctuations of the environmental factors in these buildings maintained the adaptive tension by stimulating the antigen/ antibody reactions and stabilizing the homeostatic indicators typical for their physical and chemical thermoregulation.

The monocytosis demonstrated during the different seasons might be attributed to the strong organism resistance to the THI fluctuations. Migrating in the tissues, the monocytes stimulate or suppress the

lymphocyte proliferation and differentiation. The seasonal activation observed by us might be a result of both immunological and non-immunological irritation. Abdelatif & Alameen (2012) consider that the higher monocyte percentage found in the cows reared in open spaces during the transitional periods (6.5-10) is connected to the increased cortisol secretion. Using the leukocyte indexes to analyze the different variants of intercellular ratios in the cows blood, Safonov (2013) ascertains that during the foeto-placental complex formation, the NLI decreases by 23.4%, NEI diminishes twice, LEI- by 35.4%, and the LMI increases 2.7 times. According to the author, there is an activation of the immunity effector cell unit and the microphage protection system.

CONCLUSIONS

The results of our studies displayed weak or more strongly exhibited variations of the hematological parameters and indexes in dairy cows in connection with the THI changes. Despite the high THI during the daytime in the summer, the animals retained their immunological protection by increasing their erythrocytes by 7-8%, leukocytes by 6%, hemoglobin by 10-15%, eosinophils and monocytes as well as the values of the blood cell index (BBI), the lymphocyte-granulocyte index (LGI), the neutrophil-lymphocyte index (NLI). Almost all blood cell groups and indexes tested were dependent on the season and the type of farm - $P<0.05$- $P<0.001$.

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STUDY OF THE PROFILE OF FATTY ACIDS DETERMINED FOR HUBBARD CAPONS

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Abstract

The present study looked at the effect of applying the orchidectomy operation, in Hubbard roosters, on the fatty acids profile. In this regard, two groups of roosters were formed, an experimental group (Exp. B), consisting of castrated birds at the age of 7 weeks, and a control group (C.B.) consisting of uncastrated roosters. The results obtained by reporting the values of saturated fatty acids (SFA) to unsaturated fatty acids (UFA) illustrated that in case of pectoral muscles the experimental group recorded a value of 0.58, while in the case of the whole legs, the result was 0.57. Regarding the ratio between polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA), the value calculated for the chest muscles, resulting from the capons, was 0.60, and for the whole thighs muscles, for the same batch, was 0.61. The Ω6 / Ω3 ratio was calculated to be 5.06 for the breast of the castrated roosters, respectively 4.91 for their whole thighs. Additional research in this area is recommended.

Key words*: capons, fatty acids, Hubbard, Ω6 / Ω3ratio.*

INTRODUCTION

Capon manufacturing is an ancient practice that has endured till now, with records reaching back over 2000 years (Winter and Funk, 1960; Symeon et al., 2010). Capon production is done on a limited scale, with just a small market niche, but it has a lot of room for expansion because capon meat has special sensory properties that customers like (Amorim et al., 2016). Caponization is orhidectomy, leading to androgen deficiency and consequent phenotypic and behavioral changes, such as reduced development of comb and wattles loss of aggressiveness, and reduced activity (Calik, 2014). As a result, energy that would otherwise be spent on fighting and territorial dominance is freed up, allowing for further development and fat deposition.

More variety and high-quality attributes in various poultry meat products are currently being demanded by consumers. One of these products is the capon (a male rooster with his testes surgically removed before installation of the sexual maturity). The testicles are removed, which affects the animal's metabolism, growth, behavior, tissue composition, chemical composition, and meat organoleptic quality. (Miguel et al., 2008; Sirri et al., 2009). The principal metabolic effect of caponization is the increase of fat content abdominal, subcutaneous, and intramuscular. This effect has increased meat quality by improving flavor, texture, and juiciness, as well as making it more appealing to consumers than rooster meat of the same age (Chen et al., 2005; Tor et al., 2005). A decrease in saturated fatty acids (SFA) and increase in unsaturated fatty acids (UFA) content in capon meat would be beneficial for the human diet.

The goal of this study was to compare the fatty acids content of the capons and roosters of the Hubbard hybrid.

MATERIALS AND METHODS

The biological material was thirtieth roosters from the Hubbard hybrid, divided into two: experimental batch (experimental group-Exp. B., consisting of 20 heads; control batch-C.B., consisting of 10 heads). The only difference between the two groups was that the Exp.B. males were medically castrated at the age of seven weeks. Roosters were castrated in the last intercostal area using a bilateral laparatomy

procedure, puncturing the air sacs, pulling the testicles to the fore with a special forceps, and then performing orhidectomy by unlimited torsion. A continuous thread was used to stitch the wound. All of the birds were slain when they reached the age of 20 weeks.

The applied method consisted in extracting the fat, the concentration of fatty acids was expressed in grams FAME/100 g FAME (methyl esters of fatty acids). The working method applied was in accordance with:

1. Preparation of methyl esters SR CEN ISO/ TS 17764-1: 2008;
2. Gas chromatographic method SR CEN ISO/ TS 17764-2: 2008.

The principle of the method was based on the transformation into fatty acids of methyl esters from the fat sample under analysis, followed by separation of the components on the capillary chromatographic column, identification by comparison with standard chromatograms and quantitative determination of fatty acids (g FAME/100 g total FAME).

The interpretation of the obtained data was performed using their processing in the program Microsoft Excel and GraphPad Prism 9.3.1.

The values obtained were interpreted statistically, by calculating the main estimators-descriptors such as the arithmetic mean ($\overline{X}$), the standard error of the mean ($\pm s\overline{x}$), the coefficient of variability (V%), the minimum and maximum values. The mathematical relationships underlying the estimation are:

- ***The arithmetic mean*** represents the ratio between the sum of a number of results (ΣX) and the number of samples taken in the analysis (n):

$$\overline{x} = \frac{\Sigma x}{N};$$

- ***The variance*** is the sum of the squares of the deviations from the mean:

$$\Sigma(x\text{-}\overline{x})^2$$

The sum of the squares of the deviations was not used as such to express the size of the variance, but the average square of the deviations was used, according to the relation:

$$S^2 = \frac{\Sigma(x-\overline{x})^2}{N-1}$$

- **The standard deviation of the mean** ($s\overline{x}$) is the value that shows the deviation or error of the empirical arithmetic mean from the true theoretical mean:

$$s_{\overline{x}} = \sqrt{\frac{S^2}{N}} = \frac{S}{\sqrt{N}}$$

- **The coefficient of variation (V%)** directly indicates the relative variability of the values obtained compared to the average, namely the homogeneity of the studied parameter:

$$V\% = \frac{S}{\overline{x}} \text{ x } 100$$

Depending on the value of the coefficient of variation, the homogeneity of the analyzed parameter was established, as follows:

- V% <10% for a homogeneous population;
- 10% <V% <20% in case of an medium homogeneity;
- V%> 20% a very heterogeneous population.

RESULTS AND DISCUSSIONS

The data obtained on saturated fatty acids origin from the musculature of the chest, revealed, for both batches (C.B., Exp. B.) that the main constituent is palmitic acid C16:0; thus, for C.B. the average was 26.75 ± 0.02 g/ 100 g, with variation of 26.69 g/100 g (minimum) and 26.79 g/100 g (maximum); while for the Exp. B. the average was 26.74 ± 0.01 g/100 g, with variation of 26.71 g/100 g (minimum) and 26.78 g / 100 g (maximum). The constituent with the lowest average, for both batches, was represented by C12:0, lauric acid, with a value of 0.02 g/100 g. The total saturated fatty acids resulting from the chest muscles was 36.83 g/100 g for roosters from C.B., and 36.79 g/100 g for capons (Table 1).

In case of monounsaturated fatty acids, dominant, in both cases, was oleic cis acid, C18:1n9, with an average for C.B. of 34.65 ± 0.01 g/100 g, the minimum being 34.60 g/100 g and the maximum 34.68 g/100 g; while for the Exp. B the average was 34.69 ± 0.01 g/100 g. For both groups the acid with the lowest average was erucic acid C22:1n9 (Table 1). The total of monounsaturated fatty acids was 39.33 g/100 g for C.B. and 39.56 g/100 g for Exp. B. Results on polyunsaturated fatty acids indicated for roosters (C.B.) a total value of 23.40 g/100 g, the lowest value was recorded by C22:4n6, docosatetraenoic acid, with an average of 0.07 g/100 g; on the opposite pole was C18: 2n6,

linoleic acid, with an average of 16.10 ± 0.02 g/100 g.

In case of the experimental group (Exp. B.), the linoleic acid registered a value of 16.14 ± 0.02 g/100 g, with variation of 16.10 g/100 g (minimum) and 16.20 g / 100 g (maximum).

The total of polyunsaturated fatty acids was 23.65 g/100 g for capons (Exp. B.).

Regarding the ratio between fatty acids Ω6 and Ω3 in chest, the values calculated was 5.16 for C.B. and 5.06 for Exp. B.

The SFA/UFA ratio was 0.59 for the control batch and 0.58 for experimental batch; the PUFA/MUFA ratio values was 0.59 for C.B and 0.60 for Exp. B. (Figure 1).

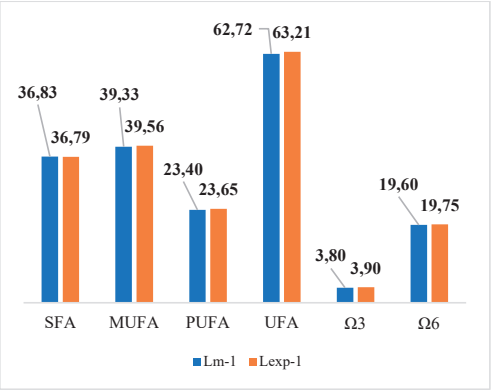

Figure 1. Fatty acids content in the pectoral musculature of Hubbard capons

Table 1. Fatty acids content in the pectoral musculature of Hubbard capons

Specifications	Breast							
	C.B.				Exp.B.			
Fatty acids	Statistics estimators							
	$X \pm s_x$ (g AG/100g)	V%	Min. (g AG/100g)	Max. (g AG/100g)	$X \pm s_x$ (g AG/100g)	V%	Min. (g AG/100g)	Max. (g AG/100g)
C8:0	0.10 ± 0.01	11.40	0.09	0.12	0.10 ± 0.01	1.94	0.08	0.13
C10:0	0.03 ± 0.00	3.65	0.03	0.03	0.04 ± 0.00	4.18	0.04	0.04
C12:0	0.02 ± 0.00	11.29	0.02	0.02	0.02 ± 0.00	10.23	0.02	0.02
C14:0	0.78 ± 0.01	3.68	0.74	0.82	0.68 ± 0.01	3.35	0.64	0.70
C15:0	0.27 ± 0.01	6.83	0.24	0.29	0.27 ± 0.01	9.94	0.23	0.30
C16:0	26.75 ± 0.02	0.17	26.69	26.79	26.74 ± 0.01	0.09	26.71	26.78
C17:0	0.09 ± 0.00	3.97	0.09	0.10	0.09 ± 0.00	2.55	0.09	0.09
C18:0	8.50 ± 0.01	0.27	8.47	8.53	8.54 ± 0.01	0.27	8.51	8.57
C24:0	0.30 ± 0.01	4.94	0.28	0.32	0.31 ± 0.01	4.78	0.29	0.33
SFA	36.83				36.79			
C14:1	0.11 ± 0.02	3.83	0.07	0.16	0.13 ± 0.01	16.42	0.10	0.16
C15:1	0.63 ± 0.01	4.26	0.59	0.66	0.66 ± 0.01	3.71	0.62	0.68
C16:1	3.58 ± 0.02	1.00	3.54	3.63	3.60 ± 0.01	0.57	3.57	3.62
C17:1	0.13 ± 0.01	2.61	0.09	0.16	0.17 ± 0.01	12.75	0.14	0.20
C18:1n9	34.65 ± 0.01	0.09	34.60	34.68	34.69 ± 0.01	0.04	34.68	34.72
C22:1n9	0.01 ± 0.00	8.94	0.01	0.01	0.02 ± 0.00	7.07	0.02	0.02
C24:1n9	0.22 ± 0.01	13.10	0.18	0.25	0.29 ± 0.01	6.26	0.27	0.32
MUFA	39.33				39.56			
C18:2n6	16.10 ± 0.02	0.33	16.04	16.16	16.14 ± 0.02	0.25	16.10	16.20
C18:3n6	0.12 ± 0.01	15.14	0.09	0.14	0.12 ± 0.01	1.87	0.09	0.15
C18:3n3	0.72 ± 0.01	2.60	0.70	0.75	0.73 ± 0.01	1.79	0.71	0.74
C18:4n3	1.92 ± 0.01	1.08	1.90	1.95	1.94 ± 0.01	1.18	1.90	1.96
C20:2n6	1.10 ± 0.01	1.29	1.08	1.12	1.12 ± 0.01	2.06	1.09	1.15
C20:3n6	0.25 ± 0.01	7.48	0.23	0.28	0.26 ± 0,01	5.70	0.24	0.28
C20:3n3	0.30 ± 0.01	4.94	0.28	0.32	0.33 ± 0.01	5.50	0.30	0.35
C20:4n6	1.42 ± 0.01	1.32	1.39	1.44	1.45 ± 0.01	2.15	1.40	1.48
C22:2n6	0.23 ± 0.01	11.55	0.19	0.26	0.25 ± 0.01	10.20	0.21	0.28
C22:3n6	0.30 ± 0.02	12.70	0.26	0.35	0.32 ± 0.01	6.85	0.29	0.35
C20:5n3	0.62 ± 0.01	3.95	0.58	0.64	0.64 ± 0.01	3.60	0.61	0.67
C22:4n6	0.07 ± 0.00	0.17	0.07	0.07	0.09 ± 0.01	0.57	0.04	0.12
C22:5n3	0.09 ± 0.01	0.49	0.06	0.12	0.10 ± 0.01	0.35	0.09	0.12
C22:6n3	0.15 ± 0.01	0.38	0.12	0.18	0.16 ± 0.01	0.30	0.14	0.18
PUFA	23.40				23.65			
UFA	62.72				63.21			
Other acids	0.29 ± 0.01	0.30	0.25	0.32	0.44 ± 0.01	0.26	0.40	0.48
Ω_3	3.80				3.90			
Ω_6	19.60				19.75			
Ω_6/Ω_3	5.16				5.06			
SFA/UFA	0,59				0,58			
PUFA/MUFA	0,59				0,60			

*SFA- Saturated fat acids
*MUFA- Monounsaturated fat acids
*PUFA- Poliunsaturated fat acids
*UFA- Unsaturated fat acids

The data obtained on saturated fatty acids from the musculature of the whole thighs, revealed, for both batches (C.B., Exp. B.) that the main constituent is palmitic acid C16:0; thus, for C.B. the average was 26.50 ± 0.01 g/100 g, with variation of 26.48 g/100 g (minimum) and 26.53 g/100 g (maximum); while for the Exp. B. the average was 26.40 ± 0.01 g/100 g, with variation of 26.36 g/100 g (minimum) and 26.44 g/100 g (maximum). The constituent with the lowest average, for both batches, was represented by C12:0, lauric acid. The total saturated fatty acids resulting from the whole thighs muscles was 36.64 g/100 g for roosters from C.B., and 36.33 g/100 g for capons (Table 2). In case of monounsaturated fatty acids, dominant, in both groups, was oleic acid, C18:1n9, with an average for C.B. of 34.69 ± 0.01 g/100 g, the minimum being 34.67 g/100 g and the maximum 34.72 g/100 g, while for the Exp. B. the average was 34.68 ± 0.01 g / 100 g. The total of monounsaturated fatty acids was 39.50 g/100 g for C.B. and 39.54 g/100 g for Exp. B. Results on polyunsaturated fatty acids indicated for roosters (C.B.) a total value of 23.87 g/100 g, the lowest value was recorded by C22:4n6, docosatetraenoic acid, with an average of 0.09 g/100 g; on the opposite pole was C18: 2n6, linoleic acid, with an average of 16.16 ± 0.01 g/100 g. In case of the experimental group (Exp. B.), the linoleic acid registered a value of 16.17 ± 0.01 g/100 g, with variation of 16.13 g/100 g (minimum) and 16.20 g/100 g (maximum). The total of polyunsaturated fatty acids was 24.13 g/100 g for capons (Exp. B.). Regarding the ratio between fatty acids Ω6 and Ω3 for whole thighs, the values calculated was 5.03 for C.B. and 4.91 for Exp. B. The SFA/UFA ratio was 0.58 for the control batch and 0.57 for experimental batch; the PUFA/MUFA ratio values was 0.60 for C.B and 0.61 for Exp. B. (Figure 2).

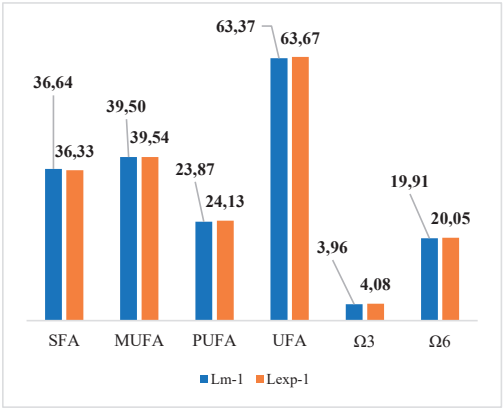

Figure 2. Fatty acids content in the whole thighs` musculature of Hubbard capons

Table 2. Fatty acids content in the whole thighs musculature of Hubbard capons

Specifications	Whole thighs							
	C.B.				Exp. B.			
Fatty acids	Statistics estimators							
	X±s_x (g AG/100 g)	V%	Min. (g AG/ 100 g)	Max. (g AG/ 100 g)	X±s_x (g AG/100 g)	V%	Min. (g AG/ 100 g)	Max. (g AG/ 100 g)
C8:0	0.11 ± 0.01	1.97	0.08	0.14	0.12 ± 0.01	2.54	0.09	0.16
C10:0	0.04 ± 0.00	2.73	0.03	0.06	0.05 ± 0.00	1.40	0.04	0.06
C12:0	0.02 ± 0.00	4.72	0.00	0.02	0.03 ± 0.01	1.94	0.01	0.05
C14:0	0.72 ± 0.01	4.28	0.68	0.75	0.61 ± 0.01	2.98	0.58	0.63
C15:0	0.27 ± 0.01	5.49	0.25	0.29	0.27 ± 0.01	5.49	0.25	0.29
C16:0	26.50 ± 0.01	0.07	26.48	26.53	26.40 ± 0.01	0.11	26.36	26.44
C17:0	0.11 ± 0.01	2.34	0.08	0.13	0.11 ± 0.01	1.86	0.08	0.13
C18:0	8.50 ± 0.01	0.17	8.48	8.52	8.38 ± 0.01	0.34	8.35	8.42
C24:0	0.37 ± 0.01	6.89	0.33	0.40	0.36 ± 0.02	10.03	0.31	0.40
SFA	36.64				36.33			
C14:1	0.11 ± 0.00	6.89	0.10	0.12	0.15 ± 0.01	1.70	0.11	0.18
C15:1	0.65 ± 0.01	2.75	0.63	0.68	0.66 ± 0.01	3.46	0.62	0.68
C16:1	3.64 ± 0.01	0.78	3.60	3.68	3.54 ± 0.01	0.64	3.50	3.56
C17:1	0.15 ± 0.01	1.45	0.12	0.18	0.20 ± 0.01	1.25	0.17	0.24
C18:1n9	34.69 ± 0.01	0.05	34.67	34.72	34.68 ± 0.01	0.06	34.65	34.70
C22:1n9	0.02 ± 0.00	0.44	0.00	0.02	0.02 ± 0.00	0.90	0.01	0.02
C24:1n9	0.24 ± 0.01	10.21	0.22	0.27	0.29 ± 0.01	1.48	0.26	0.3
MUFA	39.50				39.54			
C18:2n6	16.16 ± 0.01	0.17	16.12	16.19	16.17 ± 0.01	0.19	16.13	16.20
C18:3n6	0.14 ± 0.01	1.05	0.12	0.16	0.15 ± 0.01	1.66	0.11	0.18
C18:3n3	0.74 ± 0.01	3.87	0.70	0.78	0.76 ± 0.01	3.56	0.73	0.80
C18:4n3	1.94 ± 0.01	1.19	1.91	1.97	1.96 ± 0.01	1.11	1.93	1.99
C20:2n6	1.12 ± 0.01	2.04	1.10	1.16	1.14 ± 0.01	2.56	1.10	1.18
C20:3n6	0.28 ± 0.01	6.20	0.25	0.30	0.30 ± 0.01	7.45	0.27	0.33

C20:3n3	0.37 ± 0.01	3.08	0.35	0.38	0.40 ± 0.01	5.76	0.37	0.43
C20:4n6	1.47 ± 0.01	1.08	1.45	1.49	1.50 ± 0.01	0.99	1.48	1.52
C22:2n6	0.30 ± 0.01	9.01	0.27	0.34	0.32 ± 0.01	8.44	0.29	0.36
C22:3n6	0.35 ± 0.02	10.22	0.30	0.38	0.37 ± 0.01	6.22	0.34	0.40
C20:5n3	0.64 ± 0.01	4.94	0.60	0.68	0.66 ± 0.01	3.46	0.64	0.69
C22:4n6	0.09 ± 0.01	1.36	0.07	0.10	0.10 ± 0.01	1.65	0.08	0.12
C22:5n3	0.11 ± 0,01	1.70	0.09	0.14	0.12 ± 0.01	1.87	0.09	0.15
C22:6n3	0.16 ± 0.01	1.35	0.13	0.18	0.18 ± 0.01	1.50	0.14	0.21
PUFA	23.87				24.13			
UFA	63.37				63.67			
Other acids	0.29 ± 0.01	0.34	0.24	0.33	0.11 ± 0.01	0.36	0.09	0.12
Ω_3	3.96				4.08			
Ω_6	19.91				20.05			
Ω_6/Ω_3	5.03				4.91			
SFA/UFA	0.58				0.57			
PUFA/MUFA	0.60				0.61			

*SFA- Saturated fat acids
*MUFA- Monounsaturated fat acids
*PUFA- Poliunsaturated fat acids
*UFA- Unsaturated fat acids

CONCLUSIONS

Several factors can affect the quality of meat, some of which act during the life of the birds and others which act during the slaughter of birds (e.g. stunning, bleeding, scratching, or refrigerating the carcases). Capon meat has a number of biological features that are highly valuable, which is why their use in intensive systems has a lot of potential. As far as research is concerned, capons meat obtained from Hubbard hybrid can be considered as high quality, due it's high proportion of polyunsaturated fatty acids. One aspect reflecting the high quality of the capon meat is the Ω6/Ω3 ratio, which recorded values lower than 5,00. We recommend continuing research in this direction.

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REVIEW OF THE HEALTH BENEFITS OF LACTOFERRIN

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Abstract

Lactoferrin (LF) is a protein contained in raw milk. It was found that the highest content of lactoferrin is found in human milk, but the milk of other species also contains this protein (cow, goat, buffalo, sow, mare, mouse). Lactoferrin may improve the immune system or provide an iron supplement. It has the task of transporting iron, appreciated for the first time as a mediator of iron absorption. Potential applications of lactoferrin have prompted scientists to develop this nutraceutical protein for use in animal feed, human food and pharmaceutical applications. It is known that there are currently concerns in the world about obtaining a higher amount of lactoferrin from the milk of different animal species. This paper is a review that aims to highlight the biologically active role of lactoferrin.

Key words*: composition, lactoferrin, lactoferic milk, role.*

INTRODUCTION

Due to the high biological value of milk, the mammary gland of domestic animals has received an economic and social importance humanity continues to remain dependent on breastfeeding (Cotor & Ghita, 2012). Its nutritional value is attributed by large compounds including lactoferrin (Bondoc, 2007). Lactoferrin (LF) is found in the milk of pets. This is a glycoprotein that can achieve several biological functions (Karav et al., 2017). Lactoferrin was first detected in 1939 by Sorensen & Sorensen (1939) and later identified in human and bovine milk in 1960 by Johanson, (1939); Groves, (1960); Montreuil et al., (1960). In recent years, research has been conducted on the function of lactoferrin, much of it in areas unrelated to the areas of iron absorption and antimicrobial activity (Sanchez et al., 1992). Lactoferrin applications express importance for thermal stability, pH sensitivity, iron release and susceptibility to enzyme degradation. Lactoferrin acts as an iron transporter, considered for the first time as an intermediate in the absorption of intestinal iron or as an antimicrobial agent (Brock, 2012). Lactoferrin has the ability to supply iron to cells that need it or restricts it when it is in excess, maintaining the homeostasis of iron in the body. According to Kruzel et al. (2002), the only source of iron in milk is lactoferrin, which is a key protein for the healthy development of infants. Lactoferrin helps maintain the balance of free iron in the body to prevent the occurrence of pathological conditions. Cornish et al. (2004) through studies on the role of lactoferrin in stimulating the growth of osteoblasts, give it the probability of being used in the future in the therapy of bone diseases (osteoporosis).

In 2014, Siqueiros-Cendón et al. published a review of recent advances in understanding the mechanisms underlying the multifunctional roles of lactoferrin and provide a future perspective on its potential prophylactic and therapeutic applications. It has been established that lactoferrin is a versatile molecule that has been shaped by natural selection to be a first-line defence for mammals.

MATERIALS AND METHODS

Bibliographic sources, scientific databases, relative available articles from the literature that discuss the comparative values of lactoferrin concentrations were consulted. Graphically interpreted and analyzed data from the literature on lactoferrin content detected in milk samples from different animal species (cattle, goats, sheep, donkey and breast milk) were graphically interpreted and analyzed.

Lactoferrin from milk from different animal species as well as human milk were tested by a fast High-performance liquid chromatography (HPLC) method. The lactoferrin concentration was calculated by the standard curve method. Gas chromatography is an important fulcrum in modern chemical analysis. The method is an evolution of an older method, classical column chromatography, which was primarily used for the preparative isolation of natural compounds.

RESULTS AND DISCUSSIONS

There are a number of factors that influence the concentration of milk components in predictable ways (Cheng et al., 2008), these factors can be grouped into genetic and environmental factors. The category of genetic factors includes: breed, species, age, parity, conformation and body development, diet, lactation, lactation routine, physiological type and stage of lactation. The category of environmental factors includes: regional differences, season, climatic factors and temperatures (Welty et al., 1976; Cheng et al., 2008). Cheng et al., in 2008, stated that lactoferrin was associated with lactation (r = 0.555) and milk production per day (r = -0.472). Tsakali et al. (2014) used the main categories of milk (goat and sheep milk, 70:30) to obtain Feta cheese, the milk of these species having a high concentration of lactoferrin. An average amount of lactoferrin of 272 ± 24 µg/ml was reported in Feta whey.

Conesa et al. (2008) used a rapid method, chromatography (SP-Sepharose) to identify the purification of lactoferrin in milk from animal species: sheep, goat, camel, alpaca, elephant and gray seal but also human milk. The results of the research showed that there are some differences in the structure of lactoferrin proteins among mammalian species. The differences are observed in the behavior under heat treatment and in their antibacterial activity. The thermal behavior of lactoferrins isolated from milk was obtained by differential scanning calorimetry. The thermograms obtained the results of the values of the maximum heat absorption temperature and the change in enthalpy of denaturation were found to be higher than lactoferrins, saturated with iron as when they were analyzed as isolated, because in milk, lactoferrin is present with low iron saturation (Bezwoda & Mansoor, 1989; Nam et al., 1999). The results of lactoferrin with the highest antibacterial activity were camel lactoferrin, followed by goat lactoferrin and sheep lactoferrin. The tested concentrations that did not show antimicrobial activity were in the alpa and human lactoferrin species (Conesa et al., 2008).

The results of the highest concentrations detected in milk samples from animal species found by Tsakali et al. (2019) were found for goat milk (927.3 ± 52.1 µg/ml) and human milk (512.0 ± 35.7 µg/ml) (Figure 1).

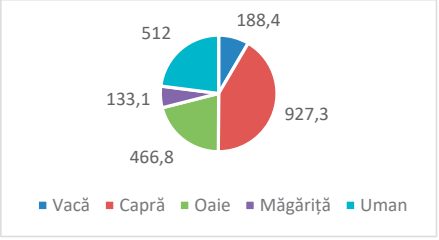

Figure 1. Mean detected lactoferrin values in milk samples from different animal species (µg/ml) (Tsakali et al., 2019)

The lowest detected results were found for donkey milk (133.1 ± 12.9 µg/ml) and cow's milk (188.4 ± 13.2 µg/ml), sheep's milk was found with a concentration of 466.8 ± 23.1 µg/ml.

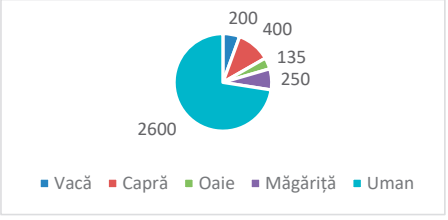

Figure 2. Mean values of lactoferrin concentrations detected in milk samples from different animal species in the literature (µg/ml) (Polidori & Vincenzetti, 2012; Queiroz et al., 2013; Gubid et al., 2015)

The results of the reference values in the literature were also found in high concentrations for goat milk (60-400 µg/ml) (Polidori & Vincenzetti, 2012) and human milk (200-2600 µg/ml) (Queiroz et al., 2013). Detected low results ranged from 20-200 µg/ml for cow's milk and 135 µg/ml for sheep's milk (Polidori & Vincenzetti, 2012). Concentrations between

120-250 μg/ml were found in donkey milk (Gubid et al., 2015)(Figure 2).
The results for sheep and goat milk were much higher than in the literature, due to re-testing with the addition of the lactoferrin standard in different concentrations, this giving a linear increase in lactoferrin and the difference in milk composition. The difference between the values obtained by Tsakali et al. (2019) and those of the literature have been reported for free fatty acids in animal milk but also human milk (Santillo et al., 2018). The results found by Tsakali et al. (2019) were satisfactory within the mentioned limits and the quantification of lactoferrin was possible, although additional tests should be performed to estimate its accuracy in each species. This rapid HPLC method could be used as a qualitative screening for the presence or absence of lactoferrin.

Uses of Lactoferrin

The use of lactoferrin in infant nutrition is an area that reflects an impressive number of studies, but the role of lactoferrin in the feeding of newborns in animals is less studied. The studies were performed generally for calves, having a role in regulating the intestinal flora (a calf that sucks 2 liters of colostrum, ingests about 2 g of lactoferrin per day).
Lactoferrin can also be used in food for fish farming, it can influence the defense system by regulating the activity of macrophages and the
Data from the literature, articles, patents with different topics on the structure, composition and properties of lactoferrin, its obtaining and purification, preclinical and clinical studies were consulted in order to obtain information on the use of lactoferrin in various fields. Commercial applications of bovine lactoferrin are shown in Table 1.

Table 1. Commercial applications of bovine lactoferrin (Miron & Macovei, 2006)

The product	Functionality
Infant formulas	Camouflage for human milk, improved resistance to pathogens
Nutritional supplements	Adjuvant in iron absorption, adjuvant to increase immunity
Functional drinks	Increases the solubility and absorption of iron
Beauty	antioxidants
Oral hygiene products	Improving oral hygiene

The best method of administering lactoferrin to infants is breastfeeding, which regulates the intestinal flora which is associated with an increased resistance to colonization by pathogens, activity of lymphocytes (Miron & Macovei, 2006). Lactoferrin is suspended in fish oil which is dispersed in the granules of the feed given to the alvevins. An experiment was performed by Kussendrager (1994), in which such feed was administered to salmon contaminated with Aeromonas salmonicide. The results showed that mortality decreased from 14% to 2% after feeding with 100 mg lactoferrin/day/kg of fish (Berkhout et al., 2003). Currently, the world produces about 20-30 tons of lactoferrin, being extracted from whey from cheese or skim milk. Active lactoferrin may be antimicrobial agent for poultry, pork, fish and marine life.

Antibacterial activity

Research by Valenti & Antonini (1998) has shown that lactoferrin has, independently of antibacterial activity and anti-invasive action represented by the ability to inhibit the penetration of bacteria into host cells. Thus, studies on *S. pyogenes* and *S. aureus* have shown that lactoferrin can be used as an adjunct in antimicrobial therapy, inhibiting the internalization of bacteria and allowing drugs to eliminate and kill bacteria on the studied pharyngeal epithelium. Wakabayashi et al. (2003), comparing the antimicrobial spectrum of bovine lactoferrin and human lactoferrin, found that bovine lactoferrin has strong antimicrobial activity, especially against bacteria and yeasts. Jahani et al. (2015), evaluating the effect of lactoferrin on two different species of Gram-negative and Gram-positive bacteria, showed that lactoferrin was more effective for Gram-positive bacteria (*Staphylococcus epidermidis, Bacillus cereus*) than for Gram-negative (*Campylobacter jejuni, Salmonella*).
A study by Lizzi et al. (2016) on the antibacterial activities of undigested and hydrolyzed lactoferrin with trypsin in the form of lactoferrin (apo-bLf) and undigested and
differentiated bovine lactoferrin (bLf) were evaluated against different species bacterial. Trypsin is an enzyme needed for bowel physiology. The results of trypsin apo-bLf

hydrolysis products showed broad-spectrum antibacterial properties with various bacterial strains. Apo-bLf was less susceptible to trypsin hydrolysis compared to the different form. Its triptych fragments had a higher activity than those obtained from diferric-bLf. Embleton et al. (2013) reviewed the antimicrobial activity and therapeutic potential of lactoferrin.

Lactoferrin is found in most body fluids. Three isoforms of lactoferrin were isolated, i.e. two with RNase activity (lactoferrin-β and lactoferrin-γ) and one without RNase activity (lactoferrin-α). Lactoferrin receptors can be found on intestinal tissue, monocytes, macrophages, neutrophils, lymphocytes, platelets, and certain bacteria (Levay & Viljoen, 1995).

Antifungal activity

Numerous studies have been performed on the antifungal activity of lactoferrin. Nikawa et al. (1993) studied the effect of human lactoferrin on 5 oral isolates of *Candida albicanis* and *Candida krusei*, costing a higher sensitivity to lactoferrin for *Candida krusei*. Most studies have been done on *Candida albicans*. Lactoferrin has been shown to inhibit the growth of *Candida albicans in vitro*, including strains resistant to azole antifungal agents, not only in the form of yeast but also in the form of hyphal (Wakabayashi et al., 1998).

Takakura et al (2003) demonstrated the antifungal activity of lactoferrin and pepsin hydrolyzate of lactoferrin, administered orally against oral candidiasis for a model of immunocompromised mice. Lactoferrin and its derivatives were added to drinking water contaminated with hydrochloric tetracillin. Mice showed oral symptoms similar to human canker sores. Lactoferrin from bovine milk can be used as a dietary supplement to support antifungal drug therapy, to prevent side effects but also to prevent oral infections, by using mouthwash and lactoferrin toothpaste (Miron & Macovei, 2006).

Antiviral activity

Research in recent years has shown that lactoferrin has antiviral activity against a large number of RNA and DNA viruses. Bovine and human lactoferrin have been shown to be potential inhibitors of human immunodeficiency virus (HIV) infections in vitro (Miron & Macovei, 2006). The antiviral action of lactoferrin against HIV is manifested in the early stages of the infection, most likely during the absorption of the virus into the host cells. Ando & Kishimoto (1996), cited by Macovei et al. (2006), patented a series of lactoferrin preparations for the prevention and treatment of opportunistic infectious diseases as a result of HIV infection. It has a synergistic action with the drugs used against cytomegavirus (CMV) and HIV.

Berlutti et al. (2011) published an article on the antiviral properties of lactoferrin. The protective effect of lactoferrin against microbial infections has been widely demonstrated in a large number of in vitro studies. However, it has been established that the antiviral activity of lactoferrin detected in monolayers of cultured cells infected with coated and empty viruses is not related to the degrees of lactoferrin iron saturation, while Zn and Mn saturated lactoferrin exerted a strong antiviral capacity against HSV (herpesvirus), HIV infection and poliovirus.

Anticarcinogenic activity

An exceptional use of lactoferrin is a non-toxic adjuvant in the treatment of cancer. Thus, lactoferrin is effective in suppressing the in vitro growth of human pancreatic cancer cells. Research by Tsuda et al. (2002) found that bovine lactoferrin (bLF) inhibits the development of colon cancer, esophageal, lung and bladder cancer in rats being administered orally in the post-initiation stage.

Japanese researchers at the National Cancer Center Research Institute in Tokyo have analyzed in vivo and in vitro animal studies showing that lactoferrin is an anticarcinogenic agent (Brik, 2000). Japanese researchers also looked at the effects of bovine lactoferrin in mice that had been inoculated with skin cancer cells or leukemia. When lactoferrin was administered, even after tumor formation, the growth of tumor cells was suppressed, and the spread of cancer cells in the lungs and liver was significantly lower than in control animals that did not take lactoferrin (Miron & Macovei, 2006).

Immunomodulatory and anti-inflammatory activity of lactoferrin

The property of lactoferrin to interact with many cell types, including most leukocytes, makes it play an excellent role in immunity. Research on various animals has shown that ingestion of lactoferrin has a direct protective effect on the regulation and modulation of the immune system. Lactoferrin can affect both the proliferation and differentiation of immune system cells (Miron & Macovei, 2006).

Lactoferrin has been shown to be essential in preventing allergic inflammation of the lungs and skin, with an increase in lactoferrin levels in the lungs of rats exposed to metal pollutants and bronchial secretions during stable asthma. Lactoferrin is a regulator of allergic skin inflammation (Cumberbatch et al., 2000), being produced in the cells of the epidermis, it has been observed that this protein can increase during the attack of allergens. The productive effect of lactoferrin against allergen-induced skin inflammation has also been increased in humans (Griffiths et al., 2000).

Prevention of bacterial colonization by lactoferrin

Specific inactivation of colonization conditions and moderation of the pathological potential of some bacteria can be observed as a third mechanism of action of lactoferrin (Shi et al., 2000). This assumption came from research on Gram-negative pathogenic bacteria *Haemophilus influenzae* (Qiu et al., 1998; Hendrixon et al., 2003).

Hendrixon et al. (2003) proved that lactoferrin has serine-protease activity and is a member of the protease family. They investigated the activity of human lactoferrin against IgA1 protease and Hap protein adhesion, with a role in the colonization of the respiratory tract by H. influenzae. It has been found that lactoferrin breaks down the two proteins in the region rich in arginine residues, thus proving to be a serine protease (Miron & Macovei, 2006). Lactoferrin is considered an important part in the prevention of dental caries in its inhibitory effect on the colonization of the tooth surface with *S. mutans*.

CONCLUSIONS

Lactoferrin has become an important topic in current research, due to its unique antimicrobial, anti-infective, anti-cancer and anti-inflammatory properties, having a special role in current medical practice. Lactoferrin has many health benefits, added in some functional foods but also in other products. The consequences of bovine lactoferrin have been shown in clinical trials in animals and humans. Favorable actions have been shown against digestive and non-digestive infections, following ingestion of lactoferrin, performed on various species of animals.

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INFLUENCE OF CLIMATE CONDITIONS AND BEE GRAZING ON THE STRENGTH AND PRODUCTIVITY OF BEE FAMILIES

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Abstract

Honey bees are extremely intelligent creatures that form an indispensable segment of the planet's ecosystem. Their lives are closely linked to environmental conditions. Mostly with climatic conditions, honey-bearing vegetation in the area of the apiary in which they are grown, cultivation technology and many other factors. Good knowledge of honey resources and proper organization of their use are crucial for the development of beekeeping and increase the productivity of bee colonies. The leading factor for the life and development of bees are the environmental conditions - climate, flora and fauna, food base, useful and harmful to bee colonies living organisms. Most of these conditions one cannot make an impact, this can be achieved through large unjustified costs. Another group of factors that determine the development of the bee family are created inside the nest as a result of the vital activity of individuals in it. These include the collection, processing and preservation of food supplies, regulation of temperature, humidity and gas exchange within the bee colony, beneficial and harmful organisms associated with the bee colony, microorganisms involved in digestion and preservation of perga and others. These factors are related to the productivity of the bee family and in them it is possible, to some extent, altered by man. Climate change affects the development and productivity of bees to varying degrees, with weak families and higher mortality. The aim is to trace the influence of climatic conditions and bee grazing on the strength and productivity of bee colonies.

Key words*: bee family, bee grazing, climatic factors, honey bees, productivity, strength of bee colonies.*

INTRODUCTION

Bees are a bioindicator of climate and human activity. The bee organism reacts to various external factors, which allows them to be used as an indicator for biomonitoring in terms of environmental quality. For the purposes of biomonitoring can be monitored: the development of bee colonies, changes in bee behavior and periodically report mortality or apply a comparative analysis of indicator characteristics of bees (heavy metals, pesticides, radionuclides and other pollutants in the bee organism), reared in areas with varying degrees of anthropogenic impact. Background environmental monitoring is conducted in areas with small sources of pollution, with insignificant anthropogenic impact. It aims to obtain complete, objective and comprehensive information about the current state of the biosphere and its individual components at the background level. It allows solving not only national tasks, but also a number of international problems related to the negative, global effects of anthropogenic activity - climate change, ozone depletion, acid rain, the spread of harmful substances over long distances. Many scientists warn that climate fluctuations and changes today are reflected now and, in the future, to significantly affect all areas of human activity. Conservation of the species diversity of a number of pollinators, including bees, is becoming a serious problem.

According to Shopova (2021), the main factor responsible for the biological development of insects is solar radiation. Of the main meteorological elements, the average values of which determine the climate of a place, solar radiation is considered the most conservative and poorly variable factor. The beginning of egg-laying by the queen bee in honey bees is associated with an increase in the length of the day. The orientation in space and during the honey harvest happens thanks to the light. Temperature changes are also responsible for the behavior of bees and their habitats. Variations in humidity, droughts and droughts

have a direct effect on vegetation and nectar production.

In recent years, agriculture in our country is losing its diversity due to the lack of an efficient irrigation system and the risk of losses in the cultivation of plants requiring higher humidity. All abiotic factors have a complex effect on the development and behavior of bees.

According to Alexandrov (2011), the last two decades of the last century and the beginning of the present show an increase in average air temperature. In our country, scientists have found an increase of up to 0.8°C compared to the period 1961-1990. The different climate models show an increase in the average value by 2050 by between 1.6°C and 3.1°C. For the region of Struma and South-Eastern Bulgaria the annual temperature should increase by between 0.9°C and 1.3°C by 2025, distributed by seasons as follows: winter - 0.6°C; spring - 1.2°C; summer - 0.9°C and autumn - 1.2°C.

Some of the models expect an increase in solar radiation in the cold half of the year by no more than 10%. Precipitation shows a declining trend at the end of the last century and an increase since the mid-1990s in many parts of the country. The model expectation regarding the annual amount of precipitation by 2025 is to decrease by between 2% and 5%, with a tendency to increase to 10% at the end of the 21st century.

Bees are a bioindicator of both climate and anthropogenic activity. Reducing them threatens the stability and sustainability of both our food and farm animal feed. The group of external factors includes: climatic and atmospheric conditions; the variety, duration and strength of bee grazing; the presence of beneficial or harmful organisms for bees. Climatic and atmospheric conditions are a result of the geographical location and relief of the area where the apiary is located. Includes: seasonality; average daily and average annual temperatures; air humidity; direction and strength of the prevailing winds; length of daylight, etc. The life of the bee family takes place depending on the changes that occur in the surrounding nature.

However, reactions to these changes are different in strong and weak families. The strong family makes better use of the favorable changes and absorbs the unfavorable ones more easily than the weak one.

The productivity of bee colonies is directly dependent, in addition to grazing, on the number of bees and their hereditary qualities. Only strong, healthy, and active bee colonies can produce a lot of honey, wax and new colonies and most effectively participate in the pollination of crops. In winter and spring, strong families retain heat better in the nest and spend less food to maintain the necessary heat (calculated per 1 kg of bees). Therefore, they overwinter more successfully and use the saved food for their better development. In addition, strong families enter the spring with bees exhausted from overwintering, which live longer and therefore better support their spring development (Terzieva, 2016).

Strong families overwinter much better than weak ones, consume less food in winter, grow much faster in spring, participate fully in the main grazing, build many cakes, and build viable, durable, and resistant to infectious diseases bees.

Many researchers in the field of beekeeping place honey plants (bee grazing) as the first factor influencing the productivity of bee colonies. Every beekeeper must know very well the area in which he is beekeeping in terms of the honey base: to know the strength of the pasture and its species composition; to know the beginning and duration of flowering of the main honey plants. In this regard, it is recommended that the beekeeper make a honey balance in the area where he raises bee colonies to get an idea of its honey potential. According to recent research, bees may not be able to adapt to changing climates by shifting their range of habitats. While other species, such as butterflies, can migrate easily, bees do not adapt to rapid change as easily, which means that they are particularly vulnerable to the unexpected effects of climate change. Unless we stabilize or reduce climate change, bees may one day disappear altogether.

Increased CO_2 in the atmosphere, heat stress, longer droughts and more intense rainfall associated with global warming continue to affect bee populations. In recent decades, there has been a drastic decline in both wild and domestic bee species worldwide. Growing disorders in bees, unusually high winter losses

of families and reduced life expectancy of queen bees have been common in recent decades. Climate change is a factor in the destruction of habitats. Climate and hydrology determine the general conditions for the emergence and prosperity of wild species. With global warming, changes are taking place in the Earth's climate zones. Climatic conditions and the condition of the honey-bearing vegetation are the main factors that determine the vital activity of bees during the different seasons and seasons (Taranov, 1987; Bilash, 1999; Nenchev, 2002).

The productivity of bee colonies is directly dependent not only on grazing, but also on the number of bees and their hereditary qualities. Only strong, healthy, and active bee colonies can produce a lot of honey, wax and new colonies and most effectively participate in the pollination of crops (Zhelyazkova, 2004).

In winter and spring, strong families retain heat better in the nest and spend less food to maintain the necessary heat (calculated per 1 kg of bees). Therefore, they overwinter more successfully and use the saved food for their better development. In addition, strong families enter the spring with bees exhausted from overwintering, which live longer and therefore better support their spring development (Radoev, 2003).

Research shows that bees raised in strong families have 5-8 days longer lives, their proboscis is 8% longer, their muscles, legs and wings are better developed, and the honey stomach is more voluminous. That is why they fly farther and carry about twice as much nectar in flight as bees raised by weak families. In the spring, the strong families accumulate an increasing number of flying bees, which use the main pasture and collect a lot of honey. Weak families continue their development during the main grazing, as most of them are engaged in raising offspring. As a result, they have few flying bees and miss grazing without collecting enough honey, even for their own feeding. Strong families build more combs, produce more wax, and their brood and bees are more resistant to disease. They are also less likely to be attacked by bee thieves and easier to deal with enemies and pests. The assessment of the development of bee colonies in the hive was determined by two criteria - the strength of the bee colony (in kg of bees) and the number of bees and the number of sealed brood.

Honey production is influenced by the season and the type of hives (Tsvetanov, 2016; Tsvetanov 2017).

MATERIALS AND METHODS

Strength of the bee family. This feature is determined by the number of frames occupied by bees in kilograms (kg). The strength of each bee colony is determined by the main spring inspection (in early April, when there are still no young bees in the family) and the autumn inspection (in early November, when egg-laying is suspended). There are differences in the weight of beekeepers in different types of hives, in Dadan-Blatt the number of beekeepers in the beehive is multiplied by 0.250 kg; at Dadan-Blatt it is multiplied by 0.250 kg;

Quantity of bee brood. It is determined by measuring the number of sealed cells with a measuring frame. In addition to the number of frames occupied by bees, the amount of sealed brood is recorded every 12 days, which is one of the indicators of the egg-laying capacity of the mother and the strength of the bee colony. The quantity is determined by means of a measuring frame, inside of which a thin wire is stretched, forming squares with a wall size of 5 by 5 cm or occupying an area of 25 cm^2. The area of each of the squares corresponded to the area occupied by 100 bee cells (4 cells per square centimeter). The measuring frame is placed on the frame with a file and the squares that cover the bee brood are counted. The measuring frame is superimposed on the bee brood frame and the squares are counted.

Honey productivity of bee families. We determined the honey productivity of bee families, individually for each hive. As the combs from each hive are removed, placed in a portable box, and weighed again with the empty combs. The difference between the two draws was used to determine the amount of honey from each hive. The determination of the individual honey productivity for each hive was performed once at the end of the honey harvest season in the year of the study.

The most important of the controlled indicators in bee colonies is honey productivity. Combined with the evaluation on all other

grounds, it is essential because it affects the efficiency of grazing and gives a definitive idea of the suitability of the bee family for tribal use. The assessment of productivity was performed on two indicators - extraction of honey and wax. The amount of honey is determined at the end of the season, taking into account the records kept for the respective family. The total honey production of the bee family was determined by summing the centrifuged honey with the honey left for winter and the honey stored outside the hive. In determining the amount of honey in the combs, we assumed that 1 dm2 of the area filled on both sides contained 350 g of honey. After determining the area of sealed honey, we calculated the total amount of honey in the honeycomb, as one filled on both sides with sealed honey 12 Dadan-Blatova pita contains 3.6 kg, sealed to the middle - 2 kg and filled to 1/3 - 1 kg. (Nenchev, 1990).

Experimental apiary 1.
The town of Ihtiman is located in the Ihtiman valley, which is surrounded by Ihtiman middle forest. The eastern part is occupied by a distinct ridge. The Ihtiman valley has an average altitude of 650 m. The Mativir River passes through the land. The climate is temperate continental (Figure 1).

Experimental apiary 2.
The town of Koynare is located in the northwestern part of Bulgaria in the Pleven region. The altitude is 78 m (Figure 1).

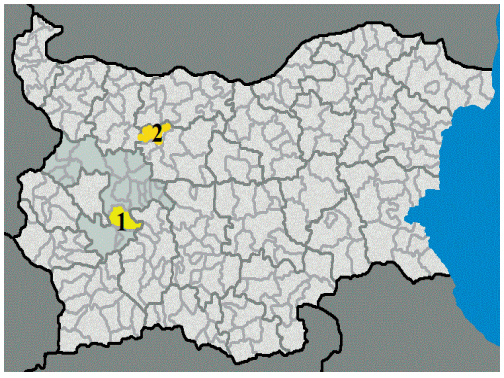


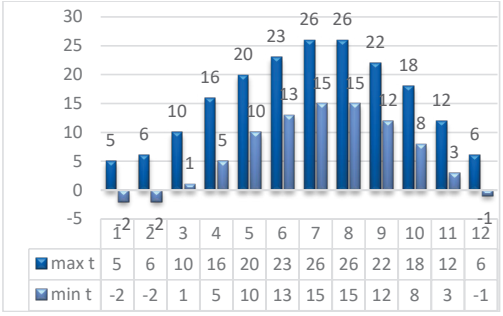
Figure 1. Map with experienced apiaries

Figure 1 shows the location of the experimental apiaries.

Number and type of hives.
For the purposes of the study, we traced 10 hives from two apiaries, system Dadan Blatt with 12 frames each.

RESULTS AND DISCUSSIONS

In terms of climate, apiary 1 falls in the temperate-continental climatic sub-region of the European-continental climatic region or in particular in the climatic region of the high fields in Western Central Bulgaria. The predominance of northwestern transport of strong ocean air masses coming from Northwestern Europe has a significant impact on climate formation. The movement of continental air masses is in the north-northeast direction and the warm tropical air masses penetrating from the south.



	1	2	3	4	5	6	7	8	9	10	11	12
max t	5	6	10	16	20	23	26	26	22	18	12	6
min t	-2	-2	1	5	10	13	15	15	12	8	3	-1

Figure 2. Minimum and maximum temperatures reported during the study period

The data in the Figure 2 show that the average annual temperature is relatively low, the coldest months of the year are January, February and December. There are also sharp drops in temperatures in spring and autumn. The warmest months are July and August. A typical spring month is April, and a typical autumn month is October. In the area of Apiary 1, there is a danger of late spring and early autumn frosts - in the second half of May and in the first ten days of September. The main reason for this is the nature of the relief. The distribution of precipitation during the year by months and seasons is uneven - a pronounced continental climate. The amount of precipitation is highest in May and June, and the lowest - in February and September. The average annual amount of precipitation is below the national average. The snowfall is in

the period November - April. This distribution of precipitation emphasizes the typical temperate-continental character, with a winter minimum and a secondary one at the end of summer and a maximum at the end of spring and the beginning of summer.

Apiary 2 falls in the temperate-continental climatic subregion of the European-continental climatic region. The continental character of the climate is very well expressed with markedly cold winters and hot summers, warm springs and sunny autumns. The average altitude of the area is about 200 m.

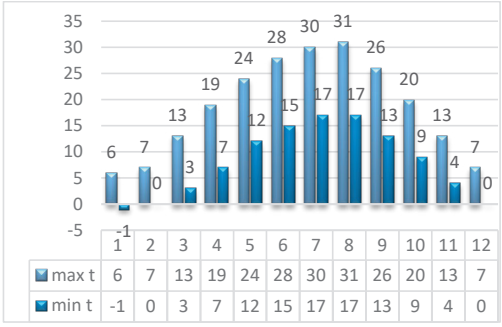


Figure 3. Minimum and maximum temperatures reported during the study period

The data in Figure 3 show that the average annual temperature is relatively low, the coldest months of the year are January and February with t -1 to 0°C. The warmest months are July and August with t 30-31°C. The highest amount of precipitation falls in March, April and May about 50 mm. The snowfall is in the period December-January.

Globally, the last 160 years have seen a trend of increasing the average temperature of the Earth by 0.5°C. Climate change is related to the general warming trend for Bulgaria, as well as the increase in the frequency of extreme meteorological and climatic phenomena, such as droughts, torrential rains, thunderstorms and hail, which greatly affect bee colonies.

The honey plants around the apiary 1 are mainly *Tilia*, less *Robinia pseudoacacia* and a variety of meadow honey plants, which are also the main source of food for bees. The cultivation of *Helianthus annuus, Brassica napus* and *Medicago sativa* in the area has also increased in recent years.

Table 1 shows the honey plants with the most burning importance for the bees in the area, the flowering period and what the bees collect from them.

Table 1 Honey-bearing vegetation determined around an apiary 1

№	Latin name	Bulgarian name	What do bees collect	Flowering period
1	*Prunus domestica*	Слива	Abundant nectar and some pollen	IV-V
2	*Prunus cerasifera*	Джанка	Abundant nectar and some pollen	IV
3	*Malus domestica*	Ябълка	Neckar, pollen	III-IV
4	*Cerasus vulgaris*	Череша	Neckar, pollen	IV-V
5	*Prunus cerasus*	Вишня	Neckar,pollen	IV
6	*Prunus spinosa*	Трънка	Nectar, some pollen	IV-V
7	*Rubus idaeus*	Малина	Nectar	V-VI
8	*Rosa canina*	Обикновената шипка	Pollen	V-VI
9	*Lamium purpureum*	Мъртва коприва	Neckar, pollen	IV-X
10	*Brassica napus*	Рапица	Neckar,pollen	IV-V
11	*Robinia pseudoacacia*	Акация	Neckar, pollen	V
12	*Tilia spp.*	Липа	Neckar, pollen	V-VI
13	*Helianthus annuus*	Слънчоглед	Neckar, pollen, propolis	V-IX
14	*Medicago sativa*	Люцерна	Neckar, pollen	V-IX

Fruit species are of great importance for the development of bee colonies and especially on the egg-laying activity of the mother and the development of the brood in the spring. *Cerasus vulgaris* are very good honey plants with abundant flowering. From them the bees

collect nectar and pollen. *Prunus domestica* and *Prunus cerasifera* are a source of copious amounts of nectar and less pollen. These species provide the bee family with good food during the period of its intensive reproduction. The flowering period is from month March to month May, consecutively. *Lamium purpureum* is a good, honey plant with a very long flowering period of 5 or more months. It supports the continuous development of bee colonies in the spring and their preparation for grazing. The honey plants of the greatest importance for apiary 1 are the predominant plantations of linden, sunflower and meadow vegetation. From *Helianthus annuus*, bees collect abundant nectar, pollen and glue. It has a long flowering period and is one of the important honey plants around the apiary.

Natural meadows are a very good honey base for bees. There are a large number of species that bloom for a long time and emit enough nectar. In many parts of the country, meadows provide the main pasture for bees. Flowering of meadow honey plants begins in spring and ends in mid-summer. It coincides with the full development of families and the most favorable external conditions. With good grass composition in the meadows, bees collect large amounts of high-quality nectar. Table 2 shows the honey-bearing vegetation around the apiary 2.

Table 2. Honey-bearing vegetation around apiaries 2

№	Latin name	Bulgarian name	What do bees collect	Flowering period
1	*Prunus domestica*	*Слива*	Abundant nectar and some pollen	IV-V
2	*Prunus cerasifera*	*Джанка*	Abundant nectar and some pollen	IV
3	*Malus domestica*	*Ябълка*	Neckar, pollen	III-IV
4	*Cerasus vulgaris*	*Череша*	Neckar, pollen	IV-V
5	*Prunus spinosa*	*Трънка*	Neckar,pollen	IV
6	*Crataegus monogyna*	*Обикновен глог*	Nectar, some pollen	IV-V
7	*Rosa canina*	*Обикновената шипка*	Nectar	V-VI
8	*Lamium purpureum*	*Мъртва коприва*	Pollen	V-VI
9	*Robinia pseudoacacia*	*Акация*	Neckar, pollen	IV-X
10	*Amorpha fruticosa*	*Черна акация*	Neckar,pollen	IV-V
11	*Tilia spp.*	*Липа*	Neckar, pollen	V
12	*Helianthus annuus*	*Слънчоглед*	Neckar, pollen	V-VI
13	*Medicago sativa*	*Люцерна*	Neckar, pollen, propolis	V-IX
14	*Onopordum acanthium*	*Магарешки бодил*	Neckar, pollen	V-IX

From Table. 2 shows that honey plants are represented by fruit species such as plums, apples *Malus domestica* and cherries and junipers. They bloom in early spring, April, May and give the bees some pollen. *Prunus spinosa, Crataegus monogyna, Rosa canina* are important for the maintenance of grazing and the development of families.

Predominant honey plants as the main food source are: *Lamium purpureum, Robinia pseudoacacia, Amorpha fruticosa, Tilia* spp., *Medicago sativa, Helianthus annuus* and *Onopordum acanthium.* In both study areas, we reported approximately the same plants, a source of food for bees with small differences.

Determining the strength of bee colonies in the spring.

The aim of beekeeping is to obtain the highest possible and constant yields. This is achieved through human intervention in the life of the bee colony, guiding and supporting it. For this purpose, it is necessary to carry out regular inspections. The size and strength of the bee colony is determined by the number of frames covered with bees. This means the number of bees that cover from top to bottom neighboring wax combs. Moreover, this number of bees in a frame is a constant number, which is the same and does not change with the seasons, the strength of the family and other factors.

The strength of the families in the spring was determined during the main spring inspection carried out on 12.03.2021 in apiary 1, the results of which are presented in Figure 4.

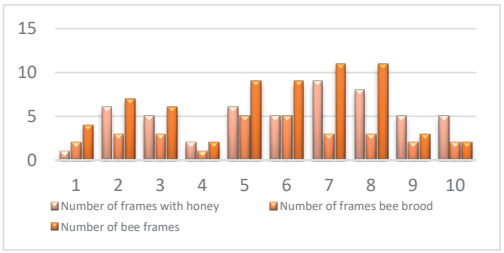


Figure 4. Number of frames with honey, bee brood and quantity of bees

In the review we found that bee colonies are relatively strong from 6 to 11 bee frames, except for three, in which we reported a smaller number of brood and frames with bees. We performed feeding in order to support and strengthen the weak bee families in order to equalize them in strength.

On apiary 2 we made the main spring inspection on March 13, 2021. The results are shown in Figure 5.

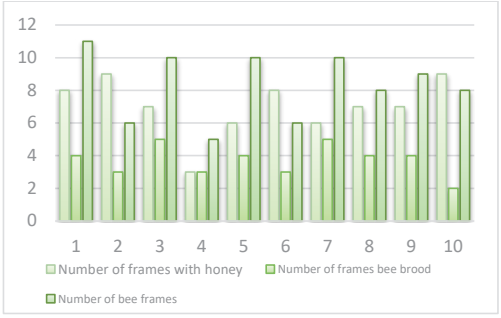


Figure 5. Number of frames with honey, bee brood and quantity of bees

The bee families in this apiary are relatively strong from 6 to 11 frames with bees, the color of the frames with a brood is from 3 to 5 pieces and a significant amount of food supplies from 6 to 9 frames with honey. We did not report weak families.

Determining the strength of bee colonies in the fall in both apiaries.

Strength of bee colonies reported in the autumn in the apiary 1. With the completion of the autumn development, the main autumn inspection of the bee families was carried out. With it we established the presence of the mother and her qualities, the amount of the pilot, the strength of the family and the health of the bees, the quality and quantity of food supplies.

Autumn inspection of apiary 1 performed on September 6, 2021. The results obtained are presented in Figure 6.

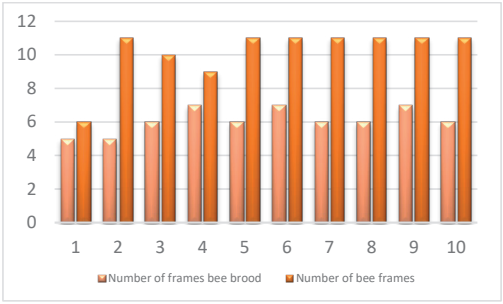


Figure 6. Strength of bee colonies reported in the autumn in the apiary 1

The results show that the ten bee families in apiary 1 are strong and values from 6 to 11 bee frames are reported. At apiary 2 we made the autumn inspection on 01.09.2021. The obtained results are presented in Figure 7.

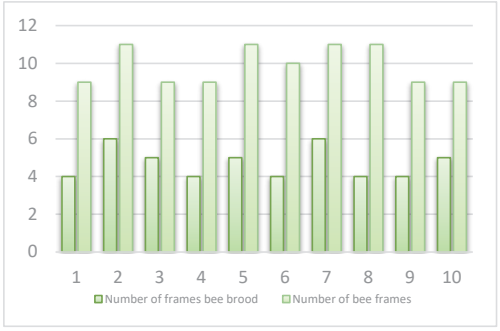


Figure 7. Strength of bee colonies reported in the autumn in the apiary 2

Figure 7 shows that in the apiary, bee colonies are strong, with values of 9 to 11 bee frames and sufficient food supplies for a good winter. Strong families overwinter much better than weak ones, consume less food in winter, grow much faster in spring, participate fully in main grazing, build many combs and raise viable, resilient and disease-resistant young bees.

Determining the honey productivity of families.

The most important of the controlled indicators in bee colonies is honey productivity. Combined with the evaluation on all other grounds, it is essential because it affects the efficiency of grazing and gives a definitive idea of the suitability of the bee family for tribal use. The assessment of productivity was performed by indicator - copper production. The amount of honey is determined at the end of the season. The total honey production of the bee family was determined by summing the centrifuged honey with the honey left for wintering and the honey stored outside the hive.

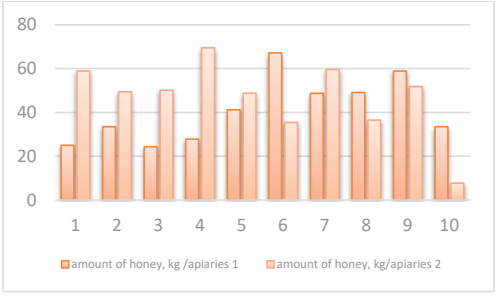


Figure 8. Determining the honey productivity of families

From the results shown in Figure 8 it can be seen that the largest amount of honey was obtained from hive № 6 and hive № 9, respectively 67.2 and 58.9 kg of honey.

The smallest quantities are obtained from beehives № 1, 3 and 4 from 24.3 to 27.8 kg of commodity honey. The other hives have relatively good amounts of honey from 33.5 to 49.1 kg. The largest amount of honey was obtained from beehives № 4, № 7, № 1 and № 9, respectively 69.5, 59.6, 58.9 and 51.8 kg of honey. The smallest quantities were obtained from beehive № 6 and beehive №8 about 17.8 and 24.3 kg of honey. The other hives have relatively good amounts of honey from 48.4 to 50.1 kg.

CONCLUSIONS

The analysis of the obtained results gives us grounds to draw the following more important conclusions:

The geographical and climatic characteristics in both regions of the apiaries are suitable for creating and raising bee families. The high temperature and humidity during the beekeeping season creates good conditions for the development of bee families and their good wintering.

The honey-bearing vegetation around the apiaries is sufficient for the development of bee families throughout the beekeeping season. There are no periods of grazing interruption. The main pasture is mainly acacia, linden and fruit species, which are a source of honey with excellent qualities. Sunflowers and meadow honey species prolong grazing and allow for the production of high-quality honey.

In both regions of the country, where the apiaries of the study are located, we found strong bee colonies during the autumn inspection. The mothers are young and very productive, which is why the strength of the families in the apiaries is due. Bee families are better developed in an apiary located in the area of the town of Koynare. Climatic conditions are more favorable and this affects development.

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PARTIAL RESEARCH ON THE EFFICIENCY OF DAIRY COW FARMS BY DIMENSION AND GROWTH SYSTEM

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Abstract

In the context in which the number of dairy cows in our country is constantly declining in recent years, a complex analysis (technical and economic) of dairy farms was necessary to determine the optimal size and exploitation mode of these categories of cattle in order to obtain a maximum profit. After conducting the three case studies and analyzing the three breeding and maintenance systems, the intensive dairy farming system seems profitable, but it requires the biggest investment, the largest number of staff and well-developed feed bases, based on generous land areas, for the realization of the vast majority of fodder within the unit, thus having the fewest inputs. Without large areas of land and high-performance dairy animals, the intensive system is losing ground to the semi-intensive system, especially as it poses major problems in terms of environmental protection, creating a lot of waste and pollutants.

Key words*: bovines farm, economic efficiency, milk production.*

INTRODUCTION

Today, raising cattle, and especially dairy cows, has become a "living factory" because these remarkable animals can process agricultural raw materials into animal products of great biological value. The most important, most complex and complete product provided by cows to man is milk.

The general demand for milk increased with the development of industry and urban settlements, developing a large number of dairy products, which led to a significant increase in the "prestige" of the cow, which is called the "nanny of mankind". Today, almost everyone consumes milk. In a lifetime, a person consumes, on average, 10,000 liters of milk (Dinu, 1996).

At the present stage, the economic agents in agriculture are permanently preoccupied with the improvement of organizational structures, production technologies, the optimization of material, financial and human resources, the efficiency of their use, to face competition and achieve a major goal - obtaining maximum profit.

Livestock farms are severely affected during this period by a number of factors such as: the restriction of the area intended for fodder production, the sharp rise in prices for compound feeds, seeds of varieties and hybrids with high productive value, water, energy and especially the increase in interest rates on bank loans is currently at a crossroads that requires in-depth studies to find out the real causes that led to the inefficiency of dairy farms in our country and the attractiveness of investors in this field.

Considering the above factors, a thorough analysis of the economic and financial activity was required at the level of the cow farm in order to develop the most appropriate decision - making solutions for the profitability of milk production.

MATERIALS AND METHODS

The main objectives of this paper are to know and study the efficiency of dairy farms depending on the size and type of exploitation. For this, 3 dairy farms were studied, as follows:

- small dairy farm, organized in a household system;
- medium-sized dairy cow farm, organized in a semi-intensive system;
- large dairy farm, organized in an intensive system.

The analysis of the technical and economic efficiency cannot be done without a presentation

of the conditions in which the milk production of these animals is registered, therefore a brief presentation of the technology of exploitation of dairy cows, as well as of the related youth was made. This specifies the breeding results of these animals as well as the way of maintenance - animal shelter, body care, movement of cows, feeding technology and milking technology.

This paper presents and makes an interpretation of the technical-economic results of the farm activity from the point of view of the following indicators:

1. Technical indicators:
- Dynamics and structure of livestock;
- Dynamics of animal production obtained: milk production (average, total); increase production (average, total); calf production;
- The dynamics of plant production and its structure;

2. Economic and financial indicators:
- The operating costs of dairy cows and their structure by categories of expenditure;
- The actual unit cost of milk;
- Revenues obtained and their structure;
- Final financial results.

These indicators have been studied in dynamics over the last three financial years, focusing on the factors that have contributed to obtaining favorable results, how to improve the activity, as well as the ways to achieve them.

The technical-economic analysis deals with the study of the economic-financial results obtained by the economic agents, of the factors and causes that generated them, as well as the ways and measures that must be taken in order to increase the efficiency of activities in all patrimonial units.

In farms and other agricultural units, the technical-economic analysis allows the manager to observe in time the shortcomings that arise during the implementation of the activity program, to know exactly the situation in each sector of activity, the resources it has, being able to take, the most appropriate measures to achieve the annual and future objectives (Oancea, 2003).

This analysis highlights the methods that are the best for management and creates the possibility of generalization, ensures their permanent adaptability in the concrete, real conditions of each economic unit.

Economic analysis allows the discovery of internal resources to increase production, as well as ways to use them as completely and efficiently as possible. It also finds ways to reduce production costs.

In conclusion, economic analysis is an important tool of scientific management in agricultural units, including livestock farms, which achieves all the attributes of management, objectives and functions of the unit as a system. It is an effective means of diagnosing, regulating, optimizing and programming the activity of agricultural units, the information provided following these analyzes being the foundation of the decision-making process.

The technical-economic analysis includes the whole set of methods and procedures used by this science to research the economic reality and evaluate the results obtained in an agricultural unit (Oancea, 2003).

The technical-economic analysis, to know all the phenomena and economic processes, uses a series of methods, techniques and procedures that intertwine with each other, due to the fact that all these aims to study causal relations, the mechanism of production and change economic phonemes and processes.

The technical-economic analysis aims at studying the different quantitative and qualitative aspects of the phenomena that take place in the economic units.

Regarding the quantitative characters, the technical-economic analysis uses a series of indicators that characterize the nature, size and evolution of economic phenomena in time and space. These indicators can be synthetic and analytical, volume or qualitative. They can be expressed in absolute quantities with the help of natural units (l, hl, pcs, heads, tons etc.) of value units (RON, convertible currencies) and conventional units (UN, UVM). They can also be expressed in relative quantities, by reporting the percentage of two indicators that express similar phenomena, a situation in which they are called indices. They can be individual, reflecting the variation of a single phenomenon, or group when they reflect complex phenomena, consisting of several simple phenomena. Compared to the base taken as a comparison, the indices can be fixed-based and chain-based (Popescu, 2007).

The most important processes that make up the technical-economic method are: The process of comparing in time and space the different indicators followed; Statistical grouping procedure; The division process; Graphic process; The process of chain substitution of factors (Popescu, 2007).
The process has two variants: a variant for the case when the relationship between factors is product and another variant when the relationship between factors is related.
If we have a product relationship, the process requires that these factors be considered variable in turn, while the others remain constant. For the correct application of the procedure, the following rules must be observed: The factors are in the order of their conditioning; Factors are replaced successively; A substituted factor remains substituted until the end; Replacement always starts with the quantitative factor.

RESULTS AND DISCUSSIONS

In terms of location and geographical conditions, the 3 units are located in plain areas, two in the northern part of the country and one in the southern area. The northern ones benefit from a milder climate and milder winters than the southern ones, which have warmer summers and colder winters.
The legal form of the three farms is different, the small one is organized in a Self-employed Person system, the medium one is an LLC and the one with the largest number of dairy cows is a commercial company.
Regarding the indicator - the size of the farm - both according to the land area owned and the number of owners, the three units are thus delimited, the small one has a number of 10 cows and an area of only 2 ha of arable land, the medium-sized one has 30 cows and an arable land of 9 ha, and the large one has 226 dairy cows and an arable area of 1540 ha.
The two small and medium-sized farms compensate for the lack of agricultural land by granting pasture that provides both green mass for dairy cows during the summer and hay for the cold season.
Given the number of animals, the land area owned, as well as the characteristics of the cow maintenance systems, the three units fall into the three classic operating systems: extensive (household), semi-intensive and intensive (Table 1).

Table 1. Unit identification data

Specification	**Farm type**		
	Small	Medium	Large
Location	Northwest (Livada)	Northwest (Salonta)	South
Geographical area	hill depression	plain	plain
The types of soil encountered	brown and peat	brown	brown chernozem – reddish forest
Climate regime	panonic type	panonic type	continentally-tempered
The destination of the farm	commercial farm which produces cow's milk		
The legal form of functioning of the farm	Self-employed Person	LLC	SA
Farm profile	mixed activity - vegetable and animal		
Farm size by surface	2 ha of agricultural land and leased pasture	9 ha of agricultural land and leased pasture	1540 ha agricultural land
Farm size by livestock	10 dairy cow heads from the Bălțată Românească breed and the related youth	30 heads of dairy cows from the Bălțată Românească breed	533 heads of dairy cows from the Bălțată Românească breed
Degree of mechanization on the farm	household system	semi-intensive	intensive

The technical equipment, as can be seen in Table 2, is different from one unit to another, while the farm needs a shelter, a haymaker, a shed and a dairy area, the other farms require larger investments, in more generous spaces, which serve units to the correct flows are ensured.

Table 2. Technical equipment of farms

Farm type		
Small	Medium	Large
Shelter; Paddocks; Dairy; Shed; Haymaker.	2 shelters; Paddocks; Dairy; Grain storage; Shed; Haymaker.	Veterinary sanitary filter; Dairy Installation for capturing solar energy Feed mill Material warehouse Shed 5 haystacks 15 silo cells Access roads to the farm 2 shelters arranged for maternity (2 shelters with 38 places each, resulting in a capacity of 76 accommodation places for cows and their calves); 2 outdoor paddocks; 5 shelters for dairy cows, each with a capacity of 104 seats; 1 youth shelter 3-6 months; 1 youth shelter 6-12 months; 1 youth shelter 12-18 months; 1 youth shelter over 18 months.

The differences in buildings and facilities are great between the three farms, if for the household system one shelter is enough, for the semi-intensive 2 shelters, for the intensive one a number of 5 shelters are needed for dairy cows, 2 for maternity and 4 for calves and youth of different ages.
The large farm, which also has a large area of agricultural land, needs, in addition to all the items listed in the table below, agricultural machinery and equipment that provides a mechanized basis for the properly work of the land. Over time, fewer and fewer people are available for employment in animal husbandry. From this point of view, the small farm is advantageous because it uses only the labor force of those within the family.
The medium-sized farm is primarily based on the permanent presence of the farmer (owner) and a small number of employees. Instead, the large farm needs a well-established staffing scheme to ensure the smooth running of the entire business within the unit. This requires both Technical, economic and socio-administrative staff and skilled workers (Table 3).

Table 3. Workforce in the units

Farm type		
Small	Medium	Large
Family and occasionally day laborers	3 employees and the farmer's family	- farm manager, economist - 2 technicians -10 milkers - 7 youth caregivers - 1 electrician - 3 mechanics - 3 night caretakers - 3 machine drivers - 1 dairy worker, - 1 forklift driver

The housing and restraint system of cows is different from one farm to another, while in all systems the cows are kept tied to the stand of medium size, the time period spent in the shelter is different. In the farm and semi-intensive farm, the cows are tied up in winter, during the summer they are free to graze during the day and sheltered at night.
In the intensive system, the cows are tied up in the shelter and only when the weather allows it are they directed to the outdoor paddocks where they are free.
In small farms, the movement of animals is easy, as the herd grows, this goal is more difficult to achieve, which can lead to foot problems.
The feeding technology is different in the three systems. In the household and in the semi-intensive system, feeding is done by grazing during the summer and by ration administered in a shelter during the winter. The winter ration includes hay, vegetables (often produced by the farmer or by-products of his household: potatoes, pumpkin, etc.) and a concentrated supplement obtained from grinding cereals.
In the intensive system, the feeding technology is complex and includes rations adapted to the age, category of animals, physiological condition and production of each animal. The unit must create a culture plan adapted to the requirements of the unit and moreover, it must purchase various products that cannot be made within it (vitamin-mineral premix, salt etc.). From this point of view, the management of the feed base becomes an important topic within the farm and has a large share in the unit's expenses.
Milking technology is similar in all units studied. In order to obtain a quality milk that can be marketed later, proper milking hygiene is required. Thus, all farms have been equipped with automatic canning milking systems, which ensure the premises of a compliant milk. How to store milk is also important, if in the small farm this is done in stainless steel vessels located in the dairy area, on medium and large farms large cooling tanks are needed to be able to store in optimal conditions the milk obtained.
Reproduction is an important component of farm management. At the farm, the reproduction is done naturally directed. This has the advantage that the date of calving, paternity is known and it is thus possible to achieve a more careful exploitation of the dairy cow. The birth rate obtained in this case was 80%. In a small herd of cows, the heat period can be easily tracked and the mounting is done at the optimal time.
On medium and large farms, reproduction was done by artificial insemination. The birth rate fell to 78% and 74% respectively for the large birth rate. The larger the number of dairy cows, the more time and attention the breeding activity requires from the farmer. In the large farm there is a calendar of uniform distribution of mounts and calving interval during a year, but even so it remains a major challenge for the manager, the optimization of the breeding activity (Table 4).

Table 4. Accommodation system analysis

Specification	Farm type		
	Small	Medium	Large
Shelter construction	- wooden shelter	- brick shelter with cement plaster and asbestos-cement roof	- brick shelter with cement plaster and roof made of galvanized sheet metal
The technology used	Tied up, medium size stand	Tied and free	Tied and free
Manure disposal	Manually	Manually	Mechanized
Ventilation	Naturally	Naturally	Naturally
Shelter hygiene	Disinfection, disinfection and rodent control		
The movement of animals	Pasture	Pasture	Outdoor courtyard
Feeding technology	Summer - pasture Winter - hay, succulents and concentrated supplement	Summer - pasture Winter - hay, succulents and concentrated supplement	Single mixture ration
Milking technology	Milking machines	Milking machines	Milking machines
Reproduction technology	Directed natural reproduction	Artificial insemination	Artificial insemination
- natality	80%	78%	84%

As can be seen in the table, the structure of the herd owned by each farm is simpler in the case of the small and medium-sized farm, the large farm has all the categories of cattle in operation. Tracking the evolution of each animal becomes a challenge for the manager, and requires a lot of time and additional human resources for each individual (Table 5).

Table 5. Livestock structure

Specification	Farm type		
	Small	Medium	Large
Livestock structure	- 10 dairy cows - 5 heifers - 5 calves - 4 calves	- 30 cows - 5 heifers - 7 calves	- 226 dairy cows - 55 heifers - 34 calves over 18 months - 34 calves 12-18 months - 44 calves 6-12 months - 32 calves 0-6 months - 28 calves 0-6 months - 80 fattening cattle

From the economic-financial analysis carried out at the level of the three farms, it can be seen that only the large one can be profitable. The small one, if the salary of the farmer and his family were considered, would have an annual deficit of -7,314 RON. At the medium-sized farm, the profit is RON 15,016.5 without considering the owner's salary, with his remuneration reaching a deficit of -112,207.5 RON. As can be seen, the deficit is increasing as the number of dairy cows kept increases.

For the large dairy cow farm, where there are both various investments and multiple expenses, the income was still considerable and at the end of 2020 a profit of +257,330 RON was registered (Table 6).

Table 6. Analysis of economic-financial indicators at farm level

Specification	Farm type		
	Small	Medium	Large
Annual production per cow (l)	3,820	4,050	5,849
Total farm production (l)	57,300	121,500	1,278,100
Total production delivered (l)	45,840	120,000	1,275,100
Total expenses (RON)	7,874 (without farmer's salary)	144,383.5 (without farmer's salary)	1,341,390
Total income (RON)	85,376	159,400	1,598,720
Profit (RON)	+77,502 (no salaries) -7,314 (with salaries)	+15,016.5 RON (no salaries) -112,207.5 (with salaries)	+257,330

CONCLUSIONS

Following the analysis of the activity carried out by the three farms under study, the following conclusions were drawn:

1. The units analysed are located in different geographical areas of the country, in the plains, being organized under different legal systems according to the number of animals and land areas owned and fall into the three classic systems of exploitation: extensive (household), semi -intensive and intensive.
2. The need for buildings, facilities and labor force show obvious differences between the three units studied. The need for labor is scarce across the country, and additional insurance measures are needed, such as involving family members on the farm.
3. While the maintenance system and the feeding technology are different in the three operating systems, milking is similar, due to all the hygiene measures necessary to ensure a high quality milk that complies with the relevant legal regulations.

4. It has been observed that the larger the number of dairy cows, the more time and attention the breeding activity requires from the farmer. On a large farm, for example, there is a timetable for the uniform distribution of mounts and calvings over a year, but in the average farm, breeding optimization is difficult.
5. From the economic-financial analysis carried out at the level of the three farms it can be seen that only the large one can be profitable. The other two are profitable only if the salary of the farmer and his family is not taken into account.
6. From the analysis of the three rearing and maintenance systems, in this case, the intensive dairy farming system seems profitable, but it requires the most investment, the largest number of staff and well-stocked feed bases that correspond to with generous land areas, for the realization of most of the fodder within the unit, thus having the fewest inputs. The problem of this system is also one related to sustainability in relation to environmental protection and the permanent assurance of quality products and integration into the food chain.

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STUDY ON THE VIABILITY OF THE YOUNG IN THE SPECIES *APIS MELLIFERA* ACCORDING TO THE SECRETORY CAPACITY OF ROYAL JELLY

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Abstract

The growth of the bee brood (the stage of the decapitated larva) is directly influenced by the ability of the nurse bees to secrete royal jelly. The secretion of the main precursors of royal jelly is made in the hypopharyngeal glands, located in the bee's head. After the nursing stage (bee capable of raising new generations of larvae), the hypopharyngeal glands regress and are responsible only for the secretion of invertases that ensure the transformation of nectar into honey. The study aimed to establish the degree of variation in the viability of the brood, correlated with the feeding with royal jelly from nurse bees, fed on diets with low protein intake (low in pollen). The study conducted in an apiary with 20 bee families, Apis mellifera carpatica, in autumn, with a lack of pollen in nature. A positive correlation established between the two factors studied. The degree of development of the hypopharyngeal glands that influence the condition of bee larvae as well as their viability. Monitoring in practice the level of protein in the feeding of nurse bees and the periods with greater or lesser need may be a criterion for determining the strength of bee families.

Key words*: Apis mellifera carpatica, hypopharyngeal glands, larval brood, viability.*

INTRODUCTION

After hatching, the young bee feeds on honey and pollen, performing various activities in the bee colony (cleaning cells for laying eggs, ventilating the hive, sanitizing, etc.). Within 6-7 days, the hypopharyngeal glands reach a maximum development (Klose et al., 2017), based on protein consumption, being capable of secretion, and the bee changes its function becoming a nurse bee (Browers, 1982) (Figure 1). This function allows the differentiated feeding of bee larvae, of different ages. Nurses also feed on the larvae of beekeepers (drones and queens), the composition of the food being different from that of the bee larvae. Usually, only perfectly formed nurse bees feed these larvae (Al-Ghamdi, 2011).

The period of maximum secretion lasts until around the age of 15-18 days, the bee changing its function into a collecting bee. Their role is to bring the nectar into the hive and then to participate in its transformation into honey, the hypopharyngeal glands having only the role of secreting an enzymatic fraction, necessary for inversion. Due to intense activity and wear, the bees die around the age of 35 days.

Bees hatched in late August and September play an important role in preserving the bee colony until next season. These bees have a lifespan of about 6 months, ensuring the growth of the brood, starting with the middle of January.

The longer life is mainly due to the lack of juvenile growth activity (high degree of wear) and the lack of long-distance flying activity outside the hive.

The hypopharyngeal glands are paired glands, acinous, with external secretion. The main function is the secretion of royal jelly, having a role in the secretion of an enzyme that participates in the inversion of nectar in honey. Each gland consists of a long tube (half the length of the body, when unfolded) to which more than 1000 acini are connected by peduncles. The structure of the acini is given by the pyramidal cells, responsible for the production of royal jelly, placed with the apex facing the inside of the acini. Elimination of the secretion from the cell is done through a

cellular lumen. The nuclei have a variable number of nucleoli, depending on the secretory state of the cell. During maximal secretion, they migrate to the base of the cell, pushed by the secretory cisterns. The increase in the volume of acini during the activity of maximum secretion is given by the accumulation of royal jelly in cisterns, which have different points of formation (similar to the formation of crystals in a metal) reaching to converge in a single cistern inside the cell, eliminating secretion. through the lumen of the cell and then through the lumen of the acini into the gland tube (Corby-Harris & Snyder, 2018).

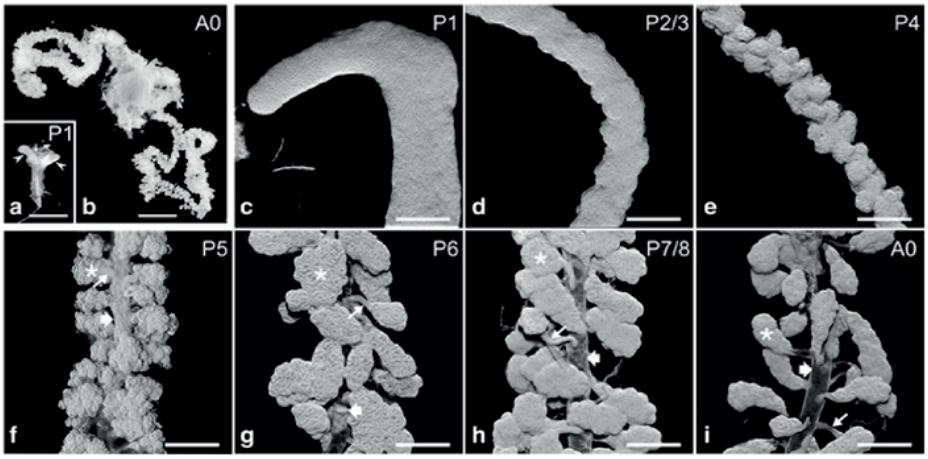

Figure 1. Hypopharyngeal gland morphogenesis (after Klose et al., 2017)

After secretion, the cell has a flabby, elongated shape, capable of secreting enzymes necessary to invert the nectar in honey.

MATERIALS AND METHODS

Carpathian bees (*Apis mellifera carpatica*) were used as a mountain ecotype harvested from bee families in full activity (active season) and from bee families entering winter (inactive season - October). Bees were also harvested during the winter from a colony located in an enclosure with controlled temperature and humidity conditions.

The summer bees were harvested from the honeycomb area with uncapped brood, and in winter, randomly from the hive.

The glands were gutted and used in observations in two ways:

- permanently prepared on the slide (hematoxylin-eosin staining);
- freshly prepared between slide and slide, in saline solution (weak Giemsa coloration).

The observations were made with an MC5 microscope, using the x20, and x40 objective lens.

In the controlled room, the conditions specific to the development period of the bee colony (spring) were simulated.

Different foods were administered. Simple pollen and honey, ad libitum, were used as feed for the Colony 1. A pollen-based recipe enriched with amino acids and vitamins, ad libitum, was used for Colony 2.

RESULTS AND DISCUSSIONS

The hypopharyngeal glands are named after Snodgrass, who in 1956 confirmed that the ducts open on the suboral plate of the hypopharynx. They act as secretions for most components of royal jelly, feeding on bee larvae (Snodgrass, 2018).

The secretion of royal jelly is conditioned by the presence of larval pheromones, by conditioned reflex. released by each larva. The study of the degree of development of these glands is closely related to the developmental capacity of the larval brood as well as the production of breeders.

The study aims to analyse the differences between the quality of the offspring obtained

during the active season and that obtained during the inactive season. Clarification of these aspects is important for the spring development period of bee families, especially in the conditions of climate change that manifests itself in recent decades (Browers, 1983; Zarić et al., 2022).
The results of this study can be capitalized on by developing new techniques for the maintenance of bee families or adapting existing techniques to the new climatic conditions. There are no recent studies showing the influence of climate change on the growth of larval broods during the spring development of bee families.

Bees harvested during the active season
The hypopharyngeal glands in 5-7 days old bees are very well developed (stage 4). But, in the developmental periods of bee families, when there is abundant feed, the hypopharyngeal glands develop even earlier (5 days) (Figure 2).

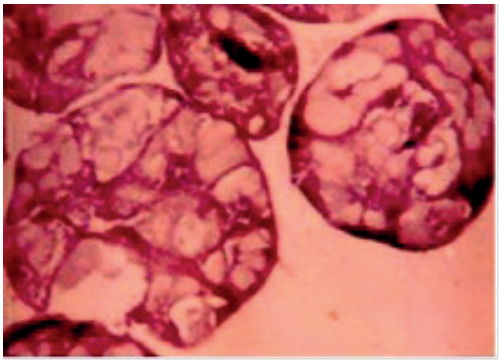

Figure 2. Glands in stage 4 (x40 objective lens)

The glands completely occupy the front surface of the bee's head, they are strongly contorted, the acini are voluminous, well developed, spherical in shape, the cells are clearly highlighted, even with a small objective lens (x20) (Figure 3). As the glands develop, the nucleus is pushed to the edge of the cell.
The bees were harvested at different times depending on the quantity and quality of pollen in the wild.
It was observed that during periods of pollen deficiency, although the hypopharyngeal glands were well developed (stage 4), the cisterns had a semi-mimicked consistency.

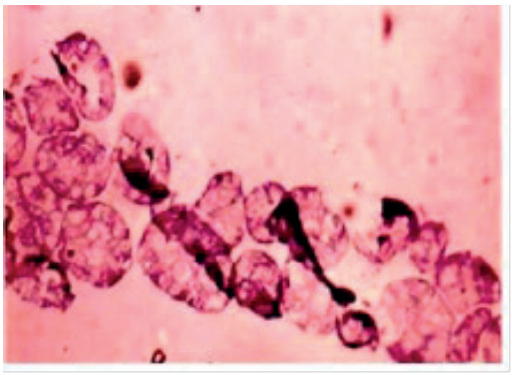

Figure 3. Glands in stage 4 (x20 objective lens)

At the age of 17-18 days, the glands underwent an involution, reaching stage 1, the secretory activity consisting only in the elimination of the enzymatic fraction necessary for the inversion of the nectar, the period corresponding to the passage of the bees in the stage of harvesting (Figure 4).

Bees harvested during the winter - in the apiary
Bees hatched in late August and the first half of September have perfectly developed hypopharyngeal glands at the age of 6 days, due to the smaller surface area of the brood.
Bees hatched in the last decades of September have incompletely developed hypopharyngeal glands (stages II-III).
The glands remain in this stage until around January 10-20, when the brood appears in the bee colony, after which it develops to its maximum capacity, there being no differences compared to the bee glands hatched during the active period.

Bees harvested during the winter - in the room with controlled conditions
Between October 15 and April 15, two bee families were maintained on the premises, simulating spring conditions (T = 15-25°C, about 80% humidity, characteristic light regime with twilight simulation).
Colony 1 was fed with honey and honeycombs at discretion, and Colony 2 was forced to consume more pollen by using an attractant.
As a result of differentiated feeding, Colony 1 started growing the brood around January 7, the brood area being smaller than in the apiary

families. Due to the growth of a smaller brood area that could not ensure generational change, it did not last until the end of the experiment (Figure 5).

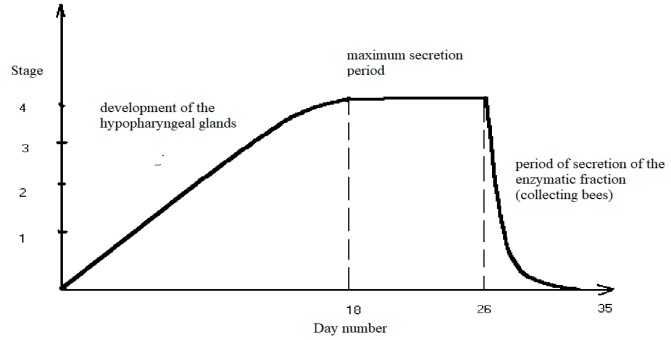

Figure 4. Development and involution of the hypopharyngeal glands in bees

Colony 2, in which an overdose of protein food was performed, started raising the young around November 20th. Although the area of the brood was comparable to that of the colony 1, a rhythmic growth of the brood was observed, the periods with brood alternating with the periods without brood (Figure 6).

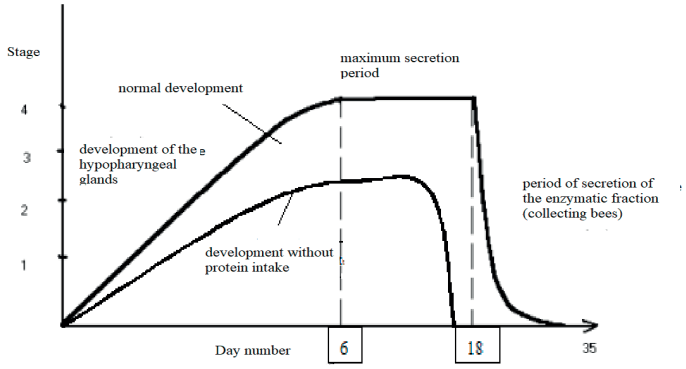

Figure 5. Development and involution of the hypopharyngeal glands in apitron with and without protein intake

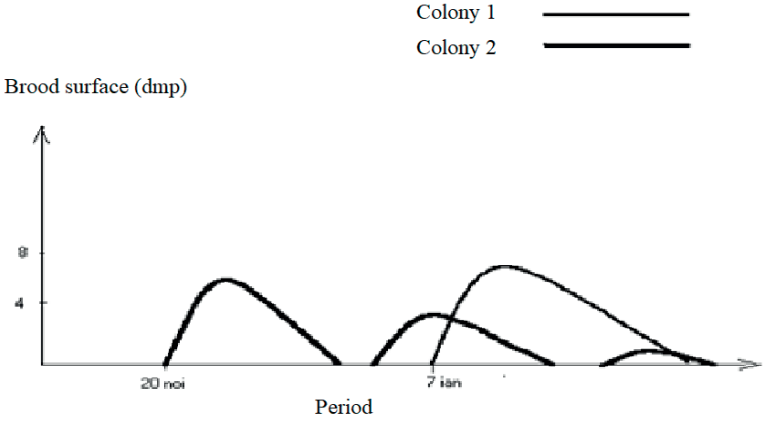

Figure 6. Raising the brood in the apitron in two differentially fed colonies (protein intake)

The colony 2 lasted until March 5, when in fact there were no more offspring.
The hypopharyngeal glands in bees of colony 1 remained incompletely developed (stages I-II) and there were no bees with glands in stage III. When the seedlings appear, the glands develop up to stage III. Acins were incompletely developed, and the cells had nuclei with fewer nucleoli. The cisterns had a semi-identical consistency.
In bees of colony 2, the hypopharyngeal glands developed up to stage 4, but the consistency of the cisterns was similar to stage 3.
The brood raised by both families had protein deficiencies, having a low survival rate, not being able to grow another generation of brood.

CONCLUSIONS

The results of the study show differences between the hypopharyngeal glands of bees hatched in the active season and those that over winter. In bees kept on the premises with controlled conditions, specific phenomena of protein deficiency were observed, although the protein feed was overdosed and the brood was present (factor that triggers the secretion of glands). The young raised by these families were crawling, showing protein deficiencies, a phenomenon materialized in the delayed development of the glands and the impossibility of raising a new generation. The change in environmental factors in apitron induced minor or insignificant changes.
The second generation of bees raised in the enclosure, although they had glands corresponding to stage 4, did not have the matte consistency of the tanks (specific to this stage) and the nuclei had a smaller number of nucleoli, indicating a low metabolic capacity.
The quality of protein food, in this case pollen, induces the proper development of the hypopharyngeal glands. This aspect can be linked to the observation that the first generations of bees in a season able to raise in turn other strong generations of bees, are those that feed on the fresh pollen from nature, the spring pollen.
Protein substitutes, widely used in beekeeping, ensure poor development of the hypopharyngeal glands.

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RESEARCH ON MORPHO-PRODUCTIVE INDICATORS OBSERVED OF AUBRAC AND ABERDEEN ANGUS CATTLE BREEDS

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Abstract

The purpouse of this paper was to highlight the morpho-productive indicators of Aubrac and Aberdeen Angus cattle breeds exploited in Romania. Data were collected from a number of 40 cattle, following the weaning weight (age of 7 months) and the average daily gain increase of the animals, which benefited from similar exploitation conditions. Also, based on the results obtained, a statistical interpretation was performed. At the age of 7 months, an average body weight of 216.6 kg was observed in Aubrac cattle, with an average daily gain increase of 912 g/day, while in Aberdeen Angus cattle an average body weight of 184.3 kg was observed, the animals recorded an average daily gain increase of 799 g/day. In conclusion, in the case of both breeds of cattle, the satisfactory parameters specific to the breeds of meat were highlighted, but making a comparison we can appreciate that the results observed in the Aubrac breed are superior.

Key words*: beef cattle, indicators, performances.*

INTRODUCTION

Beef is a high-quality protein source which can also give very satisfying dining experiences, and demand for it is growing over the world. The beef industry's long-term viability depends on high on-farm efficiency and productivity, as well as efficient value chains that reward meeting target-market standards (Jurie et al., 2015). These elements also help to lessen the environmental and animal welfare implications that are required for provenance and social license. To optimize income and restrict input costs, beef production systems that employ beef breeds should target optimal genotypes and high productivity relative to upkeep for the breeding herd and for growing and finishing cattle, especially feed, which can account for 60% or more of production expenses (Pesonen et al., 2015). Beef production may be sustained where grasslands or rangelands are the principal source of nutrients. This is especially true considering that the feed resources in these systems are unfit for human consumption (Sheveleva, 2008).

Aubrac and Angus breeds are the newest breeds of beef cows that populate farms in Romania. The qualities of adapting to the climate of our country, the profit that can be obtained both from the sale of bulls and processed products, the minimum costs of care, disease resistance, but also the fact that they are very easy to maintain have determined Romanian farmers to give up dairy cows and to raise meat breeds brought in from abroad.

The Aubrac cattle breed originates in France; This breed was imported to Romania in 2013, adapting very well to the conditions of growth and exploitation. Aubrac cattle are medium-sized animals (adult cows: height 130 cm, weight 550-800 kg, bulls: height 140 cm, weight 900-1200 kg). They are rustic animals that have special biological properties, of high resistance and adaptability to diseases and severe environmental conditions, with a very long longevity, being recognized for light calving (Mădescu et al., 2021). They are very easy to maintain, they make very good use of all categories of fodder, they adapt quickly to feeding on large, poor pastures, without significantly influencing their productive level. They are well adapted to harsh local climates and can thrive very well when fed on poor quality pastures (Valadier, 2004). Also, their body is very well adapted to store energy during

periods of abundant food and then they can reserve energy for less favorable periods. The Aubrac breed is famous for the special aroma and tenderness of the meat, having a high degree of marbling, special taste and a very good bone/meat ratio (Figure 1). This ratio of meat to bone leads to a good, consistent carcass weight with superior meat quality (Sheveleva et al., 2021).

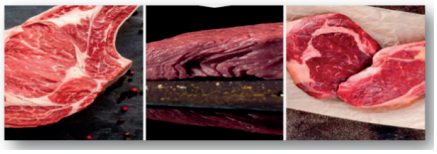

Figure 1. Aubrac beef

The Angus breed is also one of the most popular meat breeds in the country. The Aberdeen Angus breed is native to Scotland; This breed was imported to Romania at the end of 2007 (Gociman et al., 2019). Cows are animals that adapt easily to climatic and environmental conditions, being easy to exploit and meat has special nutritional proportions (Bartoň et al., 2006). It is an excellent breed for extensive growing systems, well suited for grazing, light calving. Cow weight: 500-700 kg, bull weight 800-1000 kg (Mukhamed Shakhmurzov et al., 2021). Fine, marbled meat highly prized (Figure 2).

Figure 2. Aberdeen Angus beef

The average amount of weight gained by an animal each day throughout the feeding period is known as the average daily gain (ADG). ADG is computed by dividing the weight gained by the number of days since the last weight of an animal (Nikolov & Karamfilov, 2020).

MATERIALS AND METHODS

The data collected for this research come from the records of the ANGUS RO association, registered between 2019-2021. In this study, 80 animals were studied, 40 of the Aubrac breed and 40 of the Aberdeen Angus breed, following the weaning weight (age of 7 months) and the average daily increase of the animals, which benefit from similar exploitation conditions (Figure 3).

Figure 3. Aubrac and Aberdeen Angus cattle

The database included information on the calving weight of calves, the average daily growth and the body weight of the animals at 7 months. Also, based on the results obtained, a statistical interpretation was performed. Thus, the productive performances of the studied animals were compared, depending on the breed.

RESULTS AND DISCUSSIONS

Average daily earnings (ADG) is a performance measure that many beef producers monitor.
The average daily gain is simply the rate of weight gain per day over a period of time (Bures, D. and Barton, L., 2012). Average daily earnings (ADG) are an important component of production efficiency for raising beef cattle.
The mathematical relationship between the traits that contribute to efficiency implies that for a pair of calves with the same initial body weight, the one with a faster ADG will reach a target market weight with fewer days of feed and thus could be more efficient due to allocating less food for maintenance.
Table 1 shows the values of the production indicators, registered in the case of the 80 cattle studied, during the period 2019-2021. Thus, a division was made by breeds for which the following indicators are represented: weight at the age of 7 months and average daily gain.

Table 1. The values of the production indicators, registered in the case of the 80 cattle studied

	CATTLE BREED			
	Aubrac		Aberdeen Angus	
No.	Weight 7 M (kg)	ADG 7 M (g/day)	Weight 7 M (kg)	ADG 7 M (g/day)
1	166	739	183	800
2	180	878	164	735
3	209	988	147	615
4	221	1008	210	805
5	273	1146	159	730
6	196	715	177	865
7	303	1266	209	1040
8	272	1099	180	730
9	160	730	250	965
10	172	870	193	915
11	288	1310	198	780
12	233	842	223	915
13	261	1037	181	720
14	188	796	237	1005
15	170	835	208	855
16	200	667	127	425
17	255	1027	134	700
18	162	750	170	830
19	149	710	163	730
20	194	827	173	820
21	166	739	183	800
22	180	878	164	735
23	209	988	147	615
24	221	1008	210	805
25	273	1146	159	730
26	196	715	177	865
27	303	1266	209	1040
28	272	1099	180	730
29	160	730	250	965
30	172	870	193	915
31	288	1310	198	780
32	233	842	223	915
33	261	1037	181	720
34	188	796	237	1005
35	170	835	208	855
36	200	667	127	425
37	255	1027	134	700
38	162	750	170	830
39	149	710	163	730
40	194	827	173	820

Table 2. Production indicators - Aubrac and Aberdeen Angus cattle breeds

Breed	Performance	Mean	Median	Standard Deviation	Kurtosis	Skewness	Minimum	Maximum	Count
Aubrac	Weight 7M (kg)	212.6	198	47.0291	-1.0837	0.49597	149	303	40
	ADG 7M (g/day)	912	856	186.829	-0.5300	0.68222	667	1310	40
Aberdeen Angus	Weight 7M (kg)	184.3	180.5	31.876	-0.3650	0.21685	127	250	40
	ADG 7M (g/day)	799	802.5	138.199	1.2243	-0.62016	425	1040	40

Table 2 shows that the minimum weight at the age of 7 months is 149 kg and the maximum weight is 303 kg in the case of the Aubrac breed, while in the case of the Aberdeen Angus breed there are weights less, than 127 kg minimum and 250 kg maximum, at the same age of 7 months. In 2021, Dydykina et al., observed at 7 months a weight between 151- 206 kilograms in Aberdeen Angus cows, with an average daily gain 590-960 g/day.

Sheveleva and her collaborators observed in 2021 at the age of 9 months a weight between 216 and 310 kg in Aubrac cows, registering an average daily increase of 861-1089 g/day.

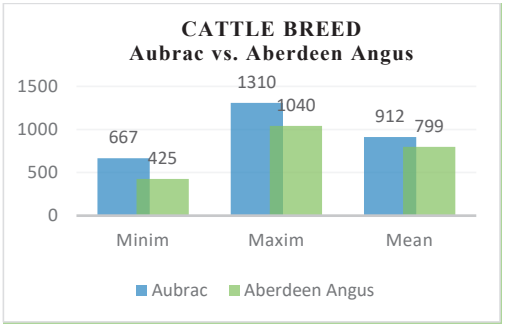

Figure 4. ADG was observed in the two breeds of cows studied (g/day)

Figure 4 shows the observed averages of the 80 animals studied in terms of ADG. The lowest value in the case of the Aubrac breed was 667 g/day at the age of 7 months, and in the case of the Aberdeen Angus breed it was 425 g/day at the same age. It can also be seen that the maximum recorded ADG value was 1310 g/day for the Aubrac breed and 1040 g/day for the Aberdeen Angus breed, respectively.

From Figure 4 it can be seen that the ADG is higher in the case of the Aubrac breed (912 g/day), compared to the Aberdeen Angus breed (799 g/day). Research conducted by Mukhamed Shakhmurzov and his collaborators in 2021, showed an average daily increase of 754 and 675 g/day recorded at the age of 7 months in Angus cows. In 2021, other researchers, namely A.I. Dydykina et al., observed an average daily increase in 590-820 g/day in Aberden Angus calves between 0-7 months and 740-960 g/day at the age 7-12 months. Other authors such as O.M. Sheveleva and her colleagues in 2021, observed an average daily increase of 1089 g/day recorded at the age of 9 months in Aubrac cows.

Table 3. Testing the differences between ADG averages in the case of Aubrac vs. Aberdeen Angus

Pooled Variance	27002.10256
Hypothesized Mean	0
df	78
t Stat	3.075350714
P(T<=t) one-tail	0.001449158
t Critical one-tail	1.664624645
P(T<=t) two-tail	0.002898315
t Critical two-tail	1.990847069

When the null hypothesis was tested, it was found that there were no significant differences between the two variables (ADG for Aubrac breed vs. ADG for Aberdeen Angus breed).

When testing the alternative hypothesis, it was observed that, with an accuracy of 95%, there are significant differences between the two variables studied. Thus, we can appreciate that the Aubrac breed has higher performances than the Aberdeen Angus breed, being observed higher values of ADG.

CONCLUSIONS

This research highlights the productive indicators of beef breeds exploited for meat production, Aubrac and Aberdeen Angus, breeds that are a trend of Romanian beef farms. The observed performances are satisfactory for the Romanian farmers, being in accordance with the exploitation conditions in our country. Research has shown that there are significant differences between the two breeds of cattle, with higher performance in the Aubrac breed. Performance can vary considerably depending on the rearing system, so it is recommended that beef benefit from a balanced diet with a high nutritional value.

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RESEARCH OVER CARCASSES QUALITY OBTAINED BY THE USE OF ROMANIAN BREEDS IN CROSSING WITH MEAT RAMS

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Abstract

The research aim was to assess the possibility of increasing the quality of carcasses provided by young sheep undergoing fattening. In this sense, the biological material used was represented by industrial half-breeds from several forms of crossbreeding between Romanian sheep breeds and meat rams' breeds. The maternal form that was the basis on suppling biological material subject to fattening was represented by F1 crossbred females resulting from the crossing of Blue faced Leicester (BL) rams with local Merino of Palas (MP) and Țigaie (TI) females. In obtaining fattened lambs, Suffolk (S) rams were used as a terminal breed. In order to meet the objectives, set by the experimental protocol, research batches were organized that benefited from the same experimental treatment. Control sacrifices were performed to determine the carcass quality at the end of fattening. The assessments made indicate that in the case of two control groups, made up of individuals belonging to the local Merino and Țigaie breeds, no situations were reported in which the exterior of the carcasses met the requirements for classification in the S.E.U. The assessment of the degree of development of the muscles on the upper line indicates clear differences between groups. In the case of determining the area of the muscle eye in carcasses obtained by slaughtering lambs from batches S x (BL x MP) shows that the average value is approximately 3 cm^2 higher than the control batch, and in batch S x (BL x TI) the difference expressed in absolute values is + 3.43 cm^2.

Key words*: carcass conformation, lamb, meat sheep, muscle mass, Romanian sheep.*

INTRODUCTION

An important index in assessing the standard of people living, along with energy consumption, is the contribution of meat, milk, eggs, butter, etc., all reported per capita. However, worldwide, for each country, more than 50% of the total amount of animal origin protein from food is provided by the daily consumption of meat. However, this percentage undergoes changes from one geographical area to another because it is strongly influenced by the social and economic factors specific to each people (Pascal, 2015).

At this time in the literature most scientific information refers to the genetic variation in the quality of sheep meat resulting from purebreds or from different batches of industrial half-breeds. The role and scientific destination of these data, although of some importance, are useful only in establishing the principles of genetic variation. On the other hand, this data is not very clear when we look at the concrete definition of breeding objectives or when we aim to identify some genetic markers that could be used in determining the quality of meat.

In general, while an increasing number of studies have investigated differences in breeds in terms of meat quality, the differences in breeds included in various scientific sources are not always consistent, concrete or convincing.

Regarding the genetic variation for some quantitative characteristics of meat production, there are some publications that present evidence of differences between breeds in terms of meat colour (Carson et al., 1999; 2001; Dawson et al., 2002; Martinez-Cerezo et al., 2005) and fat colour. Moreover, Legrand et al. (2002) suggested that differences may occur between breeds in terms of fat quality, especially fat colour. These studies show that there are differences and that lambs obtained from the use of Texel rams had a more acceptable colour score for subcutaneous fat compared to lambs produced from Charolaise rams. Crouse et al. (1981) also reported the

effects of breed on fat colour, and in Suffolk lambs' subcutaneous fat is denser and yellower than Rambouillets lambs.

MATERIALS AND METHODS

The biological material used in research was represented by several batches of young sheep half-breed obtained from systematic crosses of adult sheep belonging to Romanian breeds (Merino of Palas and Țigaie) with rams belonging to meat (Blue faced Leicester and Suffolk). The maternal form used in the crossbreeding to obtain crossbreeds intended for the research of the established objectives was a first-generation crossbreed sheep (F_1) obtained from the crossing of Blue faced Leicester rams with the local sheep Merino of Palas and Țigaie. In order to obtain the desired crossbreeds, these crossbred mothers were crossed with Suffolk terminal rams.

In order to assess the real skills specific to meat production, several batches were set up which benefited from the same experimental treatment. Subsequently, the performances of the batches of mixed lambs were compared with those recorded in the control batches consisting exclusively of young people belonging to local breeds, subjected to fattening under the same conditions of feeding and maintenance.

The applied fattening technology was of intensive type and had a total duration of 90 days, being extended on three technological phases (accommodation, growth and fattening and finishing). During the fattening, a ration was administered with an optimal structure so that the fattened youth would externalize their productive performances.

In the assessments made, in order to avoid errors calculation induced by gastrointestinal contents, the slaughtered individuals were not fed 12 hours before slaughter. The objective assessment of carcasses was made by determining the following elements: carcass mass, slaughter yield, determination of carcass physical structure, classification of carcasses by quality classes according to the methods applied in the European Union and establishing the cut portions of the carcass according to quality. The planimetry method was used to assess the development of muscle mass.

Data processing was performed using the REML (Restricted Maximum Likelihood) procedure, which guarantees that estimates are obtained in the normal range of parameters.

RESULTS AND DISCUSSIONS

The quality of the carcass is an important objective of the activities carried out in the production of sheep meat.

The carcass name is given to the compact part resulting from the slaughter of an animal after bleeding, skinning, evisceration, removal of the head, genitals, internal organs (heart, lungs, kidneys, etc.) and removal of the extremities of the forelegs and hindquarters from the metacarpal joint and the metatarsal joint.

In order to properly interpret the information used to assess the quality of the carcass, it is necessary to properly manage the data on hot and cold carcass weight, as it affects other important parameters such as fat content, carcass conformation and weight of different pieces (Díaz, 2001; Quoted, et al., 2014; Carter et al., 2008; Lambe et al., 2009).

In the case of carcass marketing, more attention should be paid to the fat content, as when it is in excess it also has a certain impact on the price of the carcass (Díaz et al., 2002). Some of the measures for this criterion are dorsal fat thickness, renal pelvic fat weight, and visual assessment of carcass fat content (Díaz et al., 2002; Carrasco et al., 2009).

Another variable that is important and used as a basic indicator in expressing the quality of the carcass is its conformation (Díaz, 2001). Conformity assessment is done on the whole carcass and involves a visual assessment and objective measurements such as chest width and depth, leg length, groin width or rib area, etc. (Díaz, 2001, quoted by Ramírez et al., 2014). To the above criteria can be added the types of pieces obtained from the carcass and the ratios between the different types of tissue; bone, muscle and fat (Díaz, 2001; Rodrigues et al., 2006).

Assessing the conformity of carcasses resulting from slaughter was a primary objective of the research conducted. According to the experimental protocol, the research included among the objectives and activities aimed at analysing factors associated with the exercise

of aspects related to the emergence of differences in carcass conformation from the slaughter of young sheep belonging to different genotypes. Conformity is appreciated and of particular importance in terms of meat trade, which often face uneven carcasses that differ greatly in shape and lack data relating to knowledge of breed data, etc. (Kirton et al., 1967).

Given the practical importance of conforming the carcass in the meat trade, a standard grid has been developed in the European Union, which is now a reference in all slaughterhouses. In order to evaluate the conformation of the carcass, the analysis of the essential parts in which there are high quality muscle masses is taken into account. This is justified by the fact that we are traditionally looking for a carcass with wide and well-filled thighs, with thick and short legs, wider than long and with a slightly developed neck in length: that is, a wide and short carcass. In addition, the butcher is looking for carcasses that will allow him to sell as much of the total carcass as possible in the form of sliced portions of the highest quality. Most often there is a tendency to associate the good conformation of the carcass with an increase in the proportion represented by the jigou and the muscle masses in the essential parts.

The conformity assessment of carcasses was based on visual assessments of carcasses resulting from control slaughter. In accordance with the working methodology applied, this assessment was based on the assessment of the degree of development of muscle mass established in relation to the profile recorded in the essential parts of the carcasses, namely the back, middle and front (Table 1). Also, given the fact that adipose tissue also plays an important role in determining the quality of carcasses, evaluations were also made according to the degree of fattening. In the latter case, the assessments were based on an analysis of the fat on the inside and outside of the carcasses (Table 1).

The assessments made indicate that in the case of the two control batches, consisting of individuals belonging to the local Merino and Țigaie breeds, no situations were reported in which the exterior of the carcasses met the requirements for classification in the S.E.U. Following the assessment of the carcasses obtained by the control sacrifices of the young belonging to the Merino of Palas breed, it was found that a proportion of over 60% met the minimum requirements for their classification in class R and only 19.67% for class U.

Table 1. Conformity classification of carcasses

Specification	Genotype			
	Merino of Palas	S x (BL x MP)	Țigaie	S x (BL x Ti)
by conformation (%)				
S	-	-	-	
E	-	33.33	-	16.67
U	19.67	50.00	-	33.33
R	60.66	19.67	50.00	33.33
O	19.67	-	33.33	16.67
P	-	-	16.77	-
by degree of fattening (%)				
1	-	-	-	-
2	16.67	-	16.67	-
3	33.33	-	66.66	-
4	50.00	50.00	16.67	83.33
5	-	50.00	-	16.67

Notes: S = Suffolk; BL = Blue faced Leicester; MP = Merino of Palas; TI = Țigaie

Figure 1. Carcass conformity in Merino of Palas (a) and in half-breeds obtained by the use of this breed (b)

Figure 2. Carcass conformity in Ţigaie (a) and in half-breeds obtained by the use of this breed (b)

There is a significant increase in the quality of carcasses in industrial crossbreeding resulting from the crossing of Suffolk rams with F1 half-breed sheep resulting from the mating of Blue faced Leicester rams with Merino of Palas ewes. Applying the same evaluation system shows that in this experimental batch approximately 88.33% of the carcasses clearly and visibly had the minimum requirements specific to classes E and U and only 19.67% for class R.

Other studies found that differences in genotype were evident and significant for conformation using the EUROP scoring system, and that merino lambs had a higher proportion ($P<0.001$) of lower scores, especially compared to Poll carcasses. Dorset × (Border Leicester × Merino) and Texel × BLM (Border Leicester × Merino) (Hopkins et. al., 1997).

The carcasses evaluation by fattening shows a different distribution of carcasses by the five quality categories (Table 1). Regarding the distribution of fat on the external surface of the carcasses, it was found that while in the local breeds the adipose tissue was thicker in the posterior area was almost absent in the anterior area. On the other hand, the carcasses obtained by slaughtering mixed individuals showed a uniformity of the fat layer and a total coverage of the carcass with this tissue.

The assessment of carcasses by fattening indicates a situation similar to that presented in the assessment of carcasses on the basis of conformity analysis. In the case of assessing the carcasses resulting from the slaughter of individuals belonging to maternal breeds, there were no situations that would allow them to be included in batch 5 (the most favourable). Of the total carcasses obtained from the Merino of Palas purebred batch, approximately 50% of them were found to meet the requirements for classification in batch 4 and 33.33% for the requirements for classification in batch 3. In the batch Ţigaie which in this experiment as a witness, it is found that at a proportion of 66.66% the minimum conditions of batch 3 were met and only 16.67% for the quality batch marked with 4.

In the batches consisting of three-breed crossbreeds, the carcasses had a different quality and had much improved features, many being similar to those specific to lambs belonging to meat breeds. In both batches of all mestizos, following the application evaluation, it was found that the minimum requirements for inclusion in the higher batches were met. The difference between the batches was represented by the fact that while in the batch S

x (BL x MP) at about 50% of the evaluated carcasses the minimum requirements to be classified in the best class were found (class 5). At the end of performing the same assessment on cross-bred carcasses S x (BL x TI) for the same classification class, only 16.67% met the minimum requirements.

These results show, first of all, that the cross-pattern applied clearly improves the conformation of the carcass, which is very close to the characteristics of the meat breeds. Secondly, these results justify the usefulness of conducting research, but also the need to expand the practice of obtaining crossbreeds for meat, but in a systematic way. All this will contribute to the increase in the export of sheep meat to carcasses, especially on the market represented by the European Union, but also to the profitability of sheep farming in the main traditional breeding grounds for these breeds.

Assessing the degree of muscle mass development is a feature that enjoys special attention to the expression of carcass quality because it is considered an objective indicator in assessing muscle mass in the carcass (Southam et al., 1971; Pascal et al., 2014). Regarding the methods for determining the surface area of the muscle eye, Hillers J.K., quoted by Murat et al., 1995 shows that there are no statistically significant differences between the planimetry method, the Polish method and the determinations performed using the graduated grid.

The determination of the surface of the muscular eye is made at the level of the space between the dorsal or lumbar vertebrae, but lately there is an increase of preoccupations that take into account other new areas for determination, so that the registered values are as representative and eloquent as possible. The surface area of the muscular eye was determined in the present studies at the level between the ribs 5-6 but also at the level of the pulp, and the data obtained for all the experimental batches are presented in Table 2.

Table 2. Average muscle surface area values

Genotype	No.	Muscle surface (cm^2)					
		Longissimus dorsi			Jigou muscles		
		$\overline{X} \pm s_{\overline{x}}$	Difference		$\overline{X} \pm s_{\overline{x}}$	Difference	
			absolute (±)	difference significance		absolute (±)	difference significance
MP	5	13.99 ± 0.08	-	-	104.14 ± 0.24	-	
S x (BLxMP)	5	16.96 ± 0.10	+ 2.97	*	157.72 ± 0.48	+ 53.58	**
TI	5	13.44 ± 0.59	-		99.13 ± 0.57	-	
S x (BLxTI)	5	16.87± 0.66	+ 3.43	*	156.17 ± 0.84	+ 57.04	**

Note: S = Suffolk; BL = Blue faced Leicester; MP = Merino of Palas; TI = Țigaie

Assessing the degree of muscles development on the upper line indicates clear differences between batches.

In the case of determining the area of the muscle eye in carcasses obtained by slaughtering lambs from batches S x (BL x MP) it was found that the average value is about 3 cm^2 higher than the control batch consisting of young belonging to the local breed Merino of Palas, and the batch metis S x (BL x Ti) the difference expressed in absolute values is + 3.43 cm^2 compared to the average value determined on the carcasses resulting from the control sacrifices of the fattened youth. All differences between batches were significant for the statistical thresholds taken into account.

Compared to other data in specialized publications, these values are close. Thus, in the profile literature, values of 16.65 cm^2, of 14.64 cm^2 for the Merino of Palas, of 12.85 cm^2 for Țigaie and of only 9.75 cm^2 for Țurcană are cited for the Carne-Palas Line (Vicovan et al., 2013; Pascal, 2011; Pascal et al., 2014).

Regarding the sections practiced in the jig muscles, the differences between the experimental and control batches are even more obvious. Thus, expressed by absolute values, the difference between the average values obtained in the hybrid batch S x (BL x MP) was higher by +53.58 cm^2 compared to the average value recorded in the control batch. In the analysis performed in batch S x (BL x

TI) the surface of the leg was superior by +57.04 cm^2.

The existence of these clear differences and the high degree of their significance indicate very good skills for meat production in batches of three-racial crossbreeds, simultaneously with an increase in carcass conformation.

CONCLUSIONS

The industrial crossbreeds resulting from crossing Suffolk rams with F1 crossbred ewes resulting from the mating of Blue faced Leicester rams with Merino of Palas ewes show a significant increase in carcass quality because after the evaluation it was found that in this experimental batch approximately 88.33% of the carcasses clearly and visibly had the minimum requirements specific to classes E and U and only 19.67% for class R.

Assessment of the carcasses conformity resulting from the batch of young sheep resulting from the pairing of Suffolk rams with F1 crossbred sheep (Blue faced Leicester x Ţigaie) found that approximately 50% of carcasses met quality requirements for E and U favourable effect due to the practice of those crossings.

When evaluating the carcass by the presence and distribution of fat layer on the inside and outside of the carcasses, it is observed that while in batch S x (BL x MP) at about 50% of the carcasses evaluated the minimum requirements for the best class (class 5), for carcasses obtained from the batch crossbreed S x (BL x TI) for the same classification class met only the minimum requirements of 16.67%.

In the case of determining the area of the muscle eye in carcasses obtained by slaughtering lambs from batches S x (BL x MP) it is found that the average value is approximately 3 cm^2 higher than the control batch (MP), and in batch S x (BL x Ti) the difference expressed in absolute values is + 3.43 cm^2 compared to the determined average value and the control batch was only from the Ţigaie breed youth.

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RESEARCH ON CURENT EVALUATION STAGE OF CURL TYPE IMPROVEMENT FOR KARAKUL OF BOTOȘANI

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Abstract

The aim of this research was to perform an objective analysis of improvement process for the type of curls on the pelt surface. The research importance is special because in improving this character the desire of the breeder is different. The biological material belonged to the Karakul of Botoşani breed and the entire herd from which the evaluated lambs came is registered in the Genealogical Register of the respective breed. The method applied in assessing the quality of skins was based on the technical norms specified in Section 1.4 and 1.5 of the MADR Order no. 22/20.01.2006. Statistical processing of the data was based on the use of the computer program S.A.V.C. (Statistics Analysis of Variance and Covariance 2003). The assessment of the degree of improvement indicates that the desired curl represented by the shape of long and medium tubes is in an advanced stage as that type increased from 65.11% in 2005 to 66.66% in lambs' subject to assessments in in 2020. During this time, as a result of the improvement, the proportion of lambs with medium and long tube curls increased by more than 1.5% in the black and greyish variety, the difference being significant for p<0.01.

Key words*: curl, genetic improvement, Karakul of Botoșani, pelts.*

INTRODUCTION

The main purpose of the planned research was to identify the degree of improvement for certain production characters specific to the Karakul de Botoșani breed.

In addition to the specific characteristics of the fibres that form the hair coat, the economic and aesthetic value of a skin is given by other features specific to the type of curl, namely: height, width, length and degree of closure. Some of these characters have a superior ameliorating role because, by expression, they define the form, shape and contour described by the curls on the surface of the skin.

In order to highlight the practical importance of these characters (Taftă, 1998) states that all these features have a great influence on the curls quality because "they affect in a certain way the commercial aspect of the curl". Therefore, in raising sheep for the production of skins, the shape or type of curl has a special connotation, being considered by many specialists and practitioners as the main objectives of improving breeds of sheep raised for skins (Pipernea, 1979), sometimes this character being influenced by the age of the parents and their state of maintenance (Florea et al., 2020)

The importance of assessing the specific characteristics of the quality of the curls and especially their shape is particularly confirmed by the fact that the direct intervention in the process of breeding sheep from skins bred in South Africa and Namibia, based on the selection applied for this character has shaped a new genotype of Karakul sheep in which the curling is slightly unfolded, flattened, and which by arrangement generates sinuous designs and have a milled appearance, very low height and intense gloss (Schoeman et al., 1992).

MATERIALS AND METHODS

The analysed biological material was represented by purebred Karakul of Botoșani lambs belonging to all colour varieties, obtained over three successive generations. The entire herd is included in the Breed's

Genealogical Register and is in the program for improving the quality of skins.

The method applied in assessing the quality of the skins was based on the technical norms specified in Section 1.4 and 1.5 of the MADR Order no. 22/20.01.2006 and in which are specified the aspects on the basis of which the official control of the skin production and the evaluation of the corporal conformity and of the constitution at Karakul are carried out.

Statistical data processing was based on the use of the S.A.V.C. (Statistics Analysis of Variance and Covariance, 2003) to determine the arithmetic mean, the error of the arithmetic mean (±s) the standard deviation (s). To test the statistical significance of the differences between the averages of the values of the studied parameters as well as the correlations between them, the algorithms Variable Analysis (ANOVA Single Factor) and Pearson Correlation were used, both included in the software used in statistical data processing.

RESULTS AND DISCUSSIONS

The shape, size and type of curl are always important objectives for the improvement of Karakul of Botoșani sheep because they have a great influence on the general appearance of the skin, influencing their aesthetic and commercial value. In the performance control activities specific to the production of skins, the quality analysis emphasizes, in particular, the main features that influence the expression and externalization of a genotype with a certain type or curl shape.

In order to create conditions for genetic expression of the desired shape and type of curls, it is necessary to increase the degree of improvement for the following parameters: length, height, width, degree of closure, direction of curl, contour and arrangement of curls on the skin surface.

When assessing the curl type character, more attention is paid to the length dimensions. The explanation for this situation is that the length is also a criterion for classifying the curls, those with a length greater than 12 mm are considered tube-shaped curls and if they are below this value the curls are bob-shaped.

Multiple evaluations were performed at each stage of assessing the degree of improvement of the character represented by the shape of the curls, so that the data processing provided objective values and highlighted the intensity and effect of the selection. The diagram described in the case of the graphical representation of the distribution by colour varieties of the average values obtained after evaluating the type of curl shows that the improvement process, although in progress, is at different levels at the five colour varieties consolidated within the Karakul of Botoșani breed (Table 1 and Figure 1). Only in the black variety the evaluation for this character allowed that for the over 1501 lambs that were subjected to the assessments the average score for the type of curl to be higher than 95 points.

Table 1. Statistical estimators obtained after evaluating the type of curl in relation to the variety of colour

Colour variety	No.	$\overline{X}$	$\pm s_{\bar{x}}$	s	V%	Minimum	Maximum
Black	1501	99.26	0.831	18.166	18.302	78	135
Greyish	1181	84.45	0.468	16.073	19.031	59	135
Brown	428	81.67	0.762	15.774	19.315	63	128
Grey	103	84.40	1.831	18.588	22.024	40	128
Pink	530	87.27	0.784	18.051	20.684	63	128

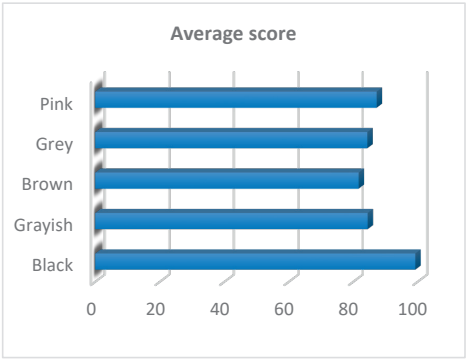

Figure 1. Average score obtained when assessing the degree of improvement of the type of curl in relation to the colour variety

In breeding programs applied to pelts breeds, high attention is paid to the uniformity of the basic characteristics of the curls, it is desirable that over 75% have approximately the same height, length and width. Regarding the uniformity of this parameter in many specialized publications it is shown that on the surface of a skin, usually the curls have different lengths, decreasing from croup to withers and from the upper line of the trunk to the abdomen (Pascal, 2001; Pascal, 2011; Taftă et al., 1998).

In black variety, the researches show that curls with tuber shape dominate, i.e. they have an average length of more than 12 mm (Table 2). In fact, this is also the main goal of improving the quality of black skin, i.e. to increase the uniformity of characters in terms of length, width and height. Improving these values influences the appearance of uniform types of curls, with a clear outline of the tubular curl.

In order to properly assess the genetic progress made, the data obtained in the three consecutive stages of evaluation of the three generations of lambs were compared with the performance recorded for the same character but in the lambs obtained in the lambing season of 2005, when it was established Genealogical Register of the Karakul of Botoșani breed. Starting from this specification we can observe that if in 2005 the proportion of lambs with predominantly tubular curl was 87.21% the average values recorded in 2018 indicate a reduction of their share to 82.25% and 79.20% and 79.61% in the evaluation seasons applied in the 2019 and 2020 campaigns.

If we take into account the fact that the interest for this character increases only when the purpose of the skins is to be used in the manufacture of coats, we consider that keeping in the active population a herd that would produce skins with wider and looser curls justified and may respond to any requests to increase demand that may occur during certain periods of time. However, it is recommended, as in this case, that the improvement be aimed not only at a better expression of gloss but also at a standardization of the design and modelling of the curl.

The analysis of the current degree of expression of the desired type of curl in the black and greyish varieties is summarized in Figure 2, and for a good interpretation of the data obtained we must specify that the goal aimed at improving the character represented by the type of curl is different from one colour variety to another. Thus, if for the black and greyish variety it tends towards an increase of the degree of uniformity of the curls, in size and height and as a way of arrangement, for the other varieties of colour it is desired to consolidate and improve some characters that can be the basis for obtaining skins to have the curl arranged in the form of low waves, associated with an intense luster.

The statistical values obtained indicate that for the character represented by the type of curl the improvement process is different. Thus, in the black variety, the proportion of lambs with a long tube-shaped curl type was significantly increased in lambs of the three generations subject to evaluations compared to the expression of the same character in lambs evaluated in 2005.

Performing the same analysis based on statistical data obtained from the evaluation of greyish lambs indicates lower average values than in the reference year, except for the average score resulting from the assessment of this character in lambs obtained in the lambing campaign 2020. The performance for this character was 23.66 points higher than the assessments made for the 2005 generation of lambs.

Table 2. Frequency type of curls for the varieties of Karakul of Botoșani breed

Colour variety	Curl type	Frequency on evaluation season							
		2005		2018		2019		2020	
		n	%	n	%	n	%	n	%
Black	Long tube	295	39.28	214	44.67	213	38.17	221	46.92
	Medium tube	188	25.03	131	27.35	145	25.98	93	19.74
	Short tube	105	22.29	49	10.23	84	15.05	61	12.95
	Intense gloss wave	98	13.04	63	13.15	110	19.71	62	13.16
	Flattened	65	8.65	22	4.60	6	1.09	34	7.23
Greyish	Long tube	152	30.52	73	18.25	85	20.48	160	36.98
	Medium tube	135	27.15	94	23.50	78	18.80	145	32.19
	Short tube	98	19.67	118	29.50	122	29.40	65	14.73
	Intense gloss wave	85	17.06	115	28.75	130	31.32	71	16.10
	Flattened	28	11.86	-	-	-	-	-	-
Brown	Long tube	12	6.43	6	4.61	7	3.62	4	3.74
	Medium tube	38	20.32	20	15.38	13	6.70	9	8.41
	Short tube	35	18.71	25	19.23	45	23.19	34	31.77
	Intense gloss wave	88	47.06	79	60.78	119	61.34	60	56.08
	Flattened	14	7.48	-		10	5.15	-	
Grey	Long tube	-	-	12	6.70	8	5.97	12	6.21
	Medium tube	-	-	64	35.75	37	27.63	28	14.50
	Short tube	-	-	34	18.99	38	28.35	57	29.53
	Intense gloss wave	-	-	55	30.72	38	28.35	85	44.07
	Bob	-	-	14	7.84	13	9.70	11	5.69
Pink	Long tube	-	-	3	7.14	-	-	-	-
	Medium tube	-	-	6	14.28	6	15.78	-	-
	Short tube	-	-	9	21.42	5	13.15	6	23.08
	Intense gloss wave	-	-	24	57.16	27	71.07	20	76.92

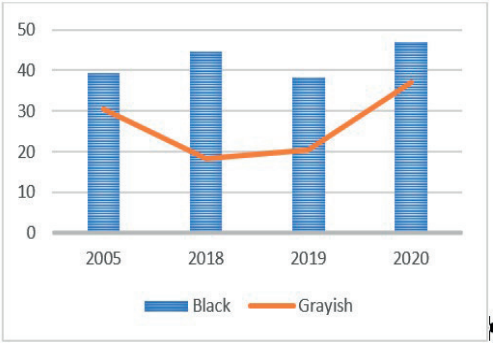

Figure 2. Rate of lambs at which was recorded the desired loop type under the shape of long tube (%)

The non-uniformity of the gloss and the modelling led to the change of the selection criteria, insisting on those characters that are associated with a better uniformity for some dimensions and shapes of the curls. Compared to the trend in 2005, when the selection insisted on obtaining skins with a flattened and a wave with a special luster curl, against the background of increasing attention for tubular curl, the proportion of lambs that had in 2015 predominant curl arranged in the form of long and medium tubes increased by 3.5%, the difference being very significant for $p < 0.01$.

For the obvious improvement of the skin mist, it is desired that the selection be based on evaluations of the modelling and the better uniformity of the colour. At least for the last aspect, the selection is difficult because the colour and its shade depend a batch on the numerical ratio and the length ratio between the fibres coloured in black and white.

Compared to other values cited in the literature, those obtained converge in meaning and intensity of expression. Thus, a study conducted on the population of lambs of the black variety, within the Karakul of Botoșani breed, shows that the proportion of those who had a different curl of the desired shape was 14.5% for the biological material of line 5 and respectively 11.8% for those in line 1557 (Marin et al., 1974). Other studies performed on the population of Karakul of Botoșani lambs show that "in terms of the shape of the curl, the most common are those whose curls are tube-shaped (34.69%)" and other forms of curl have a relatively equal proportion placed between 17% and 21% (Hrincă et al., 1991). Also, for

the black variety, in 1977, in some studies carried out by Marin it was found that in the herd of black lambs the proportion of those who had curls of tube, medium and large bob type was 70.20%, and of short bob and wave type only 20.8%.

In the case of coloured varieties, the desire of the breeder is to increase the proportion of individuals in whom the skin has wavy and very shiny curls. For these varieties of colour, the criteria used in the selection of the curl type are mainly focused on the characters that facilitate a genotype expression of undefined curls, with low height, with the longitudinal axis arranged parallel to the skin surface, slightly scattered but very shiny fibres. The presence of these characteristics determines the appearance of skins with curled and arranged curls.

In the brown variety, the evaluation shows a progressive increase in the individuals dominated by the wavy and extremely shiny curl from 47.06% to 65.42% in the lamb generation from 2015. This was possible because after the approval of this variety it was possible to pass to an intensification of the criteria that improve the curling of brown lambs. Basically, the fact that the selection difference has high values and a high degree of statistical significance, being very significant for $p<0.05$, confirms the efficiency of the selection and implicitly the fact that the improvement of this character has a degree of confidence of over 95%.

The grey variety is in the process of genetic consolidation after meeting the criteria for approval in 2018. Compared to the desired type of grey, it is intended that based on evaluations that take place in the first neonatal period to promote as breeders those individuals who allow to dominate in the genotype small curls, with silky fibres, with well-defined associated curls and with an intense luster.

Based on the research, it was found that the defined curling, represented by the tubular one, has the largest share in the lambs analysed. The exception is the type of long tube-shaped curl that holds, in each generation, less than 7%. From this perspective, the improvement must be intensified so that, in each generation, the proportion of those with a uniform curl in size, thickness and uniformity increases.

If we consider that the objective of improvement is to promote the low-height curling, with an intense luster wave arrangement, we can say that the improvement process is based on favourable coordinates because the proportion of lambs with these characteristics increases from 30.72% in 2018 to 44.07% in 2020. The difference of almost 14% indicates that the method applied in the selection is efficient and contributes to consolidating the desired type of curling for the new generations.

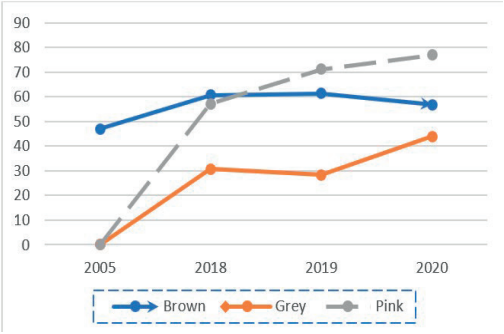

Figure 3. Proportion of lambs with the desired type of curly flattened and shiny wave (%)

Based on the research carried out, it was found that the pink variety has a small number of active population that is part of the category of those registered in the Genealogical Register of the breed. This variety is in the process of formation, and due to the beauty of the skins, it enjoys a special attention from the breeders. Being a composite colour, the uniformity of the colour shade is very difficult to obtain. The type of curl also shows increased variability. From these considerations, the main objective of the fundamental improvement is to promote the types of flattened curls, i.e. wave with special gloss.

During the analysed period, the lambs with this type of curling increase from a proportion of 57.16% in 2013 to over 75% in the case of evaluations carried out in 2015. These results confirm not only the efficiency of the selection but also the fact that the improvement is on favourable coordinates. Determining the differences and their significance indicates that for the values obtained in the curl type assessment there are significant differences for $p<0.01$ (Table 3).

Table 3. The difference and significance of difference for the score obtained in the assessing of the curl type

Genotype 1	Genotype 2	Average difference	The meaning difference	Significance threshold
Grey	Greyish	2.82	insignificant	-
Grey	Brown	5.6	significantly	0.01
Grey	Black	11.99	significantly	0.01
Grey	Pink	2.87	insignificant	-
Pink	Greyish	0.06	insignificant	-
Pink	Brown	2.73	insignificant	-
Pink	Black	14.86	significantly	0.01
Black	Greyish	14.8	significantly	0.01
Black	Brown	17.59	significantly	0.01
Brown	Greyish	2.79	insignificant	-

CONCLUSIONS

The assessment of the degree of improvement for the curl type indicates that the desired curl in the form of long and medium tubes is at an advanced stage as that type increased from 65.11% in 2005 to 66.66%. to lambs subject to appreciation in the year 2020.

Over the time period, as a result of the improvement, the proportion of lambs with medium and long tube curls increased by more than 1.5% in the black and greyish variety, the difference being significant for $p<0.01$.

The proportion of lambs in which the curling was predominantly flattened and with a low degree of closure, respectively wave and flattened, was kept within relatively constant limits placed around 20%, with relatively small variations from one generation to another.

The expression of the tubular type curl was present at approx. 50% of the lambs belong to the brown, grey and pink varieties, while in the black ones the respective type was identified in a proportion of less than 50%.

In the variety of greyish it is found that compared to 2005 the proportion of lambs that had in 2018 predominant curling arranged in the form of long and medium tubes increased by 3.5%, the difference being very significant for $p<0.01$.

The brown variety shows a progressive increase of the individuals dominated by the curl arranged in waves associated with a very good and intense luster from 47.06% to 65.42%; the fact that the selection difference has high values and a high degree of statistical significance for $p<0.05$ confirms the efficiency of the selection for this character, with a direct and positive effect on the improvement of this character.

In the grey variety because the goal of breeding is to promote individuals with low-height, wave-like curling and intense luster, we can say that breeding is on favourable coordinates as the proportion of lambs found in this form increases from 30.72% in 2018 to 44.07% in 2020.

In the pink variety, the desired type, represented by flattened curl, increased from 57.16% in 2018 to over 75% in the case of evaluations performed in 2020, results that confirm not only the efficiency of the selection but also the fact that the improvement is on coordinates favourable.

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STUDY OF PRODUCTIVE PERFORMANCE IN THE PINZGAU BREED EXPLOITED IN THE DORNELOR BASIN, SUCEAVA COUNTY

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Abstract

The paper aims to highlight the productive performance of the Pinzgau breed exploited in the Dornelor Basin, Suceava County. In this sense, a herd of 12 head of cattle, belonging to the Pinzgau breed, was studied, both the red variety and the black variety, which were followed by the productive performances at the ascendancy and descent of the nucleus from the farm. Also, the parameters regarding the quality of milk obtained from the studied cattle were analyzed. The ancestry of the studied herd is valuable with productions in the mother (M) of 5496 kg of milk, 4.22% fat and 3.39% protein, in the mother of the father (MT) of 5562 kg of milk, with 4.095% fat and 3.45% protein , and in the mother's mother (MM) of 5613 kg milk with 4.21% fat and 3.49% protein. The milk production in the offspring had an increasing evolution as follows: on total lactation 5922 Kg milk in lactation I and 6474 Kg milk in lactation II, and on normal lactation of 4820 Kg milk in lactation I and 4843 Kg milk in lactation II with average values of 4.26% fat and 3.24% protein in lactation I respectively 3.91% fat and 3.29% protein in lactation II. Regarding the quality of the milk, it had an average value of 86,000 thousand / ml milk in the total number of somatic cells (NCS) which shows us a quality milking, in hygienic conditions, respectively keeping the milk after milking in hygienic conditions, which shows the farmer's care for the cattle on the farm.

Key words: *cattle farm, Dornelor Basin, improvement program, mountain area, Pinzgau.*

INTRODUCTION

The breeds of old native animals, which were formed on the Romanian territory, have the advantage of having a special genetic structure, acquired through natural selection, which ensures an extraordinary adaptability to environmental conditions, manifested by reproductive capacity, disease resistance and recovery plant resources, being clearly superior to modern breeds. For these reasons they are considered a valuable reservoir of genetic resources, necessary to support the continuous evolution of domestic animal populations. In animal husbandry, the care for the protection and conservation of endangered breeds has been attributed to the National Agency for Animal Husbandry, subordinated to the Ministry of Agriculture and Rural Development.

On this occasion, the breeds of cattle, horses, sheep, pigs and birds were constituted, which constitute the national genetic heritage that must be protected and conserved by applying some basic rules, deduced from the science of population genetic.

MATERIALS AND METHODS

In order to highlight the productive performances of the Pinzgau cattle breed, exploited in the Dornelor Basin, Suceava County, a herd of 12 heads was studied, both from the red variety and from the black variety (Dorna cow). On this biological material, the productive performances at ascendancy and descent were followed, respectively the quantity of milk (kg), fat (%), fat (kg), protein (%), protein (kg), fat plus protein, on total lactation and normal, but also on several lactations. Milk quality indicators such as NCS (thousands/ml), fat (%), protein (%), lactose (%), urea (%), casein (%), pH (%) were also analyzed. When ascending the herd, the same indicators were analyzed for mother (M), father's mother (MT) and mother's mother (MM). The primary data were taken from the Genealogical Register of the Breed (www.registregenealogice.ro), were systematized, processed and interpreted by methods specific to such research ($\bar{X}$, ± s, s, V%, p significance test, confidence interval). The analysis and interpretation of the results was

correlated with the numerous observations made directly on the farm. Raising the genetic potential and productivity of cattle populations, while optimizing farming technologies in small and medium-sized family farms, proper management and economic management, are important ways to increase profitable milk and meat production.

RESULTS AND DISCUSSIONS

The Pinzgau breed is a breed of cattle formed in the Pinzgau region near Salzburg, Austria, and bred for mixed milk and meat production. The Pinzgau breed (Figure 1) is characterized by the red-chestnut color that covers the whole body, except for a white stripe from the withers, along the spinal cord, to the head of the sternum. Body weight ranges from 400-600 kg in cows and 850-1,100 kg in bulls. Pinzgau cattle are mainly raised in mountain areas. In Romania this breed was first introduced in Bucovina, when this region was in the Austrian Empire. The Dorna cow (black variety) (Figure 2) is a variety of the Pinzgau de Transilvania (Pinzgau red) breed, being spread in the north of Moldova (Vatra Dornei, Câmpulung Moldovenesc, Gura Humorului). The Dorna cow differs from the Pinzgau breed from Transylvania both morphologically and productively. Thus, the color is mottled black with white, with the same pattern as the white speck. The waist is 1-2 cm smaller, and the massiveness is more pronounced. Under similar operating conditions, the Dorna cow produces higher milk production, with a higher fat and protein content. Precocity, fecundity and longevity are superior to the red Pinzgau breed. Resistance to mastitis, quantified by the number of somatic cells, could be included in the breeding program, but without being mandatory, at least in a first phase. Ensuring proper maintenance conditions, even imposing a minimum standard of well-being in individual households, would have the effect of reducing environmental variation and increasing the value of heritability, which would streamline selection for udder health (Popa et al., 2021).

Figure 1. Pinzgau de Transilvania - red variety
Note: original picture

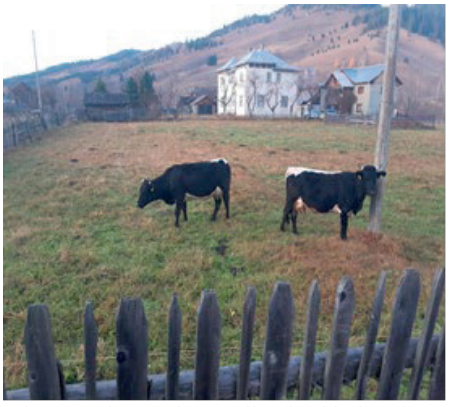

Figure 2. Pinzgau de Transilvania - black variety
Note: original picture

The Pinzgau breed from Transylvania is specific to subsistence farms, of low productivity, in the mountain area (Dragănescu, 2006). The Pinzgau breed from Transylvania is suitable for extensive and intensive exploitation. The improvement process aims to increase body weight (500-550 kg), improve the productive potential for milk production (over 4500 kg milk/lactation, with 4% fat) and meat. In order to survive, the breed must improve its characteristics (mechanical milking, production potential), in order to adapt at least to the requirements of organic farms, if not commercial ones in the mountain area (Drăgănescu, 2006).

In the analyzed animals, we studied the parameters of milk production in the ancestry of the studied cattle (mother, father's mother and mother's mother), offspring and the quality of milk production. The statistics on milk production by the number of Pinzgau cattle from Transylvania are given in Table 1.

Table 1. Statistics on milk production by the ascendancy number of Pinzgau cattle

Ascendancy	Character	n	$\overline{X}$	$\pm s_{\bar{x}}$	s	V%	Minimum	Maximum
Mother	Milk (kg)	12	**5496.58**	159.69	553.183	10.064	4200	6378
	Fat (%)	12	**4.22**	0.062	0.214	5.086	3.94	4.67
	Fat (kg)	12	231.25	7.466	25.864	11.184	177	266
	Protein (%)	12	**3.39**	0.059	0.204	6.011	3.12	3.77
	Protein (kg)	12	186	6.397	22.161	11.914	144	214
	Fat+Protein (kg)	12	417.25	13.604	47.125	11.294	321	479
Father's mother	Milk (kg)	12	**5562.25**	171.374	593.658	10.673	4012	6374
	Fat (%)	12	**4.09**	0.069	0.237	5.8	3.7	4.43
	Fat (kg)	12	227	8.005	27.729	12.216	151	257
	Protein (%)	12	**3.45**	0.058	0.2	5.806	3.12	3.76
	Protein (kg)	12	191.42	7.627	26.421	13.803	129	225
	Fat+Protein (kg)	12	422.58	15.46	53.554	12.673	280	473
Mother's mother	Milk (kg)	12	**5613.42**	161.55	559.625	9.969	4356	6466
	Fat (%)	12	**4.21**	0.077	0.268	6.359	3.9	4.83
	Fat (kg)	12	236.08	8.735	30.258	12.817	173	300
	Protein (%)	12	**3.49**	0.05	0.172	4.913	3.12	3.76
	Protein (kg)	12	195.67	6.019	20.852	10.657	155	229
	Fat+Protein (kg)	12	434	14.514	50.277	11.585	328	525

It was reported that in the mother (M) milk production was 5496 kg with 4.22% fat and 3.39% protein, in the father's mother (MT) 5562 kg milk with 4.095% fat and 3.45% protein, and in the mother's mother (MM) of 5613 kg of milk with 4.21% fat and 3.49% protein.

Therefore, the ancestry to the Pinzgau breed for the studied herd, has values of milk production within the limits of 5400-5600 kg of milk, with 4% fat and is a valuable ascendant with high genetic and phenotypic potential. The statistics of milk production in the descendants of Pinzgau cattle are presented in Table 2. Table 2 presents the productive parameters for the offspring of the studied herd. We find that milk production has an upward evolution on the total lactation 5922 kg milk in lactation I and 6474 kg milk in lactation II, and in the normal lactation of 4820 kg milk in lactation I and 4843 kg milk in lactation II. The quality of milk had average values of 4.26% fat and 3.24% protein in lactation I and 3.91% fat and 3.29% protein in lactation II (Table 2). The obtained productions are very good for this breed, bred and exploited in the Dornelor Basin, but they are lower values than the values obtained at the ancestry of the studied herd. It is recommended to make a more rigorous selection of the Pinzgau breed in order to obtain superior productions, reaching 5800 kg of milk/ lactation. This breed is a disease-resistant breed, capitalizes very well on the fodder in the mountain area, there are no difficulties in their reproduction and it is recommended for farmers for exploitation (V. Maciuc, 2006).

Table 2. Statistics on milk production in the descendants of Pinzgau cattle

Lactation	Character	n	$\overline{X}$	$\pm s_{\bar{x}}$	s	V%	Minimum	Maximum
The first total lactation	Duration of lactation (days)	14	506.14	65.426	244.801	48.366	245	1046
	Milk (kg)	10	**5922.2**	784.41	2480.521	41.885	3445	12644
	Fat (%)	10	**4.36**	0.169	0.533	12.228	3.74	5.09
	Fat (kg)	10	252.5	31.872	100.788	39.916	151	518
	Protein (%)	10	**3.27**	0.051	0.162	4.963	3.09	3.61
	Protein (kg)	10	191.5	25.871	81.81	42.721	101	412
The first normal lactation	Duration of lactation (days)	12	305	0	0	0	305	305
	Milk (kg)	10	**4820.5**	255.92	809.29	16.789	3781	6149
	Fat (%)	10	**4.26**	0.15	0.474	11.11	3.76	4.93
	Fat (kg)	10	206.8	11.92	37.694	18.227	179	303
	Protein (%)	10	**3.24**	0.052	0.165	5.085	3.09	3.61
	Protein(kg)	10	156.8	9.379	29.66	18.916	119	222
The second total lactation	Duration of lactation (days)	12	397.17	42.193	146.162	36.801	283	666
	Milk (kg)	11	**6474.27**	668.944	2218.635	34.268	3745	10277
	Fat (%)	11	**3.93**	0.133	0.441	11.237	3.21	4.66
	Fat (kg)	11	262.82	25.536	84.692	32.225	168	387
	Protein (%)	11	**3.28**	0.052	0.174	5.296	3.09	3.72
	Protein (kg)	11	220.82	23.507	77.964	35.307	152	382
The second normal lactation	Duration of lactation (days)	10	305	0	0	0	305	305
	Milk (kg)	10	**4843.1**	289.227	914.617	18.885	3254	6505
	Fat (%)	10	**3.91**	0.143	0.452	11.561	3.2	4.66
	Fat (kg)	10	189.9	14.411	45.572	23.998	111	268
	Protein (%)	10	**3.29**	0.055	0.173	5.275	3.09	3.71
	Protein (kg)	10	159.5	9.572	30.27	18.978	108	212
	Milk/day(kg)	12	14.71	0.895	3.1	21.066	7	19.7

Regarding the quality of milk production in the offspring of Pinzgau cattle, the results obtained are shown in Table 3. The number of somatic cells (NCS) thousand/ ml) in milk (Table 3) was 86,000/ml milk, which means that it falls in quality I according to the rules of the EU ICAR guideline, the percentage of fat was 4.34% and the percentage of protein was 3.25%. These results are very good for the breed under study, because it is a high quality milk.

Table 3. Statistics on the quality of milk production in the descendants of Pinzgau cattle

Character	**n**	$\overline{X}$	$\pm s_{\bar{x}}$	**s**	**V%**	**Minimum**	**Maximum**
Number of somatic cells (thousand/mililiter)	12	**86.08**	2.33	8.073	9.378	71	98
Fat (%)	12	**4.34**	0.15	0.518	11.949	3.74	5.09
Protein (%)	12	**3.25**	0.045	0.154	4.749	3.09	3.61
Lactose (%)	13	4.5	0.24	0.867	19.281	1.65	4.92
Urea (%)	13	29.23	0.995	3.586	12.268	23	36
Casein (%)	13	26.08	0.515	1.858	7.125	24	29.5
PH (%)	13	6.68	0.016	0.056	0.843	6.6	6.78
Milk/day (kg)	12	**14.71**	0.895	3.1	21.066	7	19.7

Regarding the number of somatic cells (NCS) indicating the quality of milk, it should be noted that this indicator had values in the range of a minimum of 71,000/ml milk and a maximum of 98,000/ml. There are very good values that highlight a robust breed constitution and a quality milking technology. The percentage of milk fat had an average value of 4.34%, with limits between 3.74% and 5.09%. The percentage of protein had an average value of 3.25%, with limits between 3.09 and 3.61%. These results are very good, the milk obtained from the studied cattle is of superior quality. One of the modern practices for profit in cattle is the extension of the biotechnology of artificial insemination by using material from bulls with superior breeding value in the direction of milk and meat production (Nistor-Anton & Maciuc, 2019).

In order to support the breeders of this breed of cattle in Romania, in the period 2015-2018 the ADER 5.2.3 project was started, within the Research - Development Station for Cattle Breeding Tg. Mureş which had the following objectives:

- The use of animal genetic resources in critical condition, in danger of extinction, the saving from extinction of the genetic fund of the local Pinzgau breed and the conservation of the genetic fund through a minimum number of specimens;
- Ensuring female and male breeding animals for households that want to resume the tradition of raising this breed.
- We report some activities carried out within this project:
- The study on the formation of the breed and its spread on the territory of our country and internationally;
- Identifying the existing herds, acquiring and merging 25 female and male Pinzgau de Transilvania heads at the resort farm, in order to establish the breed conservation nucleus and arrange the accommodation spaces;
- Preparation and endowment of a laboratory in the farm of the resort for natural mounting and harvesting of semen from the bulls of Pinzgau breed;
- Harvesting, processing and storage of seminal material;
- Registration of milk production, meat production and their quality;
- Suitability of the Pinzgau de Transilvania breed for mechanical milking;
- Dissemination of the results obtained by organizing a national symposium, publishing a brochure and a book on the conservation of the genetic background of the breed, its growth and exploitation in a semi-extensive system (ADER Project 5.2.3, 2018). Cattle value and transform efficiently into milk and meat natural resources (pastures, meadows, agricultural by-products), as well as different residues, participate in the intensification and profitability of traditional households both through the productions we obtain, but also through the mountain product. It also represents an important source of convertible currencies (through the export of meat, meat and milk products, live animals, frozen semen (MSC) and frozen embryos) (Ciocan-Alupii & Maciuc, 2019). The milk law project aims to regulate the marketing of dairy products, to increase consumer confidence in domestic dairy products and to eliminate falsified products (Coman et al., 2019). One of the modern practices for profit in cattle is the extension of the biotechnology of artificial insemination by using material from bulls with superior breeding value in the direction of milk and meat production.

CONCLUSIONS

From the study, the following conclusions can be drawn:

1. The ancestry of the studied herd is valuable with productions in the mother (M) of 5496 kg of milk, 4.22% fat and 3.39% protein, in the mother of the father (MT) of 5562 kg of milk, with 4.095% fat and 3.45 % protein, and in the mother's mother (MM) of 5613 kg milk with 4.21% fat and 3.49% protein.

2. The milk production in the offspring had an increasing evolution as follows: on the total lactation 5922 kg milk in lactation I and 6474 kg milk in lactation II, and on normal lactation of 4820 kg milk in lactation I and 4843 kg milk in lactation II with average values of 4.26% fat and

3.24% protein in lactation I and 3.91% fat and 3.29% protein in lactation II.

3. Regarding the quality of the milk obtained, the total number of somatic cells had an average value of 86,000 thousand / ml milk which shows us a quality milking, in hygienic conditions, respectively keeping the milk after milking in hygienic conditions, which shows the farmer's care for the cattle on the farm.

4. The Pinzgau de Transilvania breed capitalizes very well on the natural meadows in the mountain area, is resistant to weather and diseases specific to cattle, has a number of udder defects and has good skills for meat production.

5. It is necessary to develop an improvement program for this breed, which has productive potential.

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STUDY OF THE MAIN BODY DIMENSIONS USED IN THE SELECTION PROCESS, IN THE REPRODUCTIVE NUCLEUS OF PURE ARABIAN HORSES FROM NATIONAL STUD MANGALIA

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Abstract

Study of average performances in a population have a huge importance because, regarding to a population, the average of phenotypic value is equal with average of genotypic value. So, the studies of the average value of characters offer us an idea about the population genetic level. This study have the principal purpose to analyse main body dimensions who are used in selection process: withers height, thoracyc perimether and cannon bone perimether, through the integration of individs in an evaluation class, in accordance with selection methodology (Maftei et al., 2015). The biological material is represented by 73 Pure Arabian horses, 12 males and 61 females, at different ages, owned by Mangalia stood farm, representing the entire reproductive nucleus. The average performances of characters are presented in the paper. We can observe a small grade of variability with some differences between sexes. The average performances of the characters are between characteristic limits of the breed.

Key words*: Arabian, breed, height, horses, selection.*

INTRODUCTION

Regarding to a population, the study of average performances gives us an idea about the populational genetic level (Marginean, 2012). This is possible because the average of phenotypic values is equal with average of genotypic values (Maftei et al., 2015). The principal purpose of this study is to analyze the reproductive nucleus of Pure Arabian horses, from Mangalia stud, through the main body dimensions that are used in the selection process in this breed.

We analyze the wither's height, the thoracic perimeter and the cannon bone perimeter of individuals. After all this analyzes it was possible to ranking the individuals and to ingrate it in an evaluation class (a quality class), in accordance with the specific methodology (Marginean et al., 2005; Maftei et al., 2015). We must say that this study is just a part of an ample research regarding the history of Pure Arabian horses from Romania.

MATERIALS AND METHODS

The biological material is represented by entire reproductive nucleus of Pure Arabian horses from Mangalia stud, 73 individuals from which 12 sire stallions (Table 1) and 61 broodmares (Table 2). All individuals were analyzed through individual performances, through stallions average performance, through mares average performance, and, most important, through population average performance of body dimensions, related to selection criteria (Popa, 2009; Maftei et al., 2022; Pruna et al., 2021). We analyzed the withers height, the thoracic perimeter and the cannon bone perimeter using somatometrie. All measurements were recorded from genealogical register and verified by us in Mangalia stud farm in 2021 using ANIMETER measuring belt and a height measuring stick. Registered data was analyzed from statistical point of view and then was interpretated according to Romanian methodology for ranking and evaluation in Pure Arabian horses,

a methodology that respect W.A.H.O. (World Arabian Horse Organization) demands.

RESULTS AND DISCUSSIONS

Analyzing the presented data, it is easy to observe some differences, for all characters, between stallions (Table 1, Figure 1), between mares (Table 2, Figure 2) and at population level (Figure 3).

Table 1. Registered values of body measurements in stallions

No. crr.	Name	Withers height	Thoracyc perimether	Cannon bone perimether
1	HADBAN XXXVIII	152	172	18
2	SIGLAVY BAGDADY XVIII	152	174	19
3	GAZAL XX	153	180	19
4	NEDJARI XIII	154	179	19
5	MERSUCH XXVI	154	178	18.5
6	SIGLAVY BAGDADY XIX	157	176	20
7	MERSUCH XXVIII	159	194	19.5
8	IBN GALAL III	156	190	20
9	GAZAL XXIII	157	185	19.5
10	HADBAN XL	156	183	18
11	EL IMAN I	150	172	19
12	CYGAJ IV	157	185	19.5

Regarding the registered performances in stallion case, we find some significant differences between individual performances. The bigger value of withers height is 159 cm. (stallion Mersuch XXVIII), and the smallest was 150 cm for stallion El Iman I. From this point of view, stallion El Iman I is ranked in the second class (elite) but its maintenance in the reproductive nucleus is justified by the genetic value of the bloodline from which it comes.

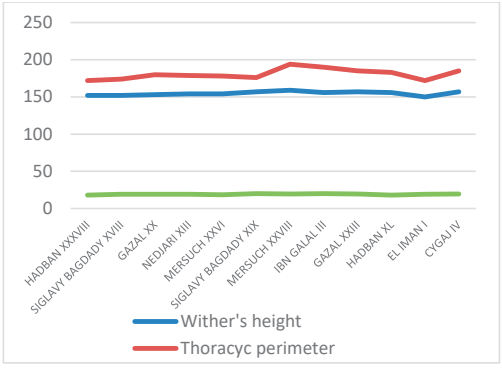

Figure 1. Registered performances in stallions

In case of thoracic perimeter character, the highest value of the character is recorded for stallion Mersuch XXVIII (194 cm), and the smallest value, 172 cm., was recorded for stallions Hadban XXXVIII and El Iman I.

Table 2. Registered values of body measurements in broodmares

No. crr.	Name	Withers height	Thoracyc perimether	Cannon bone perimether
1	833 CYGAJ 15	152	168	18
2	834 SIGLAVY BAGDADY XIV - 54	152	168	18
3	835 NEDJARI IX - 83	153	168	18
4	837 SIGLAVY BAGDADY XIV - 52	154	172	18
5	839 IBN GALAL II -3	153	168	18
6	840 IBN GALAL II 4	155	172	18
7	852 CYGAJ 42	150	172	18
8	853 IBN GALAL II 18	157	172	18
9	855 IBN GALAL II 26	155	174	18.5
10	859 CYGAJ 59	154	180	19
11	861 CYGAJ 62	152	173	18
12	863 IBN GALAL II - 34	153	177	18.5
13	879 HADBAN XXXVIII -13	150	169	17.5
14	880 SIGLAVY BAGDADY XVIII - 11	156	169	19
15	881 MERSUCH XXVI - 7	151	174	18
16	882 IBN GALAL II - 51	155	171	19
17	884 GAZAL XIX - 19	156	175	18.5
18	887 HADBAN XXXVIII - 19	155	177	19
19	889 MERSUCH XXVI - 11	157	170	19
20	891 HADBAN XXXVIII - 26	156	179	20
21	892CYGAJ I - 9	158	172	19
22	896 NEDJARI XIII-17	154	175	19
23	901 HADBAN XXXVIII - 29	155	174	18
24	902 EL IMAN I - 33	153	178	19
25	903 MERSUCH XXVI - 19	155	168	19
26	905 GAZAL XIX - 25	155	175	19
27	908 SIGLAVY BAGDADY XVIII - 27	156	172	19
28	909 CYGAJ I-17	152	169	18
29	910 GAZAL XIX - 31	155	172	18
30	911 EL IMAN I - 35	152	174	18
31	912 CYGAJ I - 15	157	179	19
32	914 NEDJARI XIII-22	158	175	18
33	915 EL IMAN I-42	157	173	18
34	916 HADBAN XXXVIII-33	157	175	18.5
35	917 GAZAL XIX-32	156	170	17
36	918 EL IMAN I-46	155	176	18
37	919 EL IMAN I-47	156	177	18.5
38	920 GAZAL XX-33	157	178	18.5
39	921 HADBAN XXXVIII-35	155	172	18
40	922 NEDJARI XIII-28	157	174	18
41	923 NEDJARI XIII-27	154	172	19
42	924 SYGLAVY-BAGDADY XVIII-33	155	169	18
43	926 HADBAN XXXVIII-36	156	174	18
44	927 MERSUCH XXVI-31	154	173	18
45	928 MERSUCH XXVI-32	150	167	17.5
46	929 MERSUCH XXVI-30	155	180	19
47	930 SIGLAVY-BAGDADY XVIII-37	156	180	18.5
48	931 SIGLAVY-BAGDADY XIX-1	156	176	19
49	932 MERSUCH XXVI-34	153	176	19
50	933 CYGAJ 82	155	185	19
51	934 MERSUCH XXVI-36 XISTA	155	175	18.5
52	935 NEDJARI XIII-33	151	174	18
53	936 NEDJARI XIII-35	154	178	18.5
54	937 NEDJARI XIII-3	152	174	17
55	938 SIGLAVY-BAGDADY XVIII-41	153	178	18
56	939 SIGLAVY-BAGDADY XIX-3	157	172	18
57	940 NEDJARI XIII-38	153	178	19
58	941 GAZAL XX-43	150	177	18
59	942 NEDJARI XIII-39	158	180	20
60	943 EL IMAN I-51	152	178	18
61	944 GAZAL XX-45	154	174	18

For cannon bone perimeter, the biggest recorded value was 20 cm. (Siglavy Bagdady XIX and Ibn Galal III).

In the broodmares case, regarding the wither's height character, we find the smallest value at 150 cm. for four mares belonging to four different bloodlines (Cygaj - 42, Hadban XXXVIII - 13, Mersuch XXVI - 32, Gazal XX - 43). The biggest value of character was 158 cm (Nedjari XIII - 39, Nedjari XIII - 22 and Cygaj I - 9). For thoracic perimeter, the biggest value was 185 cm. (Cygaj - 82), and the smallest was 168 cm., recorded at four broodmares belonging to different bloodlines (Cygaj - 15, Siglavy Bagdady XIV - 54, Nedjari IX - 83, Ibn Galal II - 3). The cannon bone perimeter has the greater value at 20 cm. (Nedjari XIII - 39, Hadban XXXVIII - 26), and the smallest at 17 cm (Nedjari XIII - 36).

Analyzing the national criteria for evaluation and ranking of Pure Arabian horses (Table 3), and the average performances of characters (Table 4), for both sexes, the stallions and also the broodmares from Mangalia stud farm has ranked in the superior class, the RECORD class (Tables 5 and 6).

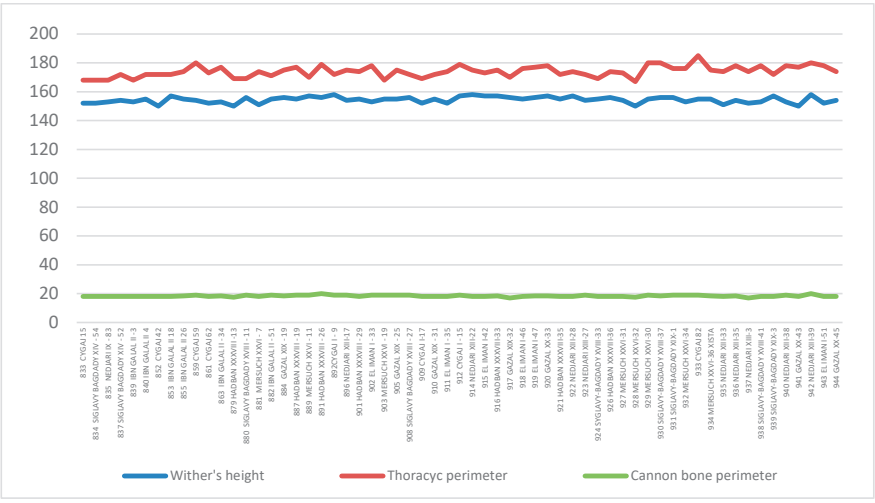

Figure 2. Registered performances in broodmares

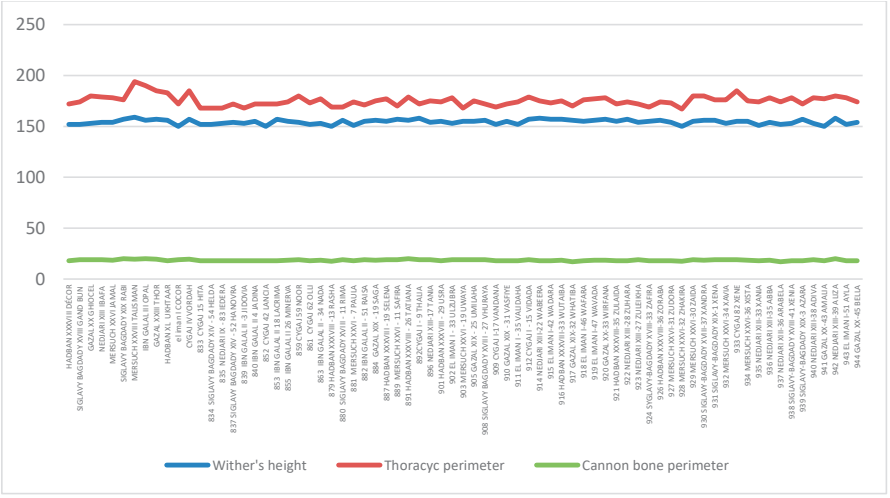

Figure 3. Registered performances in the reproductive nucleus of Pure Arabian horses from Mangalia stud farm

Table 3. Scales and notes for evaluation of Pure Arabian horses in terms of body sizes

Stallions			Note	Mares		
Wither's height	Thoracic perimeter	Cannon bone perimeter		Wither's height	Thoracic perimeter	Cannon bone perimeter
160	180	20.5	9	158	180	20
158	178	20.5	10	156	178	19.5
156	176	20	10	154	176	19
154	174	19.5	9	152	174	19
152	172	19	8	150	172	18.5
150	168	18.5	7	148	168	18
148	166	18	6	146	166	17.5
146	164	17.5	5	144	162	17.5
-	-	-	4	144	160	17

Table 4. Average values of analyzed characters (in cm) and statistics

Specifications		Wither's height	Thoracic perimeter	Cannon bone perimeter
Males	***X***	154.7500	180.6667	19.0833
	STDEV	2.6671	6.9718	0.6686
	Sx	0.8042	2.1021	0.2016
	cv%	1.7235	3.8589	3.5034
Females	***X***	154.4098	174.0328	18.3852
	STDEV	2.1477	3.8252	0.6013
	Sx	0.2773	0.4938	0.0776
	cv%	1.3909	2.1980	3.2708
TOTAL	***X***	154.4658	175.1233	18.5000
	STDEV	2.2241	5.0743	0.6614
	Sx	0.2621	0.5980	0.0780
	cv%	1.4399	2.8976	3.5753

Table 5. Grades of Pure Arabian stallions and broodmares from the reproductive nucleus of Mangalia stud farm

Specification	Wither's height		Thoracyc perimeter		Cannon bone perimeter	
	Stallions	Mares	Stallions	Mares	Stallions	Mares
Body dimensions	10	10	9	9	8	8

Table 6. Minimum marks for placing horses in the general ranking class by body dimensions

Body dimensions	Reccord class		Elite class		1st class
	Stallions	Mares	Stallions	Mares	Stallions & Mares
Record class	8	7	7	6	6

Figure 4. Pure Arabian broodmares in Mangalia stud farm

CONCLUSIONS

The results obtained after the analysis of the body dimensions of the individuals from the reproductive nucleus of the Pure Arabian breed from Mangalia stud farm, highlighted the right of these genitors to become the ancestors of future generations. Our recommendation are: increasing of reproductive nucleus, maintaining the same strong control of selection process, and why not a very strong control of selection. Also, selection of individuals with wither's height values a little bit bigger could be the answer for low performances of Pure Arabian horses in long racing. However, due to the small values of

variability we can say that the average performances of characters is able to offer us a good and correct image of Pure Arabian horse population from Mangalia stud farm.

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REPRODUCTIVE ISOLATION AND AGE STRUCTURE IN THE NUCLEUS OF PURE ARABIAN HORSES FROM NATIONAL STUD MANGALIA

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Abstract

The elaboration of strategies in the field of conservation of animal genetic resources (and not only) had, have and will be based on genetic analysis studies. Without conducting these studies, it is practically impossible to develop management strategies for inbreeding or to develop effective breeding programs. In this paper we present two important indicators of genetic analysis in horse population and not only the reproductive isolation coefficient and the age structure. This parameter has a capital importance in animal breeding because there has a directly influence in animal population evolution. More than that, To be accepted as a population, a herd must fulfill four criteria: reproductive isolation, morphological and physiological differences, environmental requirements and genetic size. The reproductive isolation is the most important criteria for population because only reproductive isolated populations have an own evolution. Regarding the age structure, this parameter has a double importance: for exploitation (influenced directly average age), and on the other hand, for animal breeding (influenced the generation interval and population variability).

Key words*: Arabian horses, genotype, reproduction, structure.*

INTRODUCTION

This research is a part of an ample project concerning the genetic analysis (history) of Pure Arabian horses from Mangalia stud farm. An important part of animal genetic resources management it is represented by the genetic analysis studies. Only starting from this analysis, it is possible to elaborate strategies for inbreeding management (Maftei et al., 2011), and to obtain some breeders of high genetic value, with maximum genetic and economic efficiency (Maftei et al., 2022). In this study we analyze two important parameters of genetic analysis: reproductive isolation level and age structure. This parameter has a capital importance in animal breeding because has a directly influence in animal population evolution (Popa, 2009).

The population acceptance criteria are four: reproductive isolation, morphological and physiological differences, environmental requirements and genetic size (Popescu - Vifor, 1990). The reproductive isolation level is the most important criteria for population acceptance, the other three being in according to them (Draganescu, 1979). This parameter is very important because only reproductive isolated populations have an own evolution, in contrary they are influenced by evolving of immigrants populations (Maftei et al., 2004).

The age structure has a double importance: for exploitation because influenced directly average age, and on the other hand, for animal breeding because is influenced the generation interval and population variability (Marginean, 2012).

MATERIALS AND METHODS

The biologic material was represented by 12 sire stallions and 61 mares Pure Arabian horse breed (Tabel 1), representing the entire reproductive nucleus from National Pure Arabian stud farm Mangalia, at this time (September 2021).

The reproductive isolation coefficient was quantified using the follow relation, developed by Wright (Draganescu, 1979):

$$R.I.C. = \frac{AA - (AI + II)}{AA + AI + II}$$

where: AA - number of individuals accepted for reproduction in analysed interval with both autochthones parents; AI - number of individuals accepted for reproduction in analysed interval with one autochthone and one immigrant parent; II - number of individuals accepted for reproduction in analysed interval with both immigrants parents.

The age structure can be described by weight of different age categories from entire population (Marginean et al., 2005). The age structure is expressed in years.

RESULTS AND DISCUSSIONS

The values for RIC (reproductive isolation coefficient), for reproductive nucleus and also for parents and grandparents of individuals that are now in the reproductive nucleus of Pure Arabian horses from Mangalia stud, are presented in Table 2. The share of immigrant and native individuals is represented graphically, for the three generations analyzed, in Figures 1, 2 and 3.

The data presented in Table 2, reveal the fact that it has a large number of fathers (stallions) of individuals which have activated in reproductive nucleus at the time of this study, and also their grandparents because of the existence of overlapping generations and of the immigrants entered in the population, as it was shown in the past (Maftei et al., 2011).

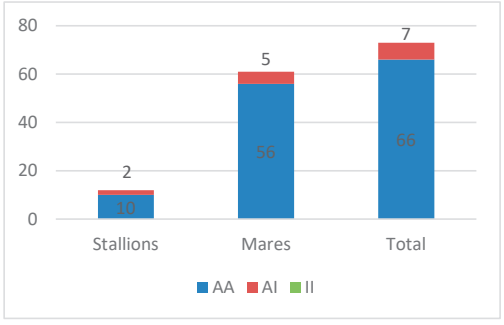

Figure 1. Share of immigrants and local individuals in the reproductive nucleus

Table 1. The biologic material

No.	Name	Sex	Spec.
1	HADBAN XXXVIII	M	AA
2	SIGLAVY BAGDADY XVIII	M	AA
3	GAZAL XX	M	AA
4	NEDJARI XIII	M	AA
5	MERSUCH XXVI	M	AA
6	SIGLAVY BAGDADY XIX	M	AA
7	MERSUCH XXVIII	M	AA
8	IBN GALAL III	M	AA
9	GAZAL XXIII	M	AA
10	HADBAN XL	M	AA
11	EL IMAN I	M	AI
12	CYGAJ IV	M	AI
13	833 CYGAJ 15	F	AI
14	834 SIGLAVY BAGDADY XIV - 54	F	AA
15	835 NEDJARI IX - 83	F	AA
16	837 SIGLAVY BAGDADY XIV - 52	F	AA
17	839 IBN GALAL II -3	F	AA
18	840 IBN GALAL II 4	F	AA
19	852 CYGAJ 42	F	AI
20	853 IBN GALAL II 18	F	AA
21	855 IBN GALAL II 26	F	AA
22	859 CYGAJ 59	F	AI
23	861 CYGAJ 62	F	AI
24	863 IBN GALAL II - 34	F	AA
25	879 HADBAN XXXVIII -13	F	AA
26	880 SIGLAVY BAGDADY XVIII - 11	F	AA
27	881 MERSUCH XXVI - 7	F	AA
28	882 IBN GALAL II - 51	F	AA
29	884 GAZAL XIX - 19	F	AA
30	887 HADBAN XXXVIII - 19	F	AA
31	889 MERSUCH XXVI - 11	F	AA
32	891 HADBAN XXXVIII - 26	F	AA
33	892CYGAJ I - 9	F	AA
34	896 NEDJARI XIII-17	F	AA
35	901 HADBAN XXXVIII - 29	F	AA
36	902 EL IMAN I - 33	F	AA
37	903 MERSUCH XXVI - 19	F	AA
38	905 GAZAL XIX - 25	F	AA
39	908 SIGLAVY BAGDADY XVIII - 27	F	AA
40	909 CYGAJ I-17	F	AA
41	910 GAZAL XIX - 31	F	AA
42	911 EL IMAN I - 35	F	AA
43	912 CYGAJ I - 15	F	AA
44	914 NEDJARI XIII-22	F	AA
45	915 EL IMAN I-42	F	AA
46	916 HADBAN XXXVIII-33	F	AA
47	917 GAZAL XIX-32	F	AA
48	918 EL IMAN I-46	F	AA
49	919 EL IMAN I-47	F	AA
50	920 GAZAL XX-33	F	AA
51	921 HADBAN XXXVIII-35	F	AA
52	922 NEDJARI XIII-28	F	AA
53	923 NEDJARI XIII-27	F	AA
54	924 SYGLAVY-BAGDADY XVIII-33	F	AA
55	926 HADBAN XXXVIII-36	F	AA
56	927 MERSUCH XXVI-31	F	AA
57	928 MERSUCH XXVI-32	F	AA
58	929 MERSUCH XXVI-30	F	AA
59	930 SIGLAVY-BAGDADY XVIII-37	F	AA
60	931 SIGLAVY-BAGDADY XIX-1	F	AA
61	932 MERSUCH XXVI-34	F	AA
62	933 CYGAJ 82	F	AI
63	934 MERSUCH XXVI-36 XISTA	F	AA
64	935 NEDJARI XIII-33	F	AA
65	936 NEDJARI XIII-35	F	AA
66	937 NEDJARI XIII-3	F	AA
67	938 SIGLAVY-BAGDADY XVIII-41	F	AA
68	939 SIGLAVY-BAGDADY XIX-3	F	AA
69	940 NEDJARI XIII-38	F	AA
70	941 GAZAL XX-43	F	AA
71	942 NEDJARI XIII-39	F	AA
72	943 EL IMAN I-51	F	AA
73	944 GAZAL XX-45	F	AA

The genetic persistence of immigrants individuals can be easily seen as a result of maintaining in population of a large percent of stallions and mares with one immigrant parent or even immigrants themselves.

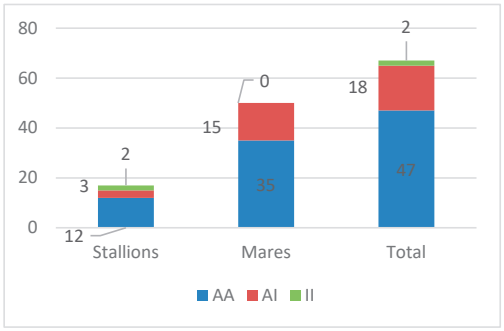

Figure 2. Share of immigrants and local individuals in parents of reproductive nucleus

The obtained values of reproductive isolation coefficient, give us the right to say that the Pure Arabian horses from Mangalia stud is still a population with an independent evolutionary path to other similar populations. Current number of sire stallions (RIC = 0.6667) with a big percent of autohtone stallions - both parents (N = 10) and only two stallions with one immigrant parent, plus the number of brood mares (RIC = 0.9107) that have a significant share of autohtone mares - both parents (N = 56) and only a small proportion (N = 5) with an immigrant parent, shows that population still maintain their own evolutionary way, but with clear genetic influence of populations from which come the immigrants (Germany, Egypt , Poland, etc.).

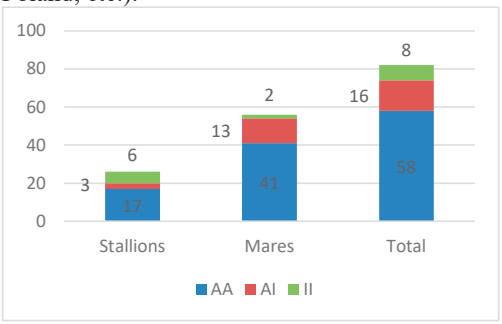

Figure 3. Share of immigrants and local individuals in grandparents of reproductive nucleus

The largest number of immigrants it was recorded in the generation of grandparents of RN (N = 8, 6 stallions and 2 mares), but it is easy to observe an evolution of reproductive isolation coefficient (Figure 4). We consider that the use of immigrant stallions it was made for the desire to expand the number of bloodlines.

Table 2. Reproductive isolation coefficient values

Specifications		No.	Immigrants (I)	Parents			R.I.C.
				AA	AI	II	
Reproductive nucleus (RN)	♂	12	-	10	2	-	+0.6667
	♀	61	-	56	5	-	+0.9107
	Total	73	-	66	7	-	+0.8082
Parents of RN	♂	17	2	12	3	2	+0.4118
	♀	50	-	35	15	-	+0.4000
	Total	67	2	47	18	2	+0.4030
Grandparents of RN	♂	26	6	17	3	6	+0.3077
	♀	56	2	41	13	2	+0.4643
	Total	82	8	58	16	8	+0.4146

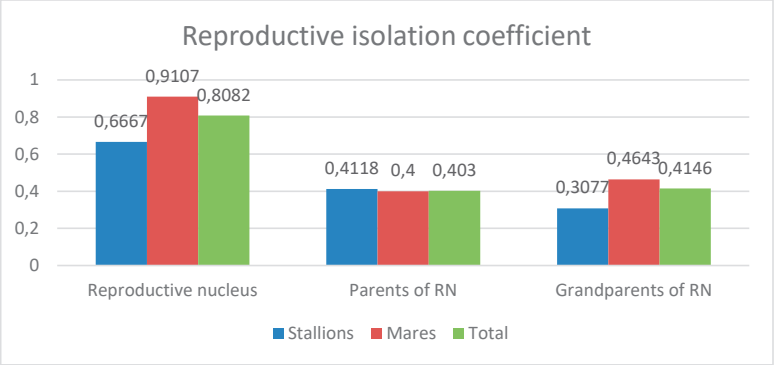

Figure 4. The dynamics of reproductive isolation coefficient

Table 3. Age structure in the reproductive nucleus of Pure Arabian horses from Mangalia stud farm

Sex	Total	Year of birth																										Average age
		1995		1996		1999		2000		2001		2002		2004		2005		2006		2007		2008		2009		2010		2011		
		N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	
M	12	1	8.33	1	8.33	2	16.67			1	8.33	1	8.33							1	8.33			1	8.33			2	16.67	17.5
F	61	2	3.28	1	1.64	3	4.92	2	3.28	1	1.64	3	4.92	2	3.28	1	1.64	2	3.28	1	1.64	1	1.64	3	4.92	3	4.92	3	4.92	4	6.56	5	8.20	7	11.48	8	13.11	7	11.48	7	11.48	1	1.64	12.67

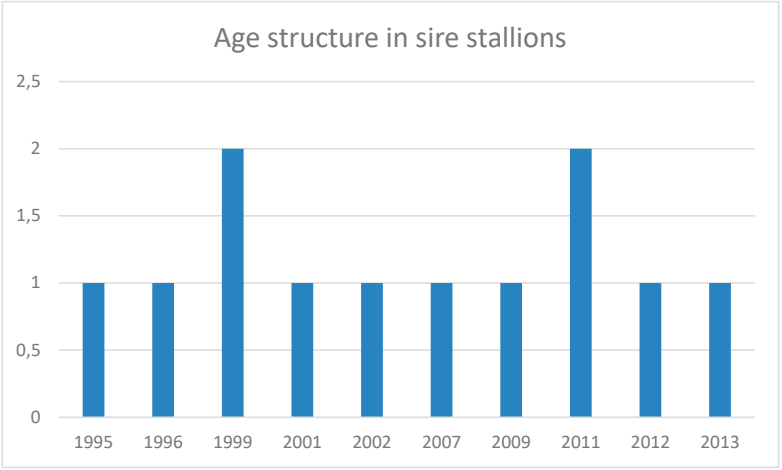

Figure 5. Age structure in sire stallions

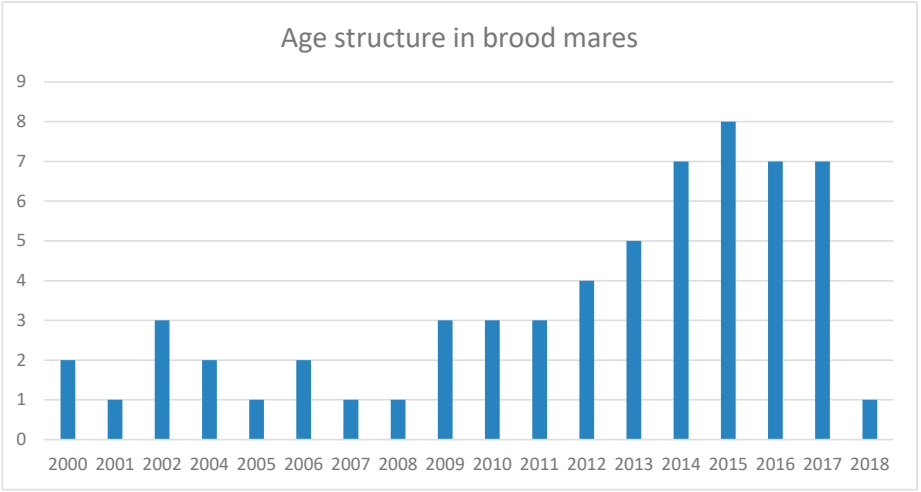

Figure 6. Age structure in the reproductive nucleus of Pure Arabian horses from Mangalia stud - broodmares

The age structure is presented in Table 3 and in Figures 5 and 6. It is obvious that we have here an unbalanced age structure. Age of stallions fluctuates between 9 and 27 years old, with an average age of 17.5 years old. In case of mares, due to the admittance in the reproductive nucleus to a significant number of young mares in the last years, the age structure can be more easily balanced than in the case of sire stallions. For broodmares the average age is 12.67 years old, with limit at 4 and 22 years old. All this age structure is not properly from genetically and economically.

CONCLUSIONS

The Pure Arabian horses from Mangalia stud is a population having his own evolution. The value of reproductive isolation coefficient has an ascendant trend which suggest that the desire of breeder was only to come with other bloodlines and maybe to "correct" some external characters or energetic.

Maintaining or continued use of immigrant stallions it is very possible to have a negative impact on the Pure Arabian horses from Mangalia stud because the evolution of the breed will be disturbed being influenced by the genetic influence of immigrant populations. In this case it will be possible to talk about the absorption process. And this would be inconceivable, and even shameful, given that the specimens of the Pure Arabian horses, bred in the Mangalia stud farm, are considered representative of the classic type of Arabian horse.

The age structure is improper for increasing genetic progress because is increasing the generation interval as following the existence of a significant share of old parents (in specially in the sire stallions rank). A half from the sire stallions are too old.

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TECHNOLOGIES OF THE AGRO FOOD PRODUCTS PROCESSING

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STUDY OF ADDITION OF PURPLE SWEET POTATO FLOUR (*IPOMOEA BATATAS* L.) ON ANTIOXIDANT ACTIVITY AND QUALITY CHEMISTRY OF CHICKEN NUGGETS AS FUNCTIONAL FOOD

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Abstract

This study aims to examine the effect of adding purple sweet potato flour (Ipomoea batatas L) on antioxidant activity and chemical quality of chicken nuggets as a functional food. The research was carried out in 3 stages, namely (1) making purple sweet potato flour, (2) making chicken nuggets with the addition of purple sweet potato flour, (3) testing chicken nuggets including antioxidant activity tests, and chemical quality consisting of water content tests, content tests. protein, fat content test and dietary fiber test. This research was carried out experimentally using a completely randomized design with 4 additional treatments of purple sweet potato flour, namely P1 = 10 g, P2 = 20 g, P3 = 30 g and P4 = 40 g, with 4 replications. The results showed that the addition of purple sweet potato flour had a very significant effect (P<0.01) on the antioxidant variables and the chemical quality of chicken nuggets. In conclusion, the addition of purple sweet potato flour up to 30 g resulted in antioxidant activity and good chemical quality of chicken nuggets as a functional food.

Key words*: antioxidant, chemical quality, chicken nuggets, purple sweet potato.*

INTRODUCTION

Consumers' interest in foodstuffs, not only those that have complete nutritional content and good taste, but also must have certain physiological benefits for the body.

Nugget is one of the practical fast food products and is currently well known by the public.

The fat content of broiler chicken is quite high as the main ingredient for processing nuggets. Consumption of nugget products containing fat must be balanced with the presence of dietary fiber in purple sweet potato flour. Another advantage is the high anthocyanin content in purple sweet potato flour. Anthocyanins have a high ability as an antioxidant because of their ability to scavenge free radicals and inhibit lipid peroxidation, the main cause of damage to cells associated with aging and degenerative diseases (Cevallos-Casals & Cisneros-Zevallos (2002) in Suda et al., 2003).

Generally, the flour used as a binder in the manufacture of chicken nuggets is starchy material such as tapioca flour, rice flour, maezena flour, sago flour and wheat flour. On the other hand, staple food for the community should ideally be sourced from local raw materials so that costs can be reduced.

Purple sweet potato has a nutritional content that is rich in vitamins (B1, B2, C and E), minerals (Ca, Mg, K and Zn), dietary fiber and carbohydrates (Antarlina & Utomo, 1999). Due to its high water binding ability, purple sweet potato flour can be used as a binder in the manufacture of processed food products. In addition, purple sweet potatoes have a fairly dense purple color due to the purple pigment anthocyanin which functions as an antioxidant and contains dietary fiber (Darmadji, 2005).

The advantages that can be taken through substitution and addition of biological materials include improving nutrition, as health food and increasing the preference value of these processed products. Research on the addition of purple sweet potato flour up to 30 g in chicken nuggets resulted in the physical properties and sensory quality of chicken nuggets that were favored by panelists (Ratulangi & Rimbing, 2021). The study of the use of vegetable products with purple sweet potato flour added to processed meat products such as nuggets is an interesting side to study. It is hoped that the

addition of purple sweet potato flour will produce nugget products that contain dietary fiber and antioxidants, so that this product can be used as a source of functional food.

MATERIALS AND METHODS

The ingredients used in this study were 1200 g of fresh chicken breast and 100 g of purple sweet potato flour, milk powder, butter, egg yolk, tapioca flour, breadcrumbs, garlic, onion, pepper, flavoring, DPPH Himmedia 50 mg, methanol proanalysis, vitamin C, hexane, potassium sulfate, sulfuric acid, NaOH-$Na_2S_2O_3$, boric acid, HCl, acetone, mannanase enzyme, phosphate buffer & acetate buffer, coomasie brilliant blue G250, BSA.

Data was collected by measuring variables through proximate analysis and antioxidant activity. The tabulated data is then analyzed by analysis of diversity. If there are differences between treatments, it is continued with Duncan's Multiple Region Test (Setyaningsih et al., 2010).

RESULTS AND DISCUSSIONS

The results of the analysis of antioxidant activity, water content, fat content, protein content and dietary fiber content of chicken nuggets added with purple sweet potato flour are as shown in Table 1 below:

Table 1. Average antioxidant activity, water content, fat content, protein content and dietary fiber content of chicken nuggets added with purple sweet potato flour

Variable	Treatment			
	P1	P2	P3	P4
Antioxidant activity (µg/ml)	3356.99^{a}	3108.10^{b}	2578.90^{c}	2398.30^{d}
Water content (%)	58.36^{a}	58.35^{a}	56.60^{b}	56.79^{c}
Fat content (%)	7.35^{a}	7.81^{b}	8.01^{bc}	7.43^{ab}
Protein content (%)	14.17^{a}	14.75^{b}	14.81^{bc}	14.21^{a}
Dietary fibercontent (%)	0.66^{a}	0.53^{b}	0.84^{c}	0.92^{d}

Note: Different superscripts on the same line show significantly different (P<0.05).

Antioxidant activity

The results of the observation of the antioxidant activity of chicken nuggets with the addition of purple sweet potato flour ranged from 3356.99 to 2398.30 µg/ml. The more the addition of sweet potato flour to chicken nuggets, the lower the antioxidant activity value, which means the better.

The results of analysis of variance, the addition of purple sweet potato flour 10-40 g to chicken nuggets gave a very significant difference (P<0.01) on the value of antioxidant activity. Based on the DMRT further test, it was shown that treatment P1 was significantly different (P<0.05) with treatment P2, P3 & P4. Treatment P2 was significantly different (P<0.05) with treatment P3 & P4, as well as treatment P3 was significantly different (P<0.05) with treatment P4. The difference in the value of antioxidant activity in the treatment of adding purple sweet potato flour to chicken nuggets was due to the presence of anthocyanins as natural antioxidants contained in purple sweet potato flour. The higher the treatment with the addition of purple sweet potato flour, the higher the anthocyanin content causes the value of antioxidant activity to increase as measured by the Inhibiting Concentration (IC50) (Tritantini et al., 2016). The antioxidant activity contained in purple sweet potato flour which was used as a treatment in this study was based on the results of the examination of the Medical Laboratory of the Health Polytechnic of the Ministry of Health of Manado (2021) of 46.9 ug/ml. Anthocyanins are a type of natural antioxidant. The natural antioxidants contained in purple sweet potatoes can stop the chain reaction of free radical formation in the body which is believed to be the mastermind behind premature aging and various accompanying diseases such as cancer, heart disease, high blood pressure, and cataracts. Anthocyanins isolated from purple sweet potato have strong antioxidant activity (Pokorny et al., 2001).

Water content

The results of observations of the water content of chicken nuggets with the addition of purple sweet potato flour at different levels ranged from 56.60 to 58.36%. The more the addition of sweet potato flour to the chicken nuggets, the lower the water content.

The results of analysis of variance, the addition of purple sweet potato flour 10-40 g to chicken nuggets gave a very significant difference (P<0.01) on the percentage of water content. Based on the DMRT further test, it was shown

that treatment P1 was not significantly different (P>0.05) with treatment P2, but significantly different from treatment P3 & P4. Treatment P2 was significantly different (P<0.05) with treatment P3 & P4, as well as treatment P3 was significantly different (P<0.05) with treatment P4. The difference in water content values in the results of this study was due to the addition of purple sweet potato flour to chicken nuggets, causing differences in the water content of each treatment. The higher the addition of purple sweet potato flour to chicken meat nuggets, the water content decreases. The value of the water content in this study was about 56.60% to 58.36% lower than the recommended water content of SNI 6683:2014, which is a maximum of 60% specifically for combination chicken nuggets.

Water content is one of the most important characteristics of foodstuffs, because water can affect the appearance, texture, and taste of foodstuffs. The water content in foodstuffs determines the freshness and durability of these foodstuffs, high water content makes it easy for bacteria, molds, and yeasts to breed, so that changes will occur in foodstuffs, the lower the water content, the slower the growth of microorganisms to breed, so that the decay process will take place more slowly, the limit of the water content of microbes that can still grow is 14-15% (Winarno (2004) in Rijal et al., 2019).

Fat level

The results showed that the average fat content of chicken nuggets with the addition of purple sweet potato flour ranged from 7.35% to 8.01%. The results of analysis of variance showed that the addition of purple sweet potato flour 10-40 g to chicken nuggets had a very significant effect (P<0.01) on the percentage of fat content. Based on the DMRT further test, it was shown that treatment P1 was significantly different (P<0.05) with treatment P2 & P3 but not significantly different from treatment P4. Treatment P2 was not significantly different (P>0.05) with treatment P3 & P4, as well as treatment P3 was not significantly different (P>0.05) with treatment P4. The difference in the fat content value from the results of this study was caused by the addition of purple sweet potato flour to chicken nuggets, causing differences in the fat content of each treatment. The higher the addition of purple sweet potato flour in chicken nuggets, the fat content decreases. The fat content value in this study was about 7.35% to 8.01% lower than the recommended fat content of SNI 6683:2014 with a maximum quality requirement of 20% both chicken nuggets and combinations. This chicken nugget product with the addition of purple sweet potato flour can be a reference for consumers who want chicken nugget products with low fat content. Consumption of excess fat can harm consumers, where fat is stored in the body so that it can cause various diseases such as stroke, heart, coronary, obesity and so on. Winarno (2002) in Rijal et al. (2019) states that fats and oils are found in almost all types of food with different contents. The fat content in foodstuffs is crude fat and is the total lipid content in actual amounts. Fats and oils are food substances that are important for maintaining the health of the human body. In addition, fats and oils are also a more effective source of energy than carbohydrates and proteins.

Protein level

The results of the observation of the protein content of chicken nuggets with the addition of purple sweet potato flour ranged from 14.17% to 14.81%.

The results of analysis of variance, the addition of purple sweet potato flour 10-40 g to chicken nuggets gave a very significant difference (P<0.01) on the percentage of protein content. Based on the DMRT follow-up test, it was shown that treatment P1 was significantly different (P<0.05) with treatment P2, P3 but not significantly different (P>0.05) with treatment P4. Treatment P2 was not significantly different (P<0.05) with treatment P3 but significantly different (P<0.5) with treatment P4, as well as treatment P3 was significantly different (P<0.05) with treatment P4. The addition of purple sweet potato flour to chicken nuggets resulted in different protein levels for each treatment. This difference in protein content values was caused by the addition of purple sweet potato flour in each treatment. The value of protein content in this study was 14.17% to 14.81% higher than the quality requirements set by SNI 6683:2014, a minimum of 12% for chicken meat nuggets and 9% for combination chicken nuggets. Thohari (2017) said that the additives used were very influential, the higher the protein content of the additives, the higher

the protein content of the nuggets. According to the Indonesian National Standard 6683-2014, the nutritional quality requirement for chicken nugget protein in 100g is 12% and with the addition of purple sweet potato flour, the protein content of chicken nuggets increases between 14.17% to 14.81%.

Protein is a food substance that is very important for the body, because this substance in addition to functioning as fuel in the body also functions as a building and regulatory substance. The main function of protein for the body is to form new tissue and maintain existing tissue (Winarno, 1997). The high protein value of meat is due to its complete and balanced content of essential amino acids. Essential amino acids are the building blocks of body proteins that must come from food (cannot be formed in the body) (Wijayanti et al., 2013).

Dietary fiber level

The results of observations of the fiber content of chicken nuggets with the addition of purple sweet potato flour ranged from 0.53% to 0.92%. The results of analysis of variance, the addition of purple sweet potato flour 10-40 g to chicken nuggets gave a very significant difference ($P<0.01$) on the percentage of dietary fiber content. Based on the DMRT further test showed that treatment P1 was significantly different ($P<0.05$) with treatment P2, P3 P4. Treatment P2 was significantly different ($P<0.05$) with treatment P3 & P4, as well as treatment P3 was significantly different ($P<0.05$) with treatment P4. The difference in dietary fiber content in the results of this study was due to the addition of purple sweet potato flour treatment. The higher the addition of purple sweet potato flour to chicken nuggets, the higher the dietary fiber content. The dietary fiber content of this study was about 0.53% to 0.92% higher than the water soluble dietary fiber content of Tutut snail nugget with 75% sweet potato flour substitution was 0.82% (bb) and 1.59% (wk), and higher than the water soluble dietary fiber content in mandalung duck nuggets with the addition of 20% carrots, which is 0.71% (bk) (Patriani (2010) in Nurhidayah, 2011).

Dietary fiber is a component of plant tissue that is resistant to hydrolysis by enzymes in the stomach and small intestine. Dietary fiber is divided into two groups, namely soluble fiber (soluble dietary fiber) and insoluble fiber (insoluble dietary fiber). Soluble Dietary Fiber (SDF) is a pectin, gum, mukilase which is widely found in oatmeal, nuts, vegetables and fruits. Insoluble fiber such as cellulose, hemicellulose and lignin are found in cereals, legumes and vegetables (Winarno, 2008). Purple sweet potatoes contain high levels of dietary fiber, minerals, vitamins and antioxidants. Pectin, hemicellulose, and cellulose compounds which are dietary fiber are found in sweet potatoes and play a role in determining their nutritional value. Dietary fiber is a polysaccharide that cannot be digested and absorbed in the small intestine so that it will be fermented in the large intestine (Murtiningsih & Suyanti, 2011). According to Sarwono (2005), sweet potatoes contain a lot of carbohydrates ranging from 75-90%, consisting of 60-80% starch, 4-30% sugar, cellulose, hemicellulose, and pectin.

CONCLUSIONS

Based on the results and discussion, it can be concluded that the addition of purple sweet potato flour up to 30 g resulted in antioxidant activity and good chemical quality of chicken nuggets as a functional food.

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IMPROVING THE QUALITY OF PRODUCTS IN FOOD INDUSTRY. APPLICATION OF QUALITY FUNCTION DEVELOPMENT METHODOLOGY FOR CHICKEN LIVER PÂTÉ

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Abstract

Quality Function Development (QFD) is a systematic approach specific to quality management that facilitates product development by ensuring consumer requirements meeting "customer voice", these being taken into account from the design phase, then during the entire technological process, being reflected in the quality characteristics of the finished product. The purpose of this study was to apply the QFD methodology to improve the quality of products in the food industry, taking into account the technological process of chicken liver pâté (designing a new product that meets the requirements of consumers), thus providing a synthetic model. The working method consisted in the participation of a number of 250 consumers, aged between 20-24 years, who provided the list of consumer requirements, prioritizing and weighting them based on a standardized score from 1 to 5 points. The following stages were represented by the transposition of consumers' voice in quantifiable technical requirements, their correlation using predefined symbols, establishing the direction of improving the quality of the new product, assessing current competition and determination of target values. Following the analysis, the most important consumer requirements for chicken liver pâté were: the taste (15.63%), the smell (15.63%), the appearance/ color (15.63%), the small amount of saturated lipids/ without added lard (12.5%), the fine texture and spreadable (12.5%), without synthetic colorants and preservatives (12.5%), good price (9.37) and without flavor enhancers (6.24%). Thus, in order to meet consumer requirements, the replacement of sodium nitrite with turmeric powder (Curcuma longa L.), as alternative natural colorant and preservatives (curcumin the principal bioactive substance of turmeric) led to a healthy product, but which will have a higher price compared to the products currently available on the market. However, applying the level II/ III of QFD methodology the low cost was provided by mitigation of price of raw material's.

Key words*: chicken liver pâté, Quality Function Development.*

INTRODUCTION

In the last years, traditional meat-based products were the target of the food industry for innovation and improved products development. This trend is consistent with recent concerns about the consumption of these products due to their high saturated fat content, which is linked with disorders such as heart, diseases, cancers and obesity (Bis-Souza et al., 2019; Martins et al., 2020). The meat industry is paying attention to those strategies that aim to change the fat source to promote healthier dietary intake (Paglarini et al., 2022). However, despite the pâté being a popular food in many countries, its components, specially backfat, provide a caloric product with high saturated fatty acids (SFA). Consequently, these types of products are questioned by government agencies and consumers (Domínguez et al., 2017; Marin et al., 2019). Therefore, with the purpose to encourage the consumption of this product, the lipid profile improvement (lower SFA and high of PUFA) of pâté emerged as a promising approach to obtain a healthier product (Vargas-Ramella et al., 2022).

Pâté is a homogenized food product with a predominant content of meat or liver, the manufacturing being based on combining of various types of ingredients and methods of their processing (boiling, blanching, sautéing, frying, homogenization etc.) depending on the recipe (Marudova et al., 2018; Kabdylzhar et al., 2022). The composition of meat pâté can provide a significant impact on their nutritional characteristics with a wide variety of recipes, where chicken offal is added, that are sold on the world market. Chicken pâté are becoming more and more popular due to the increase in the global production of poultry meat; the production of by-products obtained after the poultry slaughter is increasing simultaneously

(Mokrejs et al., 2017; Carpes et al., 2020; Mielnik et al., 2002; Singh et al., 2013; Biswas et al., 2007).

Many companies compete to create new products that can speed up their marketing time. A commonly used method for product management is QFD (Rujito et al., 2020). Its double purpose is to ensure that the true needs of customers are properly developed and implemented throughout the design, "construction" and delivery of a new product, whether assembled, processed, maintained or even software, and to improve the product development process itself (Akao & Mazur, 2003). QFD is a comprehensive quality system that aims primarily at customer satisfaction (Pop et al., 2020). Since 2015, the QFD methodology has been transposed into the international standard (ISO 16355), which includes eight parts structured in several editions, the latest from 2021, and others that are still in progress, making QFD much more credible and practical. Conventional QFD consists of the following four phases (Sayadi et al., 2017; Dvoryaninova et al., 2020; Isharyani et al., 2019):

1. the first stage translates the marketing requirements into technical attributes;
2. the second phase translates the technical attributes into the characteristics of the parts;
3. the third phase transposes the characteristics of the part into manufacturing operations;
4. the fourth phase translates the manufacturing operations into production requirements.

The main planning tool used in QFD is the Quality House (HoQ). HoQ is a house-shaped matrix that connects the customer's wishes (WHAT?) and how the product will be designed and made to meet the customer's wishes (HOW?).

The purpose of this study was to apply the QFD methodology to improve the quality of products in the food industry, taking into account the technological process of chicken liver pâté (designing a new product that meets the requirements of consumers increasingly informed and more concerned about nutrition and health), thus providing a synthetic model. In this study, the replacement of carmine and of sodium nitrite or other food colorants and preservatives with turmeric was the novelty for technological production of liver pâté. Turmeric is a natural antiseptic. Due to its extra-ordinary molecular structure it shows strong anti-oxidative, as well as anti-inflammatory properties. It is extensively used for imparting color and flavor to the food. In traditional medicine, turmeric is used to treat a wide variety of diseases (Dada Khalandar et al., 2018). The active component of turmeric, a common Indian spice, which is derived from the dried rhizome of the *Curcuma longa* (a member of the *Zingiberaceae* family plant) is curcumin, a yellow substance belonging to the polyphenols superfamily. Numerous studies have demonstrated that curcumin possesses anti-oxidant, anti-inflammatory and anticancerous properties. Curcumin and its analogues have been demonstrated to possess various anticancer properties in a series of cancer cell lines, such as pancreatic, lung, ovarian, oral, colorectal, breast carcinoma and even in melanoma cells. In the future, further research will certain or not the potential of curcumin analogues as effective chemotherapy agents (Vallianou et al., 2015).

MATERIALS AND METHODS

The methodology consisted in the participation of a number of 250 chicken liver pâté consumers, aged between 20-24 years (students from three different Food Engineering specialties', because the QFD methodology recommends the use of multidisciplinary work teams), which provided the list of consumer requirements, prioritizing and weighting them based on a score from 1 to 5. The next steps were to transpose consumers' voice into quantifiable technical requirements (design requirements), establishing measurement units for each requirement, correlating them to "the roof" of HoQ to identify possible technological problems, establishing the relationship between technical measures and the customer's voice using pre-defined symbols, establishing the direction to improve the quality of the new product (which are technical criteria that require a decrease or increase to meet customer requirements), the *Benchmarking*-assessment of current competition (establishing the strengths and weaknesses of the newly designed product, X) and determining the target values (which need to be improved). Next stapes for the level II and III of QFD methodology are: exploring the product and processes alternative, selecting

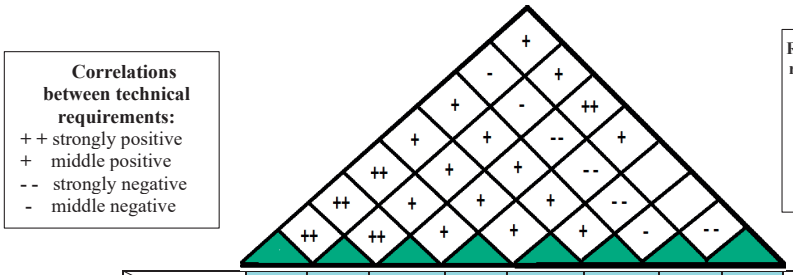
the best alternative after application of process flowchart with the parameters, the CPs, the CCPs and the critical limits established for each stage of the chicken liver pâté.

RESULTS AND DISCUSSIONS

The consumers are increasingly informed and more concerned about health and nutrition.
The consumer's requirements (Figure 1), were mainly represented by the sensory properties *(good taste; pleasant, characteristic smell; appearance/color; fine texture and spreadable)*, nutritional quality from the perspective of the positive/negative influences on the consumer's health after ingesting the product *(small amount of saturated fat/ without added lard; without synthetic colorants and preservatives; without flavour enhancers)* and its price.
The "customer's voice"/consumer requirements (WHAT?) was translated practically in technical criteria with related measure units (HOW?):

- % of liver, onion, salt;
- % of liver and spices;
- % of liver, turmeric, paprika;
- % walnuts oil;
- sieve diameter (Ø=2mm), % of liver broth;
- % turmeric, paprika and salt;
- Low Production cost (RON);
- % of protein, liver, spices, oil.

To meet these requirements, a new product has been designed, with a higher proportion of liver (72%) compared with current option of Romanian market (between 20÷45% liver), innovative in terms of ingredients traditionally added to chicken liver pâté manufacturing technology (Figure 2), replacing the chicken skin/saturated fats with walnut's oil, the food dye (carmine) and preservative (sodium nitrite) with turmeric and paprika; alongside, the flavour enhancers were eliminated also by the use of a higher proportion of liver (72%).
Predetermined symbols are used to highlight the relations between the customer's voice and quantifiable technical measures (WHAT vs. HOW?) placed in the cell located at the intersection of each row vs. column.
At the level of the "foundation" of the HoQ, the higher the values obtained, the more important those characteristics are (the good taste and without flavor enhancers (both with 16%) and very close the pleasant/characteristic smell (15.9%) and the appearance/colour (15.5%) and without synthetic colorants and preservatives (10.8%) because there are strong correlations between the ingredients of chicken liver pâté and the specific parameters/ technical criteria of the stage of technological flow (HOW MUCH?).
The results from the first house (HoQ 1. product planning) are further used in the following matrices specific to the QFD methodology (level II/III process design).
The room on the right side of the HoQ is the assessment of current competition (*Benchmarking*) used to measure the success of the newly designed product that competes with those on the market; thus a scale from 1 to 5 is used for the assessment (1 indicates a requirement that is not met and 5 indicates a requirement that is fully met). By averaging the numbers in each column, depending on the score obtained, a measure of the degree of customer satisfaction for each product under study is obtained.
Following the comparative analysis of the newly designed X product, with products of five competing companies (*Ardealul, Bucegi, Capricii și delicii, Sadu, Scandia Sibiu* randomly coded with A, B, C, D and E), a good position of product X was obtained (36 points), compared to the current competitors (20 points for product C, 21 points for product A and B, 27 points for product E, respectively 30 points for product D); the weaknesses being represented by the price of the product. This score from *Benchmarking* reflects a concrete/quantifiable customer requirements satisfaction.
The determination of target values is based on the values established by the evaluation of competing products and product X, establishing strategies to maintain strengths and improve weaknesses.
The strengths of product X are transposed into the technical criteria represented by the elimination of saturated fat/ added lard, of flavor enhancers, synthetic colorants and preservatives from the ingredients vs. use of carefully selected quality of chicken liver, walnuts oil, turmeric and paprika from controlled origin, certified with quality standards (with declaration of conformities and analysis bulletin from the stage of qualitative reception).



Technical requirements (HOW?) / (WHAT?) Consumer requirements	Importance 1-5	Importance %	Improvement direction ⇧ % of liver, onion, salt, pepper	⇧ % of liver and spices	⇧ % of liver, turmeric, paprika	⇩ % walnut oil	⇩ sieve Ø, % meat broth	⇧ % turmeric, paprika and salt	⇩ Production cost (RON)	⇧ % of protein, liver, spices, oil	Benchmarking NEW PRODUCT= X 1p	2p	3p	4p	5p
Good taste	5	15.63	●	●	●	O	▽	●	O	●		A, B	C, E	D	X
Pleasant smell	5	15.63	O	●	O	▽	▽	O	▽	●		A, B	C	E	D,X
Appearance/ colour	5	15.63	●	●	●	▽	O	O	▽	O		B	A, C	D,E	X
Small amount of saturated fat	4	12.5	O	▽	▽	●	●	-	O	●		B, A	C,E	D	X
Fine texture and spreadable	4	12.5	●	O	O	▽	●	▽	O	O		C	A,D	E	X,B
Without colorants and preservatives	4	12.5	O	O	●	-	▽	●	●	O	A,C	B	E	D	X
Good price	3	9.37	●	●	●	●	O	O	●	●	X	D	C, E	B	A
Without flavour enhancers	2	6.24	●	●	●	▽	O	●	●	●		B, C	A, E	D	X
	32	100%	656	650	631	293	363	444	406	656	=4099				
		%	**16**	**15.9**	**15.5**	**7.1**	**8.8**	**10.8**	**9.9**	**16**	100%				

Figure 1. House of Quality (HoQ) for chicken liver pâté

⇧=*parameter increase*; ⇩=*parameter decrease*

The ingredients of the products under the study was:

1. For product A: water, chicken liver 20% (EU origin), chicken meat, chicken skin, sunflower vegetable oil, palm vegetable oil, soy vegetable protein, potato starch, poultry animal protein, wheat starch, sugar, salt, onion, tomato paste, spices, extract of yeast.
2. For product B: chicken liver 20% (origin: EU), water, chicken meat, sunflower oil, soy protein, iodized salt, sugar, onions, white wheat flour, spices, dextrose, emulsifiers: monoglycerides and diglycerides of fatty acids, stabilizers: sodium diphosphates and triphosphates, yeast extract, thickening agent: xanthan gum, aroma, colouring: carmine, preservative: sodium nitrite.
3. For product C: chicken liver (20%), water, poultry meat, non-hydrogenated sunflower vegetable oil, soy vegetable protein, corn starch, iodized salt, sugar, onions, spices, dye: carmine, preservative: sodium nitrite.
4. For product D: chicken liver 45%, water, vegetable oil, salt, onion, spices and spice extract (paprika, pepper, mustard, thyme, coriander, rosemary), flavor enhancer (yeast extract).
5. For product E: chicken liver (20%), chicken meat, vegetable oil, non-hydrogenated sunflower, soy protein, water, iodized salt, sugar, corn-starch, sour cream powder, milk powder, onion, spices, garlic.
6. For product X: liver 72%, onion, water, walnut oil, pepper, nutmeg, ***turmeric***, paprika and salt.

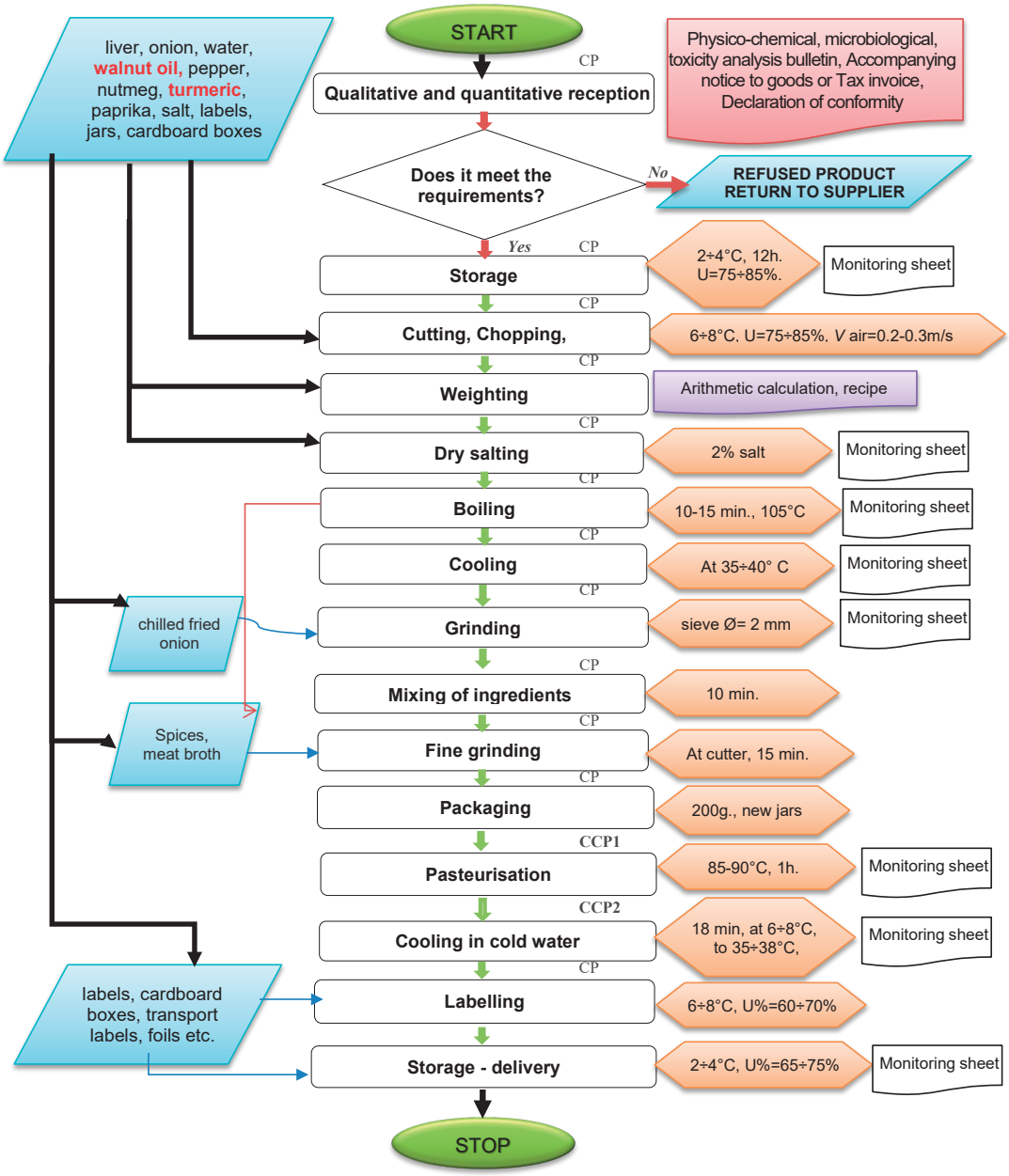


Figure 2. The flowchart for the new design product (*chicken liver pâté*, X product)

For maintaining the quality characteristics of new X product/chicken liver pâté, the technological parameter and monitoring sheet will be periodically checked and completed by the qualified and trained human resources. Next stapes for the level II and III of QFD methodology are: exploring the product and processes new solution, selecting the best alternative after application of process flowchart with the parameters, the CPs, the CCPs and the critical limits previously established for each stage of the chicken liver pâté technology (Figure 3).

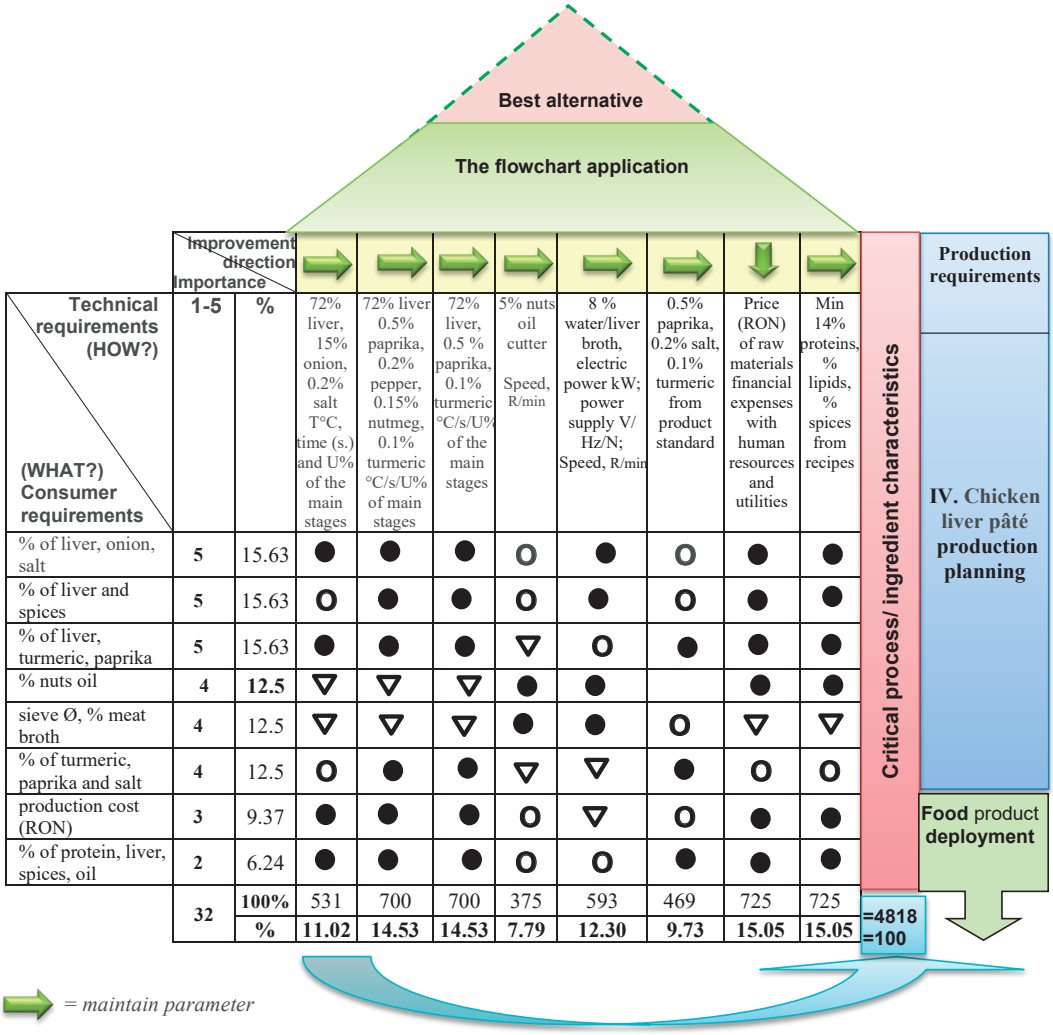


Figure 3. QFD matrix level II/ III and IV, design requirements for identify key design characteristics of chicken liver pâté, synthesis

The most important relation was found for production cost (RON) and of protein content, liver, spices and oil proportion/*without flavour enhancers* (15.5% for booth), followed by the proportion of liver and spices/*pleasant smell* and turmeric and paprika/*appearance-colour* (14.53% for booth).

Critical process- ingredient characteristics/ the proportion of ingredients for the voice of consumer satisfaction were represented by: 72% liver, 15% onion, 8 % water/liver broth, 5% walnuts oil, 0.5% paprika, 0.2% salt, 0.2% pepper, 0.15% nutmeg, 0.1% turmeric (from product standard/recipe's); temperatures/T°C,

time (s) and humidity/(U%) of the main stages; electric power kW; power supply V/Hz/N; Speed, R/min for grinding machine; Cutter Speed, R/min; Price (RON) of raw materials; financial expenses with human resources and utilities; minimum 14% proteins, the proportion of lipids and spices from recipes.

Maintaining and improving the critical parameters (after several attempts in production) ensures the success of the sale and the high profit obtained for the new design product (X product).

CONCLUSIONS

In order to meet consumer requirements, the replacement of sodium nitrite with turmeric powder (*Curcuma longa* L.), as alternative natural colorant and preservatives, led to a healthy product, but which will have a higher price compared to the products currently available on the market. However, applying the level II/ III of QFD methodology the low cost was provided by mitigation of price of raw material's.

What should be noted in the QFD methodology applied to the food industry (and of particular importance) are the inter-correlations between the ingredients and processes (that cannot be considered separately), with the influence on the quality characteristics specific to the finished product obtained.

The correct management of the technological processes, of the parameters related to each technological stage, respectively of the critical limits and the compliance of the technical specifications are the key elements that lead to the satisfaction of the consumers' requirements and to the improvement of the quality of the finished products.

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RESEARCH ON THE GELLING AND EMULSIFYING CAPACITY OF PECTIN OBTAINED BY SEMISYNTHESIS AND USED IN THE FOOD INDUSTRY

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Abstract

The chemical composition and nutritional value of food products are determined both by the raw materials from which they come and by the contribution of auxiliary materials. These substances, for the most part, are added in order to improve some properties of the products. Respecting the legislation regarding the allowed limits of additives in food products, it is necessary to pay special attention in order to maintain the safety and health of the population. The generic term pectin includes polygalacturonic acids (acid polysaccharide) whose carboxylic groups are esterified in varying proportions with methyl alcohol and partially neutralized with calcium or magnesium ions. The specific properties of pectic substances, due to which they have uses in the food industry, are the gelling capacity and the stabilizing capacity of emulsions. Also, the gel does not show the phenomenon of syneresis, does not absorb moisture from the external environment and is resistant to sugaring. In our country, pectin is obtained industrially from apple pomace, a by-product resulting from obtaining juices and has multiple uses. The functional properties are largely determined by the degree of methoxylation of polygalacturonic acid, which determine the degree of esterification of this polysaccharic acid.

Key words*: emulsifying, food industry, gelling, pectin.*

INTRODUCTION

The generic term pectin includes polygalacturonic acids (polysaccharide) whose carboxylic groups are esterified in varying proportions with methyl alcohol and partially neutralized with calcium or magnesium ions (Mohnen, 2008).
The specific properties of pectic substances due to which they are used in the food industry are: the ability to gel and the ability to stabilize emulsions. Also, the celtic gel does not show the phenomenon of synerese, does not absorb moisture from the outside environment and is resistant to sugaring.
Pectin is used in the manufacture of jams, marmalades, jellies, as a stabilizer of emulsions, in the manufacture of margarine, mayonnaise and ice cream, in the preparation of aspic for meat or fish products, in addition to prolonging the shelf life of fresh pastries, at obtaining edible protective coatings for cold-preserved products, as thickeners for creams, in obtaining various preparations for diabetics (Laurent & Boulenguer, 2003; Liu et al., 2003).
The functional properties are largely determined by the degree of methoxylation of polygalactouronic acid (Dergal et al., 2006).
Totally esterified pectin lacks the ability to gel. Pectins with a high degree of esterification, with a methoxyl group content over 9% are obtained from apple pomace or citrus peel (albedo). A normal gelation in the case of these pectins is obtained for a concentration of 65% sucrose in the gel and a pH = 3 (Pilgrim et al., 1991).
Low esterification pectins (less than 60%) containing less than 7% methoxyl groups are extracted from sugar beet borer. In these pectins, gelling occurs for a lower percentage of sucrose (less than 35% sucrose in the gel) or even in the absence of sugar, but in the presence of calcium ions and in a wide pH range between 2.5-6. Calcium ions make the

connection between carboxyl groups of polygalactouronic acid molecules (BeMiller, 1986).
The fruit processing industry uses exclusively highly esterified pectin. These pectins are partially desesterified in a controlled manner, using various demethoxylation processes (Willats, 2001).
Pectins have industrial uses as gelling and stabilizing agents for food and cosmetics. They have been used in the synthesis of biofilms, adhesives, paper substitutes and medical products for implants or drug carriers (Thakur, 1997).
Many studies indicate its benefits for human health, as it has been shown to help lower blood cholesterol and glucose levels, in addition to boosting the immune system.
Pectin as a finished product can be obtained in different forms:
- Pectic extract, which is obtained by extracting hot apple pomace, followed by concentration. The extract contains 10-12% soluble dry matter and 3-4% soluble pectin;
- Pectin powder, obtained by drying pectic extracts or by precipitating aqueous extract, followed by conditioning and then drying;
- Pharmaceutical pectin, characterized by an advanced purity (Thakur, 1997).
Because the manufacture of pectin powder requires complex installations and higher fuel consumption, pectic extracts can be used for short-term use after manufacture.
The technological scheme for the manufacture of apple pectin is shown in Figure 1.
Fresh apple pulp contains 0.7-1.2% pectic substances and 60-70% water. Therefore, it cannot be stored as such, in addition, in order to ensure the use of pectin for several months, it is necessary to preserve the pomace. Two methods can be used: chemical preservation (with 0.15-0.20% SO_2 - for short duration) and drying (up to 5% humidity in drum dryer - the most commonly used method for long-term preservation).
In the absence of drying facilities, pomace can also be preserved by adding SO_2 (5% solution in proportion of 10% compared to pomace), by keeping it cold (more expensive).

Washing the pomace is practiced to remove the accompanying substances of pectic substances (sugars, acids, tannins, dyes, mineral salts) with water at a maximum temperature of 40°C.

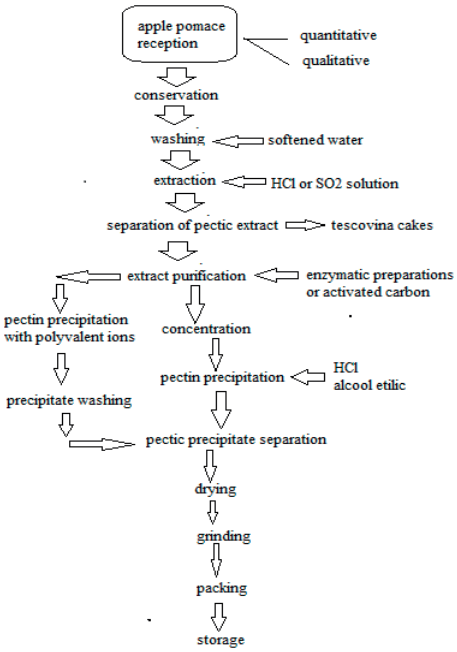

Figure 1. Technological scheme for the manufacture of apple pectin

It is used a volume of washing water of 5 to 6 times larger than the amount of pomace, the duration of the operation being 1-3 hours. The soaking and washing time depends on how the pomace was preserved (12 hours for dry pomace).

The soaking and washing process is performed in an extractor, in which the pectin is subsequently extracted, with the possibilities of stirring and heating the mixture. Sugar-rich wash water can be used.

The extraction of pectin consists in the transformation of the existing protopectin into apple cider vinegar and its transition into solution. The most advantageous hydrolysis takes place with acidified, softened hot water. The added water is 5-6 times higher than the amount of fresh pomace and 10-12 times the amount of dehydrated. It is recommended that the extractive water be added in three stages. The acidification to pH = 2-3 is done with

hydrochloric acid or sulfuric acid. In the last case, which allows to obtain a light pectin, the working parameters are: temperature (86°C), duration (2.5-3 hours) and in the last phase water without acid is added. The extractive process must be conducted in such a way that the efficiency of the operation is as good as possible and the pectin is not degraded. The three solutions of pectic extract, obtained by draining, are cooled to 40°C and mixed with the liquid obtained from pressing the pomace (Adetunji et al., 2017).

Purification of pectic extracts, an operation necessary especially for the processing of insufficiently ripe apple pomace, can be done by two methods: treatment with enzymatic preparations (from *Aspergillus oryzae*) or absorption on activated carbon. The first method is based on the hydrolyzed action of enzymatic preparations added proportion of 0.03-0.05%, at 40-50°C, for 30-40 min, on starch and proteins. In some cases, in order to avoid the hydrolysis of pectin, the pectic extracts are purified by adding 0.1% active carbines and 0.3% kieselgur, followed by filtration (Robledo & Vázquez, 2018).

Concentration of pectic extracts. The purified extracts have a low pectin content, between 0.15 and 0.4%, therefore they are concentrated in vacuum installations at a maximum temperature of 60°C, until the volume is reduced by 8 to10 times, reaching 12% soluble dry matter.

Pectin precipitation. Two methods can be used to separate pectin from solution:

- Alcohol precipitation of the concentrated extract with approximately 3% pectin; the extract is mixed with 1.5% concentrated hydrochloric acid (d = 1.18) and then with 1-1.2 parts of 95% ethyl alcohol, the pectin precipitating as a fibrous mass which separates by filtration. Pectin is washed with ethyl alcohol in a ratio of 1:1. The alcohol used for precipitation and washing is recovered by distillation and reintroduced into the circuit, the method being the most used.
- Precipitation with polyvalent ions, which is done directly on unconcentrated pectic extracts, at pH = 3.6-3.7 and at temperatures of 30°C. The obtained curd is washed with a mixture of alcohol and hydrochloric acid for the complete removal of the metal ions from the curd. The washing liquids are separated from the curd by centrifugation, and the alcohol is recovered. The best results were obtained using anhydrous aluminum chloride.

Pectin drying. The pectic precipitate is dried in vacuum drying instalations at a maximum temperature of 75-80°C, up to a product humidity of 4-5%.

The pectin grindind is done to a maximum size of 2 mm, with the help of hammer-mills. To obtain a product with the same degree of gelling, pectin powder is mixed with powdered sugar in varying amounts.

Pectin packaging is done in shipping containers of different capacities, made of materials intended for dehydrated and hygroscopic products (for example, polyethylene bags or sacks).

Storage will be done in warehouses where temperatures should not exceed 25°C (Shalini R. and Gupta D.K., 2010)

MATERIALS AND METHODS

This paper presents three laboratory methods of pectin semisynthesis.

Method I

Principle of the method:

A method for obtaining pectin from apple pomace is presented, using the process of transforming insoluble protopectin into soluble pectin. This process is carried out with the help of sulfuric acid, followed by the extraction, concentration and purification of the obtained semisynthetic substance.

Procedure:

In a 250 ml flask, provided with ascending refrigerant, thermometer and mechanical stirrer, a quantity of 20 g of dried apple cider vinegar and approximately 150-200 ml of water heated to 45°C is introduced. The contents of the flask are stirred for 30 minutes, after which the liquid containing soluble ballast substances (sugars, mineral salts, tannin, and corrosive substances) is removed.

After removing the liquid, 150 ml of distilled water, heated to 90°C, is introduced into the flask, the pH is corrected to the value of 2 by adding sulphuric acid. The reagent mass is stirred for one hour at 90°C.

The resulting pectin extract is drained and stored, after which two more extractions are

successfully performed, working in conditions identical to those used in the first extract (150 ml distilled water, temperature 90°C, pH = 2 by adding sulphuric acid, stirred for one hour).
The three extracts obtained are mixed and cooled to a temperature of 45°C, after which the gross suspensions are removed by centrifugation.
To clarify the liquid, it is filtered through a kieselgur filter, after which it is concentrated in vacuo at 65°C to a content of 12% dry matter.
The concentrated extract is vigorously mixed with concentrated HCl (d=1.18) in a proportion of 1.5% compared to the obtained solution and then with an equal part of ethanol.
The curd formed is separated by filtration and washed in two steps with ethanol of 95% concentration in a ratio of 2:1 to the precipitate.
The purified pectin is crushed, dehydrated by drying at a temperature of 75-80°C, cooled, grinded and conditioned by the addition of powdered sugar.

Method II

Principle of the method:
A method of obtaining pectin from apple pomace or orange peel (albedo) is proposed. A combined process of acid hydrolysis with enzymatic treatment is used.
An amylolytic preparation obtained from *Aspergillus oryzae* is used.
Procedure:
A quantity of 20 g of apple pomace or dried orange peel, finely chopped, are placed in a 250 ml flask provided with an ascending refrigerant, a thermometer and a mechanical stirrer.
Sulfuric acid solution is added until the pH of the reagent mass is 2.5-3.5.
Pectin extraction takes place at a temperature of 85-92°C for one hour.
The obtained extract is separated from the solid residues by filtration, then the pH is corrected to the value of 4.5-5 by the addition of Na_2CO_3.
The crude pectyl extract is further treated with 0.5% amylolytic preparation (*Aspergillus oryzae* mycelium, grown on wheat bran), at a temperature of 40-50°C for 30-60 minutes. At the end, the temperature of the reagent mass is raised to 80°C to inactivate the enzymes.
For clarification and classification of the extract, it is mixed with 0.02% kieselgur (relative to the amount of extract) after which it is filtered.
The clear filtrate is concentrated under reduced pressure at 50-60°C to a content of about 3% pectin (15 degrees refractometric).
The concentrated extract is mixed with 95% ethanol, in a ratio of 1:1.2 and acidified with concentrated HCl in a proportion of 1.5%. A pectin clot is formed which separates by filtration. The curd is washed on a filter with 95% ethanol. The purified product, having the consistency of a thick paste, is dehydrated by drying at a temperature of 60-70°C.

Method III

Principle of the method:
Pectin is obtained from sugar beet borer using the acid hydrolysis (HCl) reaction to convert insoluble protopectin to soluble pectin. The precipitation of soluble pectin is done by treating the pectin extract with aluminium sulphate.
Procedure:
In a 250 ml flask equipped with an ascending refrigerant, thermometer and mechanical stirrer, a quantity of 15 g of sugar beet marc (with a humidity of 1%), 4.5 ml of 35% HCl solution and 16 ml of water are introduced.
The contents of the flask are heated with continuous stirring at 70°C for two hours. The resulting extract is passed through a centrifugal separator to separate the pulp particles.
The clear solution obtained is partially discoloured and deodorized by mixing with activated charcoal in a proportion of 0.5% carbine compared to the extract, for 25-30 min. under stirring, after which it is filtered. To the clear solution of pectin there was added a 10% ammonia solution to pH = 4 and then with continuous stirring, aluminium sulphate in a proportion of 3 g and aluminium sulphate 9% concentration. Pectin changes into aluminium pectinate, which precipitates.
A new pH correction is made at the value of 4 by adding ammonia.
The precipitated pectin is separated by filtration, after which, together with a solution of citric acid of 56% concentration (in the ratio of curd/citric acid of 4:1), it is introduced into a flask provided with a stirrer and mixed at room temperature until obtain a homogeneous viscous fluid. By adding water, the

concentration is corrected to about 10% dry matter. 0.12% SO_2 or 0.15% sodium benzoate is added to preserve the solution.

RESULTS AND DISCUSSIONS

The gelling capacity of pectin was checked as follows: a mixture of 0.26 g pectin, 17.5 ml water and 3 g sugar flour, put in a Berzelius glass and boil for about 2 minutes, until the pectin is completely dissolved. 22.7 g of caster sugar were added to the obtained solution and heated and stirring for 7-8 minutes. Because pectin is difficult to dissolve in concentrated sugar solutions, not all sugar is added from the beginning.

After the boiling was stopped, the solution was left to stand for one minute, after which 1 ml of tartaric acid solution was poured over the obtained solution (tartaric acid solution contains 488 g of tartaric acid/11 g solution).

The resulting product was allowed to stand again at 25°C until a gel was obtained. The gelling power of pectin was expressed in US-SAG degrees.

In Tables 1, 2 and 3 there are presented pectin characteristics and pectin LM, LMC and LMA gelling conditions.

Table 1. Pectin characteristics

Parameter	Pectine HM	Pectine LM-LMC	Pectin LM-LMA
DE	58-85	25-50	23-50
DA	0	0	≤ 25
pH	2.8-3.5	3.2-4.7	3.5-4.7
MM	140000-190000	70000-140000	70000-140000

DE- degree of esterification, DA- degree of acetylation, MM-molas mass
Pectine HM - High methoxylated pectins
Pectine LM - Low methoxylated pectins
LMC - Low calorie methoxylate
LMA - Amidated low methoxyl

Table 2. Pectin LM gelling conditions

Parameter	Pectine LM		
DE	40 - 37	33 - 30	27 – 24
COO^-	+	+ +	+ + +
Ca^{2+} sensitivity	Low	Medium	High
Reaction speed	Low	Medium	Fast
Ca^{2+} requirement	High (20)	Medium (12)	Low (7)

COO^- - Carboxylate ion
Ca^{2+} - Calcium (mg/g)

Table 3. Gelling conditions for CML and AML pectins

Parameter	Pectine LM-LMC	Pectin LM-LMA
DE	30	30
DA	0	17
COO^-	70	53
$COOCH_3$	30	30
$CONH_2$	0	17
Ca^{2+} requirement	High	High

$COOCH_3$ - methyl acetate
$CONH_2$ - amide group

Figure 2 shows the strength of the gel formed with LM as a function of Brix and the addition of Ca^{2+} in mg/g of pectin.

The mechanism of pectin gel formation involves the presence of calcium ions, structurally the gel having the configuration of the egg shell (Figure 3).

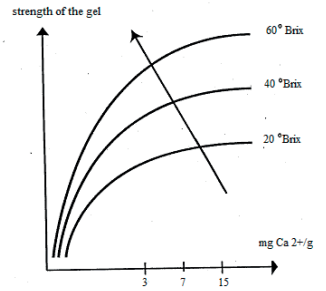

Figure 2. The strength of the gel formed, depending on the Brix and the addition of Ca^{2+}.

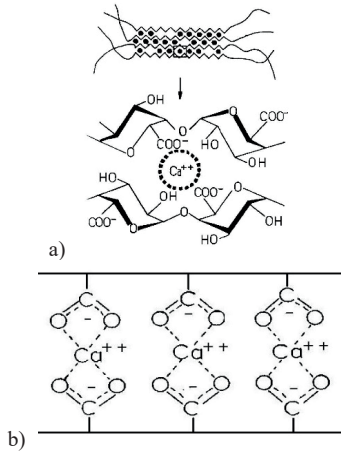

Figure 3. Mechanism of gel formation with low methoxylated pectins:
a) "egg formwork" mechanism;
b) Ca^{2+} intervention in the binding of two polygalacturonic chains

The mechanism of gel formation with highly methoxylated pectins involves the formation of hydrogen bonds and hydrophobic interactions, which support the formation of the three-dimensional network of the gel.
The conditions for the formation of the gel are: total soluble substance, minimum 60° Brix, sugar having the role of reducing the acidity, the acidity of the medium must ensure a pH<3.5.
The acidity decreases the electrostatic repulsion between the HM pectin chains. HM pectin gel is irreversible and its strength depends on pH value (Figure 4) and Brix value (Figure 5).

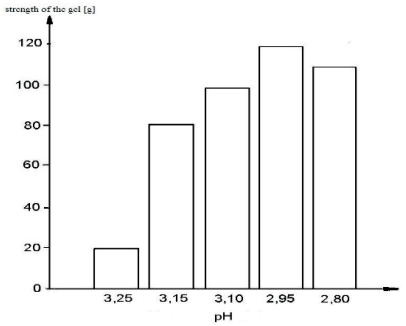

Figure 4. HM pectin gel strength depending on pH

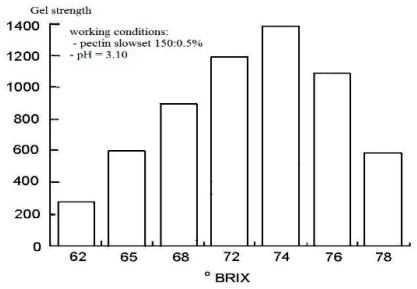

Figure 5. HM pectin gel strength depending on °Brix

CONCLUSIONS

Pectins are an important source of fiber that is present in a large number of vegetables and fruits consumed daily by humans, as it is a structural part of the cell walls of most green plants.
Due to their composition, pectins are very soluble in water molecules, which is why they have multiple applications, especially in the food industry.
Pectins can be used as gelling, stabilizing or thickening agents for many dishes, especially jellies and jams, yogurt drinks, milk and fruit milkshakes and ice cream.
Apple pectin is pectin that is derived from apples and is usually sold in powder form. It can be used as a gelling and thickening agent, as well as a food stabilizer. It is also used in medicine, as a supplement, in chewers such as throat lozenges or as a laxative additive for its natural purgative qualities. Apple pectin is full of healthy carbohydrates, dietary fiber, sodium, manganese, copper and zinc.
NH pectin is an apple pectin commonly used for fruit glazes and fillings. It is a modified type of LM pectin. NH pectin needs calcium to gel, like any other type of LM pectin. It is also thermally reversible, which means it can be melted, set, remelted and then reset again.

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RESEARCH ON THE MILK SECTOR TO REALIZE ROMANIA'S RESILIENCE

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Abstract

Ensuring security and resilience in the Romanian dairy and dairy sector is possible only through thought, planning and exercise to absorb, return and then adapt existing production capacity and warehouses to disruptive threats. As it is known, milk is a universal food that is administered both in the first days of birth and throughout life, to children, adolescents, the elderly and adults, to people with various diseases but also to the healthy. Ensuring the safety and resilience of milk is linked to a highly developed animal husbandry, located as close as possible to large urban agglomerations. Investments in this sector are the key to successfully overcoming threats of any kind. The aim of the paper is to analyze the current situation regarding the territorial distribution, by development regions, of the investments made in the milk and dairy products industry, as well as their use, in order to establish areas of interest for possible new investments in this field.

Key words: *development regions, processing capacity, production achieved, resilience.*

INTRODUCTION

Food security and resilience are closely linked to the emergence of a new world, dominated by risks and threats. In the face of various risks: natural disasters, pandemics, zoonoses, damaged infrastructure, war, etc., leading factors recognize that risks and threats cannot be avoided, so food security can never be fully ensured (Marin et al., 2019).

The main purpose of the paper is to establish and interpret the current situation regarding the territorial distribution, by development regions, of the investments made in the milk and milk products processing industry, as well as their use, to establish areas of interest for possible new investments in this area. respectively in the upstream and downstream areas of this processing industry - farms for raising and exploiting domestic animals and respectively – storage / marketing units for dairy products so as to achieve the uniform and efficient provision of the necessary food Romanian adders throughout the national territory.

MATERIALS AND METHODS

The data were taken from the National Agency for Veterinary Sanitary and Food Safety (ANSVSA) - the projected capacity of authorized economic agents, the County Agricultural Directorates (DAJ) - the number of animals and milk and dairy products marketed in 2021 and the latest data published by the National Institute of Statistics (INS).

The data were statistically processed by counties, by development regions and then at the national level.

Data on herds and milk production were grouped according to the national specifics of their exploitation into two groups: "Cattle" species, including cows and buffaloes exploited mainly for milk, and "Sheep + goats" species, including sheep and goats. also exploited for milk production.

The researches followed the productions made and the potential to provide dairy products to the population under normal living conditions, but also in case of risks and threats that cannot be avoided.

In particular, the ability to achieve resilience at county, regional and national level in the milk and dairy sector was pursued, as well as the possibilities for intervention to cover the desired needs.

RESULTS AND DISCUSSIONS

Table 1 and Figure 1 show the distribution by development areas of the herds of the main animal species exploited mainly for milk production in Romania (INS, period 2020-2021)

Table 1. Distribution of livestock on the dairy sector of Romania (heads)

Development regions	"Cattle"	"Sheep+goats"
N-E	253171	1245269
S-E	159236	1325088
S	124890	964973
Bucharest-Ilfov	3302	31009
S-V	129251	773987
V	121070	1633583
Center	251278	1874549
N-V	198969	1734536
National total	**1241167**	**9582994**

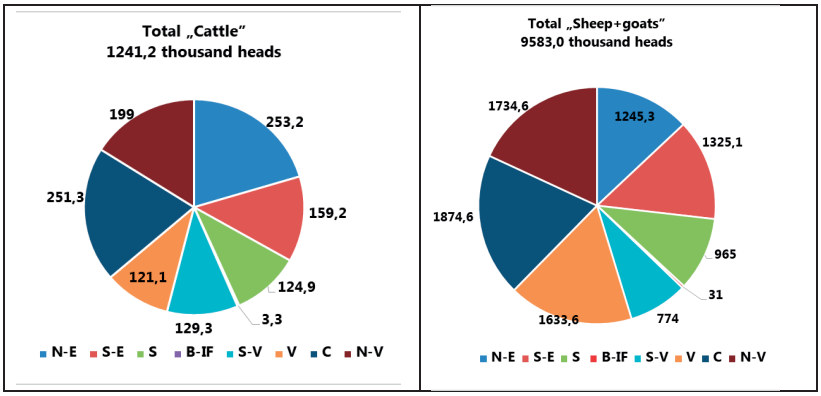

Figure 1. Territorial distribution of the number of animals exploited in the Romanian dairy sector

It can be seen that out of the national total of 1241167 heads, most animals of the "Cattle" species exploited mainly in the direction of milk production are raised in the N-E region (253171 heads), followed by the Central regions (251278 heads) and the N-W regions. (198969 heads), S-E (159236 heads). The rest of the development regions register less than 130 thousand heads/region. The region with the lowest number of animals is Bucharest-Ilfov with only 3302 heads.

In the group "Sheep + goats" at national level there are a number of 9282994 heads raised and exploited and in the direction of milk production. By development regions, the situation is as follows: the Central region has the highest number (1874549 heads), the N-V region has 1734536 heads, the V region has 1633583 heads, the S-E region has 1325088 heads and the N-E region 1245269 heads. The rest of the developing regions recorded less than one million heads per region. The smallest number of animals (31009 heads) is registered in the Bucharest-Ilfov region.

In region V, a significantly higher numerical weight can be observed in the group of "Sheep + goats" species than in the numerical weight of "Cattle", an aspect certainly determined by the tradition of sheep breeding and exploitation in the western part of Romania.

In general, it can be seen that the areas with the highest share of dairy animals are Transylvania, Northern Moldova and Banat.

Table 2 and Figure 2 show the distribution by development areas of the milk products of the two groups of animal species, productions capitalized on the market of milk and dairy products in Romania (DAJ period 2020-2021).

The territorial distribution of merchandise milk production shows a logical correlation directly proportional, in both groups of species, with the herds of animals raised and milked in the areas of development pursued.

Table 2. Territorial distribution of capitalized productions on the Romanian milk market in 2021 (tons)

Development regions	"Cattle" milk production	"Sheep + goats" milk production	Total production of milk
N-E	384379.8	32291.4	416671.2
S-E	197182.9	56895.1	254078.0
S	307813.4	56449.1	364262.5
Bucharest-Ilfov	20864.5	2847.0	23711.5
S-V	119697.6	33427.3	153124.9
V	247332.6	51779.4	299112.0
Center	722732.5	63356.7	786089.2
N-V	625308.0	29519.1	654827.1
National	**2625311.3**	**326565.1**	**2951876.4**

As a result, the highest total milk production is as a result registered in the Centre, N-V, N-E, S, V, S-E regions, and the lowest were recorded in the S-V and Bucharest-Ilfov regions.

Comparing the distribution by development areas of milk production obtained on the two groups of species "Cattle" and "Sheep + goats" (Figure 2) there is a more uniform distribution of milk production products obtained in the group "Sheep + goats" and a relative change in the ranking of developmental areas compared to the ranking obtained based on the number of animals (Figure 1) within each group of species.

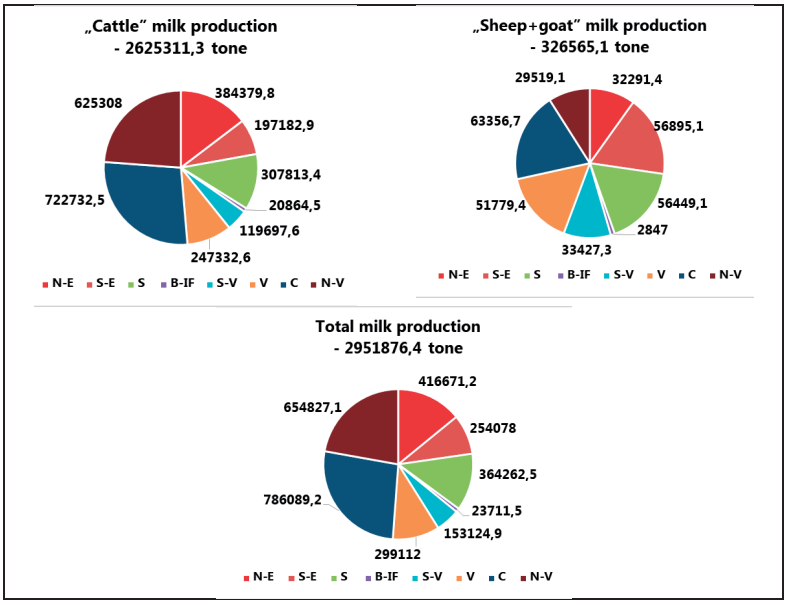

Figure 2. Territorial distribution of capitalized productions on the Romanian milk market in 2021 (tons)

Table 3. Distribution of estimated quantities of milk and milk products per capita by production of milk goods and authorized capacity processing in 2021

Development areas	Milk production	Authorized processing capacity	The difference: 2 - 1	Population INS/2011 (number)	The amount of milk estimated/inhabitant (kg)	
	tons				After production	After capacity
N-E	416671.2	258632.4	- 158038.8	3302217	126.18	78.32
S-E	254078.0	47187.4	- 206890.6	2545923	99.80	18.53
S	364262.5	172360.8	- 191901.7	3136446	116.14	54.95
B-IF	23711.5	96252.0	+ 72540.5	2272163	10.44	42.36
S-V	153124.9	5189.6	- 147935.3	2075642	73.77	2.5
V	299112.0	216814.0	- 82298	1828313	163.60	118.59
Centru	786089.2	626860.0	- 159229.2	2360805	332.98	265.53
N-V	654827.1	224166.8	- 430660.3	2600132	251.84	86.21
National	**2951876.4**	**1647463.0**	**- 1304413.4**	**20121641**	**146.70**	**81.88**

At 31.12.2021 at national level, the authorized capacity of dairy factories was 1647462.96 tons (Table 3) of which the highest authorized production capacity was registered in the Center region, N-E region, N-W region, V region, region S, and the lowest capacities were registered in the regions of Bucharest-Ilfov and S-V. It should be mentioned that in the southern area of Romania, respectively the S-V region, in Dolj and Olt counties and respectively in region V, in Caraș-Severin county, there are no milk processing units. From the point of view of the resilience of these areas, it can be seen that this product can be made exclusively on the basis of collaboration with neighboring counties in case of adverse and disruptive events.

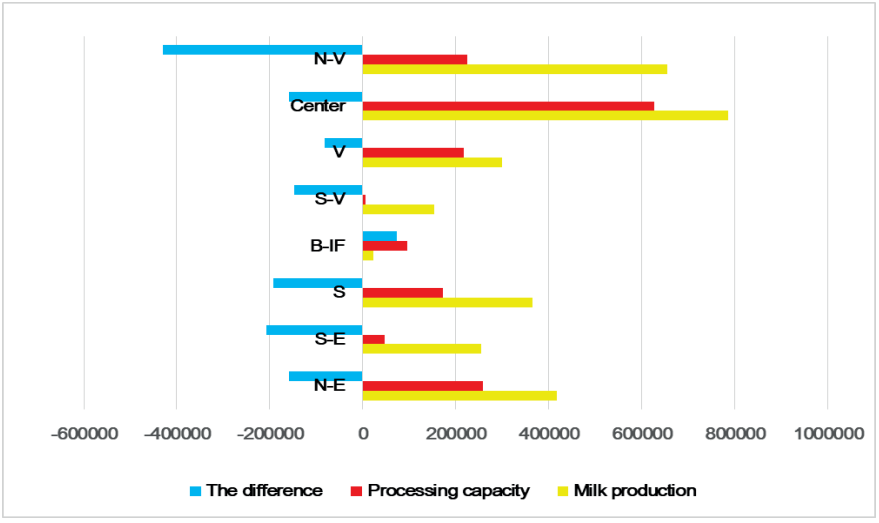

Figure 3. Territorial distribution of milk production and authorized processing capacity in 2021 (tons)

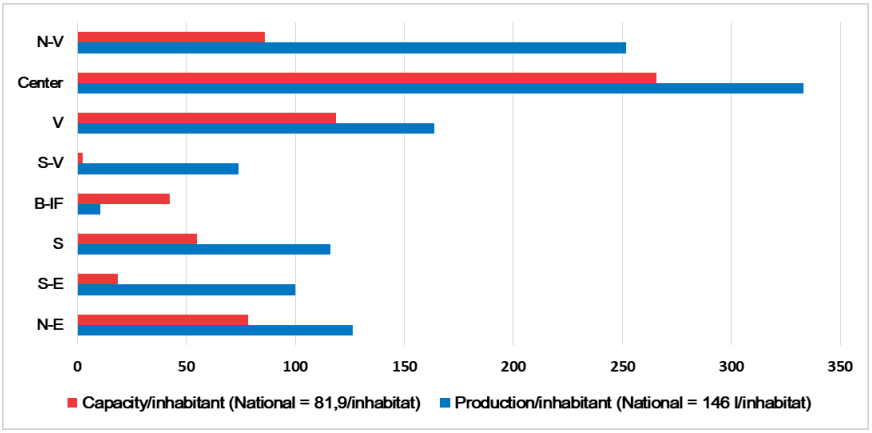

Figure 4. Distribution of quantities of milk and dairy products per capita estimated by production of milk goods and authorized processing capacity in 2021 (kg/capita/year)

From the statistical data processing it can be seen that at the national level a quantity of 146.70 liters/inhabitant is ensured (INS, for the period 2020-2021). From the point of view of the evolution of the estimated quantity of milk per inhabitant on the development areas, it can be observed that it is closely related to the areas with an advanced animal husbandry.

The highest quantities of milk per capita (Table 3) are found in the Center, N-V, West, N-E region, and the smallest are found in the Bucharest-Ilfov and S-V regions.

From the point of view of the estimated quantity of milk/inhabitant (Figure 4) it can be seen from the statistically processed data are very small, the lowest estimated quantities were recorded in the SV region (2.50 kg/inhabitant), SE region (18.53 kg/inhabitant) and even B-IF region (42.36 kg/inhabitant).

The highest estimated quantity was recorded in the Center region (265.53 kg/inhabitant).

As a result, the implementation of the resilience program in the milk and milk products sector cannot be achieved at national level, mainly due to the small quantities of milk and dairy products, with the exception of the Center region.

CONCLUSIONS

Comparing the capitalized productions, estimated from the level of farms and the authorized processing capacities in the milk and dairy products sector, a very big difference can be observed, we can say even double at national level.

The application of the resilience program on the basis of the capitalized production at the farm level can be appreciated as it could be achieved quantitatively but not with efficiency and practical almost impossible given the perishable nature of raw milk.

As regards the authorized processing capacity in the milk and milk products sector, the resilience program cannot be implemented.

As a result, in the current situation, if Romania were subjected to threats of any kind, it cannot apply an efficient resilience program through the authorized production capacities in the dairy sector.

The way out of this situation can be achieved only through investments in new processing capacities, correlated with investments in the production of raw milk, in the areas of deficient development areas.

Until the implementation of this program, the solution may be to build warehouses with sufficient storage capacity to fill the deficit (*Regulation 854/2004)*. It should be noted, however, that this solution may not be viable for fresh dairy products.

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Operating dates the *County Agricultural Directorates (DAJ)* for the period 2020-2021.

Operating dates the *National Institute of Statistics (INS)* for the period 2020-2021.

Regulation (EC) no. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the principles and general requirements of food law, establishing the European Food Safety Authority and laying down procedures in the field of product safety feeding.

Regulation (EC) no. Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs.

Regulation 853/2004 - Laying down specific hygiene rules for food of animal origin.

Regulation 854/2004 - Rules for the organization of official controls on products of animal origin intended for human consumption.

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IMPACT OF USING DEHYDRATED FRUITS POWDER AS NATURAL ANTIOXIDANT ON SENSORY PROPRIETIES OF NITRITE-FREE SALAMI FORMULAS

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Abstract

The present study aims to evaluate the effects of dehydrated fruits powder as natural antioxidant used to substitute the nitrites in the manufacture recipe of a cooked and smoked salami type on its sensory proprieties. For this purpose were used sour cherries (SC), cranberries (C) and black currants (BC) which in advance were subjected to a dehydration process for 15 hours at a moderate temperature of 55-60°C, 5 hours daily, three days in a row. The powder obtained from each dehydrated fruit was used at three levels of concentration in order to ensure a dose of polyphenolic compounds equally with 90, 200 and 300 mg gallic acid equivalents (GAE)/kg raw processed meat. The minimum dose of polyphenolic compounds coming from dehydrated fruits powder was chosen according to the nitrites content added to a kg of raw minced meat (90 mg nitrites/kg processed meat). Thus, nine salami formulas were prepared by addition of SC, C and BC powder at the three established concentrations. The sensory properties such as appearance, taste, odor, aroma of designed salami formulas were investigated in relation with those of the salami control samples prepared with the nitrites addition, respectively without nitrites. The substitution of nitrites by dehydrated fruits powder led to changes in the section appearance of obtained salami formulas, whereas the taste, odor and aroma were not affected. The information derived from this study is useful for the development of innovative nitrite-free meat products, in accordance with the consumer requirements, by exploiting the bioactive potential of some local fruits.

Key words*: cranberries and black currants, dehydrated fruits powder, natural antioxidants, nitrite-free salami formulas, sensory properties, sour cherries.*

INTRODUCTION

Since the middle of the last century , the food industry start to use nitrite as a food additive in meat products. Its use to stabilizing the typical color of cured meat products and as an antioxidant in meat products (Dominguez-Hernandez et al., 2018; Fraqueza et al., 2021).
The International Agency for Research on Cancer (IARC) reveals that there is a relationship between the consumption of processed meats and the occurrence of colon cancer.
Therefore based on scientific knowledge of the benefits and potential health risks, the limits for addition to meat products have been set (IARC Working Group, 2018). In order to reduce or eliminate nitrites from meat products, alternative measures should be considered (McAfee et al., 2010; Haugaard et al., 2014).
The impact of nitrites on the sensory properties of meat products is closely connected to color stabilization due to the formation of nitrosylmioglobin by the binding of nitric oxide to the iron of that hemoprotein (Savu et al., 2002). Nitrite also participates in flavoring through its antioxidant action (Patarata et al., 2022).
To guarantee the strategy's efficacy when we reduce or eliminate nitrate from meat products

during manufacture, we must consider an alternative measure (Papuc et al., 2013; Predescu et al., 2018).
The stabilization of color due to the formation of nitrosylmyoglobin by the bond of nitric oxide with the iron of that hemoprotein is mainly related to the effect of nitrite on meat products' sensory attributes (Huang et al., 2020).
Sodium nitrite is an additive that fulfills several functions, among which the hardening of the meat, offers a unique pink-reddish color and aroma, prolongs the shelf life of the products and provides microbiological safety. However, there has been a high consumer demand for low-nitrite or nitrite-free products (Engel et al., 2004; Pogorzelska-Nowicka et al., 2014; Wakamatsu et al., 2020).
A partial or total substitute of nitrite in meat products with a natural antioxidant would be a valuable choose, without compromising the sensory attributes of the products (Stoica et al., 2022). Also, an alternative is to use packaging in a controlled atmosphere (Petcu et al., 2014).
A major problem that decreases the shelf life of meat products is oxidation, and nitrite as a synthetic compound is used to delay oxidation reaction, but also natural bioactive components can be used instead synthetic compounds (Jiménez-Colmenero et al., 2001; Kamala et al., 2019).
Natural sources with antioxidants compounds that can be used in meat products are plants, herbs and spices extract, fruits and vegetables, and oilseeds (Vitaglione et al., 2008; Khan et al., 2010; Metzner Ungureanu et al., 2020; Raba et al., 2020).
Nowadays the association of fruits with meat products shows major interest to the meat industry, fruits as natural antioxidants, manage to improve sensory properties and improve biologically active properties (Jiménez et al., 2001; Popa et al., 2011; Raba et al., 2021).
Nour (2022) used sour cherry and plum juice marinades on pork loin to study the effects of these fruits on quality charactheristics and oxidative stability. The results shows that sour cherry and plum juice may be used as marinating ingredients as they promote interesting sensory proprieties and improve the storage stability of porc loin.
Lee et al. (2006) reveal that cranberry powder in addition with mechanically separated turkey and ground cooked pork reduced TBARS values by 81% over a storage period of 7 days at 2ºC in the cooked pork and by 84% over a period of 6 days at 2ºC in the mechanically separated turkey.
Jia et al. (2012) indicated that antioxidant activity of black currant extract in raw pork patties, significantly increase lipid and protein oxidation during chilled storage.
Cranberries have many phenolic compounds such as phenolic acids, flavonoids, anthocyanins, p-hydroxybenzoic acid and their derivatives (Vattem et al., 2005; Caillet et al., 2012).
The value of mature cranberries total phenolic content are 4745 mg/kg in gallic acid equivalents and total monomeric anthocyanin content of 111.0 mg/kg (Çelik et al., 2008; Wu et al., 2008).
The antioxidant capacity of cranberries is associated with their phenolic and anthocyanin-anthocyanidincontents.
Taking into consideration the mentioned data, the aim of this study was to evaluate the effects of three dehydrated fruits powder addition, sour cherries (SC), cranberries (C) and black currants (BC), in order to substitute the nitrite in the recipe of a cooked and smoked salami type on its sensory proprieties.

MATERIALS AND METHODS

Fruit powder obtaining

The fruits used in this study, sour cherries (SC), cranberries (C) and black currants (BC), were dehydrated at a moderate temperature of 55-60°C in a forced air oven (Froilabo AC60/France, 1000 W), for 15 hours, 5 hours daily, three days in a row. The dehydration process allows the fruit bioactive compounds preservation. The dried fruits were ground in a laboratory mill (Grindomix Retsch GM 2000), then, passed through a 60 mesh sieve. The obtained fruit powders were used as a nitrite substitut in salami formulas.

Evaluation of total phenolics content of fruits powder

The total phenolics content in the fruits powder was assessed using the Folin-Ciocalteu colorimetric method (Singleton et al., 1999). The evaluation of total phenolics content was

performed on the extract obtained as follows: 1 g fruits powder was mixed with 10 mL ethyl alcohol, 70% (v/v) under stirring for 1h, at a temperature of 25°C using the horizontal shaker Heidolph Promax 1020 (Germany). The mixtures were filtered and the resulted fractions were used for analysis. The absorbance was measured at 750 nm using the gallic acid as a standard.

The determinations were performed in triplicates and the results, were expressed as mg gallic acid equivalents (GAE)/g dry weight (d.s). The polyphenol content of fruits powder was reported as average value ± standard deviation.

Establishing the dehydrated fruit doses

The powder obtained from each dehydrated fruit was used at three levels of concentration in order to ensure a dose of polyphenolic compounds equally with 90, 200 and 300 mg gallic acid equivalents (GAE)/kg raw minced meat. The minimum dose of polyphenolic compounds coming from dehydrated fruits powder was chosen according to the nitrites content added to a kg of raw minced meat (90 mg nitrites/kg minced meat).

Salami formulas preparation

The preparation of the salami is done strictly respecting the manufacturing stages, weighing the raw material, weighing the auxiliary material, mixing the raw material by adding the auxiliary material, filling in membranes, heat treatment and storage.

At the mixing stage, SC, C, BC fruit powders were added at three levels of concentrations 90, 200, 300 mg GAE/kg in raw minced meat in order to replace the nitrite.

The nitrite-free salami formulas were investigated in relation with those of the salami control samples prepared with the nitrite addition, respectively without nitrite.

The salami samples were cooked and smoked in a smocking cell with a closed smoke flap according to a technological diagram, until it reached 62°C in the technological center of the product.

After cooling the salami formulas were stored in refrigeration conditions, 2-4°C, for 21 days until the sensorial analyzes were performed. The sample coding is shown in Table 1.

Table 1 Sample coding

Sample name	Code
Salami control sample with nitrite	CS
Nitrite-Free Salami control sample	NFCS
Nitrite-Free Salami with SC powder to ensure a polyphenol content of 90 mg GAE/kg raw minced meat	1SC
Nitrite-Free Salami with SC powder to ensure a polyphenol content of 200 mg GAE/kg raw minced meat	2SC
Nitrite-Free Salami with SC powder to ensure a polyphenol content of 300 mg GAE/kg raw minced meat	3SC
Nitrite-Free Salami with C powder to ensure a polyphenol content of 90 mg GAE/kg raw minced meat	1C
Nitrite-Free Salami with C powder to ensure a polyphenol content of 200 mg GAE/kg raw minced meat	2C
Nitrite-Free Salami with C powder to ensure a polyphenol content of 300 mg GAE/kg raw minced meat	3C
Nitrite-Free Salami with BC powder to ensure a polyphenol content of 90 mg GAE/kg raw minced meat	1BC
Nitrite-Free Salami with BC powder to ensure a polyphenol content 200 mg GAE/kg raw minced meat	2BC
Nitrite-Free Salami with BC powder to ensure a polyphenol content 300 mg GAE/kg raw minced meat	3BC

Sensory characteristics evaluation

A key criterion for the successful using of fruit powder in a food system is whether the sensory properties of the food are not adversely affected. Finally, the quality and desirability of a food product is determined by the reaction of consumers to the appearance, taste and smell of food. For this reason, the sensory properties of new or improved foods are usually tested using human taste panels to ensure that foods have acceptable and desirable properties before they are placed on the market (Lawless & Heymann, 2010).

Foods are often tested using consumer panels, large groups of untrained consumers, to determine their reaction to an improved new product before completing large-scale marketing for further development, or alternatively select trained persons to be able to reliably detect small differences in specific qualities of certain foods.

The sensory analysis was performed using the hedonic scale method (STAS 12656-88), for salami, using a group of 10 tasters.

The sensory characteristics evaluated were appearance, taste, odor and aroma, thus the scores that were assigned for each characteristic ranged from 0-5, by comparison with sample formulas. A maximum of 20 points must be obtained by summing the maximum approved score for each sensory characteristic. Each taster assigns a score to a sensory characteristic of one sample, and then a medium score is made. The sum of the medium scores of the 4 characteristics analyzed for an analysis test leads to the formation of the total medium score. Each sample has a total medium score, which helps us to identify which concentration level of the added fruit powder is better to ensure the optimal level of natural antioxidant, but also which is the best fruit of the three analyzed SC, C, BC as a substitute for nitrite.

RESULTS AND DISCUSSIONS

The total phenolic content recorded in the investigated fruits powder in order to calculated the doses incorporated in the manufacture recipes were in the range 10- 16 mg GAE/g d.s, as is shown in Table 2.

Table 2. Total phenolic content of fruits powder

Fruits powder	Total phenolic content (mg GAE/g d.s)
SC	10.01±0.28
C	12.36±0.34
BC	16.01±0.42

The salami formulas obtained by SC powder incorporation are presented in Figure 1.
The highest score (19.6) was recorded for the CS formula, and respectively the lowest score (12.6) was recorded for NFCS.
Analyzing the addition of SC powder, the highest score was recorded for 1SC (15.9), followed by 2SC (15.7), and the lowest score was registered for 3SC (15.3).
In terms of appearance, the highest score was recorded for the 1SC, followed by 2SC and the lowest score was obtained for 3SC. The smell and aroma did not change significantly.
Another sensory characteristic that had a slight influence is the taste that had decreasing values as the amount of SC increased.
As a result, the values of the total scores of the samples with SC added (1SC, 2SC, 3SC) were the lowest compared to the other fruits C and BC, respectively, due to the values of the medium scores of the salami appearance, the SC granulation offers a less acceptable appearance (Figure 1).
The salami formulas obtained by C powder incorporation are presented in Figure 3 and the sensory analysis results of prepared formulas against control samples are displayed in Figure 4. The highest score is the 3C (18.5) sample, followed by 2C (18.3) and the lowest 1C score (17.8). The scores recorded for the salami formulas were in the range of 12.6-19.6, as can be seen in Figure 2.

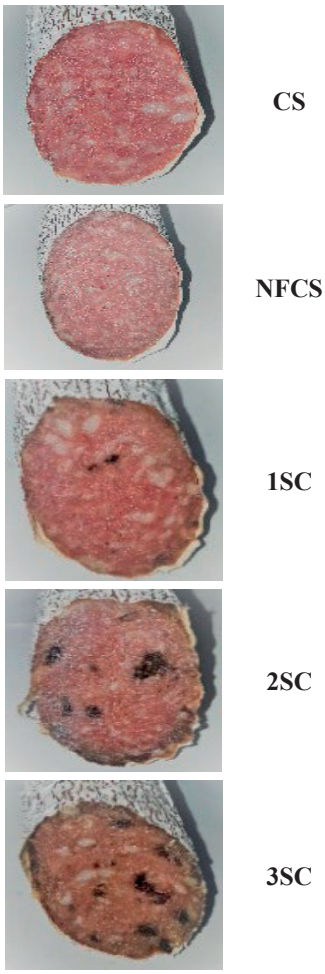

Figure 1. Salami control samples and salami formulas with addition of SC

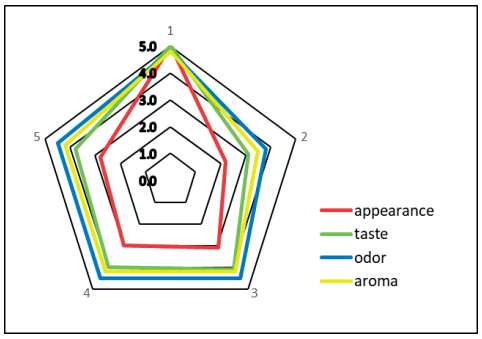

Figure 2. The sensory analysis results of salami with SC against control samples
(1-CS, 2-NFCS, 3-1SC, 4-2SC, 5-3SC)

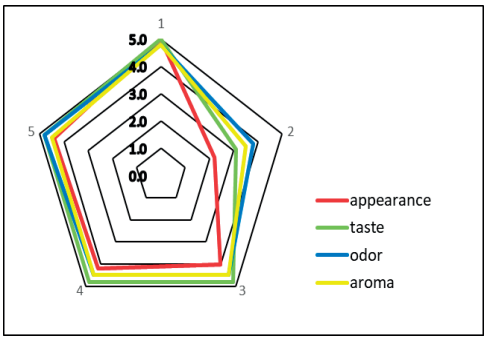

Figure 4. The sensory analysis results of salami with SC against control samples
(1-CS, 2-NFCS, 3-1C, 4-2C, 5-3C)

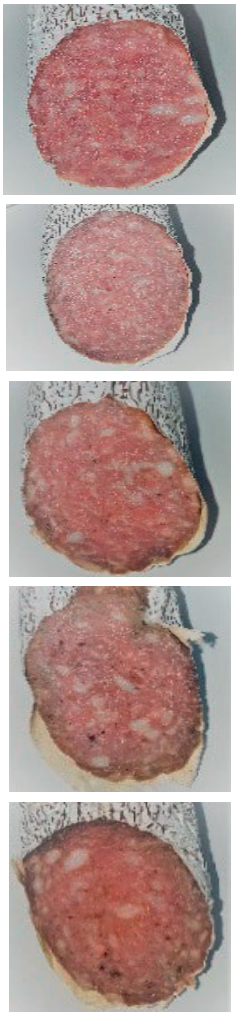

Figure 3. Salami control samples and salami formulas with C added

At the sensory analysis of the appearance, the highest value was registered at the salami sample 3C (4.4) respectively 2C (4.2) and the lowest value 1C (4.0), and these can be observed in Figure 3. The other sensory features did not undergo significant changes (Figure 4).

The salami formulas obtained by BC powder incorporation are presented in Figure 5 and the sensory analysis results of prepared formulas against control samples are displayed in Figure 6.

The salami formulas prepared with BC addition recorded the highest values in the 3BC samples (17.3), followed by 2BC (17.2) and the lowest value 3BC (16.8).

A sensory characteristic that has undergone slight changes is the appearance, with a medium score of 4.0 for the 1BC sample, 4.2 for the 2BC sample, and 4.3 for the 3BC sample (Figure 5). The other characteristics, has no significant changes.

The results of the sensory evaluation have the highest values of the total medium scores, compared to the other fruits proposed in this paper, SC respectively BC.

As a result of the sensory analysis performed for the 9 salami samples, the highest total but also individual score for each characteristic was obtained for the 3C sample (Figure 6).

The addition of C in the highest concentration level (300 mg GAE/kg raw processed meat), best fulfills the role of natural antioxidant as a substitute for nitrite, having a color as close as possible to the control test with nitrite (CS), without negatively influencing the other sensory aspects of salami.

Figure 5. Salami control samples and salami formulas with BC added

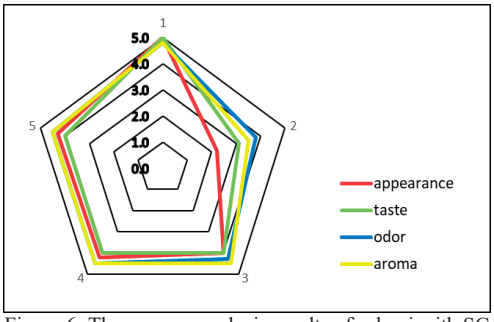

Figure 6. The sensory analysis results of salami with SC against control samples
(1-CS, 2-NFCS, 3-1BC, 4-2BC, 5-3BC)

CONCLUSIONS

Beyond the effects of improving the nutritional and sensory properties of meat products, the addition of fruit can lead to unwanted changes, which limits the application of fruit in certain categories of food. In addition to the changes of the appearance of salami, the inclusion of fruit powder in their manufacturing recipe may induce other changes in sensory properties, such as the generation of the sour taste. The results of sensory analysis of salami with dehydrated and ground fruits indicates that all three fruits sour cherries, blackcurrants and cranberries, can partially and even completely replace the nitrite added in the initial recipe. The best values are the samples with the addition of cranberries, followed by blackcurrants and sour cherries. We consider that the tested fruits can be used as natural antioxidants to create nitrite-free meat products in accordance with the consumer requirements.

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STAS 12656-88, Food products. Sensory analysis. Scoring methods.

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ANTIOXIDANT AND NUTRITIONAL CHARACTERISTICS OF TWO INNOVATIVE SUGAR FREE FRUIT JELLIES

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Abstract

Fruit jellies are very popular sweets for all ages, but due to the significant amounts of sugar added in classic jellies (54-58%), they have begun to be avoided by more and more consumers, either for health reasons or to prevent certain diseases or to control weight. The first aim of this paper was to obtain two varieties of jellies: one from orange (OJ) and the other from kiwi fruit (KJ), using dried Stevia rebaudiana leaves powder as sweetner. A second aim of the paper was to determine the content of vitamin C (titrimetric iodometric method), total polyphenols (Folin-Ciocalteu assay), antioxidant activity (CUPRAC method) of the finished products compared to the raw materials, as well as the analysis of the nutritional and sensory characteristics of the two types of jellies. Among the raw materials, kiwi fruit was noted for the highest content of vitamin C (90.82±3.22 mg/100 g) and for the strongest antioxidant activity (9.68±0.31mg Trolox/g). From the two finished products, KJ was the richest in ascorbic acid 80.25±2.44 mg/100g and have had the higher antioxidant activity (8.98±0.28 mg Trolox/g). In terms of total polyphenols, they were present in larger quantities in oranges (4.93±0.08 mg gallic acid/g) and in OJ (7.90±0.12 mg gallic acid/g). Stevia rebaudiana used as a sweetener has a very high content of total polyphenols (34.22±0.83 mg gallic acid/g) and a very good antioxidant activity (112.75±2.28 mg Trolox/g). Both types of jellies are distinguished by a low energy intake (53.82 kcal/100 g for KJ, respectively 41.76 kcal/100g for OJ), a significant dietary fiber intake (8,58 g/100 g for KJ and 6.03 g/100 g for OJ) and a low sugar content (8.35 g/100 g for KJ, respectively 7,89 g/100 g for OJ - and this is natural fruit sugar). The two types of jellies were very well appreciated from an organoleptic point of view (hedonic scoring method from 1 to 5), OJ having slightly higher scores in terms of taste and aroma, compared to KJ.

Key words: antioxidant activity, jelly, kiwi fruit, oranges, polyphenols, *Stevia rebaudiana.*

INTRODUCTION

Conventional fruit jellies are part of the category of products processed from fruit and preserved with sugar. They are gelled products obtained from fruit juices mixed with sugar, pectin, with or without the addition of organic acids. The added sugar is 54-58 kg/100 kg jelly (Banu et al., 2013).

Because the sugar used in the manufacture of conventional jellies can trigger many diseases that affect the human body, such as obesity, diabetes, tooth decay, it has become necessary to replace it with other natural sweeteners safe for the consumers health (Reissig et al., 2016; Saveski et al., 2015; Khouryieh et al., 2005).

Among the natural sweeteners, the leaves of *Stevia rebaudiana* Bertoni (*Asteraceae* family) are gaining more and more interest nowadays. It is estimated to be about 300 times sweeter than sucrose, due to the content of specific diterpene glycosides called steviosides and rebaudiosides, which although they taste very sweet, are non-nutritive (calorie-free) and non-toxic (Salehi et al., 2019; Suresh et al., 2018; Marcinek & Krejpcio, 2015). Some studies have even shown that *Stevia rebaudiana* Bertoni leaves have antidiabetic effects, increasing insulin

production and stabilizing blood sugar levels (Xiao & Hermansen, 2005; Chen et al., 2006). Stevia is also an important source of antioxidants (vitamin C, polyphenols, flavonoids, carotenoids) but also of mineral elements (potassium, iron, calcium, magnesium, phosphorus) (Absan et al., 2020).

Fruits are highly revered for their nutritional properties, serving as a primary source of vitamins and minerals in many people's diets. Recent attention has focused on phyto-nutrients naturally present in many fruits. These compounds include polyphenols, anthocyanins, carotenoids, and other antioxidant compounds that play an important role in preventing cancer and heart disease (Baker et al., 2005).

It has been experimentally determined that jellies contain flavonoid profiles similar to those of the natural fruits from which they are obtained. The content of total polyphenols is influenced by the variety of fruit, the degree of ripeness of the fruit and the industrial process used (Tomas-Lorente et al., 1992).

Although fruits are thermally processed to obtain jellies and some of the thermolabile antioxidants are destroyed (especially vitamin C), it has been found that fruit jellies also have a good antioxidant activity (Kim & Padilla-Zakour, 2004).

Kiwi fruits belong to the genus *Actinidia* and originate from China. There are over 60 species belonging to the genus *Actinidia*, but few of them are of economic importance. *A. deliciosa* and *A. chinensis* are the most common species in the world (Sanz et al., 2021). Kiwi fruit contains high amounts of antioxidants such as vitamin C, vitamin E, carotenoids and polyphenolic compounds, but also folate, minerals K, Mg, and Cu as well as dietary fiber (Pinelli et al., 2013; Skinner et al., 2011).

Oranges, tasty and juicy fruits, belong to the *Rutaceae* family and are botanically known as *Citrus sinensis.* Significant amounts of antioxidant compounds are also found in oranges: vitamin C, carotenoids, polyphenols, flavonoids, as well as dietary fiber, calcium, potassium (Parle & Chaturvedi, 2012). Some studies have shown that the antioxidants present in oranges provide protection against several degenerative diseases, reduce the risk of developing cardiovascular disease and cancer (Benavente-García et al., 1997; Parle & Chaturvedi, 2012; Adenaike & Abakpa, 2021).

The first purpose of this paper was to obtain two varieties of fruit jelly (one from kiwi and the other from orange) sweetened with *Stevia rebaudiana* Bertoni dried leaves powder and minimally heat processed. The second aim of the paper was to analyze the obtained jellies in terms of vitamin C content, total polyphenols, antioxidant activity, as well as their nutritional and organoleptic characteristics evaluation.

MATERIALS AND METHODS

Jellies preparation

All the raw and auxiliary materials used to jellies preparation were purchased from the Romanian market. Recipes used to obtain the two assortments of finished products (kiwi jelly - KJ, respectively orange jelly - OJ) are presented in Table 1

Table 1. Recipes used to jelly assortments preparation

Finished product / Raw and auxiliary materials (g)	KJ	OJ
Kiwi juice	400	-
Orange juice	-	400
Stevia leaf powder	6	6
Agar-agar powder	20	20
Vanilla pods powder	1	1

In order to prepare the jellies, the kiwi and respectively orange fresh juices were obtained first, using a Tefal ZE 585H38 Easy Fruit centrifugal juicer. Then, stevia powder, agar-agar and vanilla were dissolved in each type of juice. The mixture, for each product, was brought to a boil and kept for 1 min, stirring constantly. Then, the hot jelly was poured into molds, allowed to cool and then refrigerated (4°C) for at least 4 hours. From the jellies thus obtained as well as from the raw materials and stevia powder, samples were taken to determine the content of vitamin C, total polyphenols and antioxidant activity.

Vitamin C determination
The titration iodometric method was used to determine the vitamin C content of the samples, based on the color reaction between starch and a solution of iodine in potassium iodide. Ascorbic acid was determined quantitatively by titration with a solution of potassium dichromate in the presence of starch and potassium iodide. The working method was performed as presented by Dumbrava et al. (2016), the results being expressed in mg vitamin C/100 g.

Assessment of the total polyphenol content
The Folin-Ciocâlteu method was used to determine the total polyphenols in raw materials and finished products. This method is based on the reducing properties of polyphenols compared to the hexavalent molybdenum from the poly-phosphomolybdate contained in the reagent Folin-Ciocâlteu (Folin & Ciocâlteu, 1927). Hexavalent molybdenum is partially reduced by polyphenols, in a strongly acidic environment at lower states (+4, +5) which in an alkaline environment are colored in blue, having absorption bands at approx. 750 nm (Huang et al., 2005; Prior et al., 2005). The working methodology was the same as the one presented by Dumbrava et al. (2020), the results being expressed in mg gallic acid/g.

Assessment of the antioxidant activity
CUPRAC (cupric ion reducing antioxidant capacity) method was used to determine the antioxidant activity of both raw materials and finished products. In this method, the reagent copper (II) - neocuprine is used as the chromogenic oxidizing agent. The samples absorbance is read at 450 nm. Since the bis (neocuproine) copper (I) cation chromophore is soluble in both organic solvents and water, the CUPRAC method can determine the antioxidant activity of both fat-soluble and water-soluble antioxidants present in the test sample. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), an antioxidant that mimics the structure of vitamin E but is both fat-soluble and water-soluble, is used as a reference substance (Apak et al., 2007). The working method used was similar to the one presented by Dumbrava et al. (2020), the results being expressed in mg Trolox/g.

Statistical analysis
All determinations were performed in triplicate and the results were expressed as a mean values ± standard deviation (SD). Microsoft Excel 2010 was used for statistical data processing.

Assessment of the proximate composition and energy value
The Nutritional Data Base USDA was used to determine the proximate composition and energy value of the two types of obtained jellies.

Sensory evaluation
Sensory analysis of KJ (kiwi jelly) and OJ (orange jelly) was performed by 30 untrained panelists aged 14 to 51 years (18 females and 12 males), non-smokers, no known food allergies. The KJ and OJ samples were presented to the panelists in a single sensory session, placed in disposable white plates, labeled with four-digit characters. Panelists were asked to evaluate the following sensory characteristics of the samples: appearance, color, odor, texture, taste and aroma. Between two sample evaluations, participants were asked to rinse their mouths with plain water. The five-point hedonic scale was used as follows: 1 = extremely dislike; 2 = slightly dislike; 3 = neither like nor dislike; 4 = slightly like; 5 = extremely like (Lim, 2011). The level of acceptability and the range of scores were classified in the same way as presented by Dumbrava et al. (2020).

RESULTS AND DISCUSSIONS

Vitamin C content
Results on vitamin C content in raw materials, stevia powder and finished products are presented in Table 2.

Table 2. Ascorbic acid content in raw materials, stevia powder and finished products

Sample	Ascorbic acid content (mg/100 g)
Kiwi pulp	90.82±3.22
Orange pulp	65.22±2.14
Stevia powder	12.89±0.14
Kiwi jelly (KJ)	80.25±2.44
Orange jelly (OJ)	52.34±2.16

Among the raw materials, kiwi pulp was richer in vitamin C (90.82±3.22 mg/100 g) than orange

pulp (65.22±2.14 mg/100 g). Nangbes et al. (2014) found lower values for ascorbic acid content in orange pulp, 50.23 mg/100 g for ripe fruit and 56.26 mg/100 g for unripe fruit. Najwa and Azrina (2017) also reported a lower orange pulp vitamin C concentration of 58.30±0.53 mg/100 g.

For kiwi pulp Elhefian et al. (2019) reported a similar value: 90.20 mg/100 g, while Tyagi et al. (2015) found a higher concentration of ascorbic acid: 105 mg/100 g. An ascorbic acid concentration of 12.89±0.14 mg/100 g was determined for the dried leaf powder of *Stevia rebaudiana* Bertoni, a slightly lower value than reported by Kim et al. (2011): 14.98 mg/100 g DW.

In the case of finished products, the concentration of ascorbic acid is slightly lower than in the raw materials, during the heat processing of the jellies there are losses in this thermolabile vitamin. Because the thermal processing was minimal, the boiling time of the products during the production process being only 1 min, the concentration of vitamin C in the jellies is high, KJ being richer in vitamin C (80.25±2.44 mg/100 g) with 53.32% than OJ (52.34±2.16 mg/100 g).

Total polyphenols content

Table 3 shows the experimentally obtained data for the total polyphenol content in the raw materials, stevia powder and finished products.

Total polyphenol content was higher in orange pulp (4.93±0.08 mg gallic acid/g) than in kiwi pulp (2.61±0.04 mg gallic acid/100 g) and stevia powder was noted by a very high amount of these compounds (34.22±0.83 mg/100 g).

Table 3. Total polyphenols content in raw materials, stevia powder and finished products

Sample	Total polyphenols (mg gallic acid/g)
Kiwi pulp	2.61±0.04
Orange pulp	4.93±0.08
Stevia powder	34.22±0.83
Kiwi jelly (KJ)	3.98±0.06
Orange jelly	7.90±0.12

Sir Elkhatim et al. (2018) analyzing the total polyphenol content in the mixture of orange pulp and seeds, reported higher values ranged between 19.2 and 16.2 mg of gallic acid equivalent/g.

For kiwi pulp, Alim et al. (2019) determined values of 9.5 mg gallic acid equivalent/g dry weight, also, Pal et al. (2015) studying the variation of total polyphenols concentration in the kiwi pulp of different kiwi fruit varieties at various degrees of maturity, found values between 0.84 and 2.15 mg gallic acid equivalent/g fresh weight, our data being slighty higer than superior limit of this range.

Kaushik et al. reported in stevia leaves higher total polyphenol values of 42 mg/100 g dry leaf. OJ (7.90±0.12 mg gallic acid/g) was distinguished by an almost double content of total polyphenols compared to KJ (3.98±0.06 mg gallic acid/g), both being richer in these compounds than the pulp of the fruits from which were obtained.

Antioxidant activity

The experimental data resulting from antioxidant activity analysis presented in Table 4, show that kiwi pulp had higher antioxidant activity (9.68±0.31 mg Trolox/g) than orange pulp (7.05±0.14 mg Trolox/g).

It was also found that stevia powder had a very strong antioxidant activity (112.75±2.28 mg Trolox/g), so that the finished products, although subjected to a short heat treatment, kept a good antioxidant activity (8.98±0.28 mg Trolox/g for KJ respectively 6.86±0.11 mg Trolox/g for OJ), very close to that of the fresh fruits from which they were prepared.

As it is known, the antioxidant composition of fruits depends a lot on the variety, soil and climatic conditions, degree of ripeness, storage conditions, so that the values found in the literature for the antioxidant activity of kiwi and orange pulp, respectively, have presented some differences. Thus, Sharma et al. (2012) reported a lower antioxidant activity for kiwi pulp (1.175 mg Trolox/g FW), with an increase in 1-methylcyclopropene-treated fruit during the post-harvest life (3.125 mg Trolox/g FW). Wang et al., (2008) found an antioxidant activity of 9.02 mg Trolox/g FW for oranges, while Park et al. (2014) reported 1.39 mg Trolox/g FW. Covarrubias-Cárdenas et al. (2018) reported for *Stevia rebaudiana* Bertoni leaf powder aqueous and ethanolic extracts respectively, values of antioxidant activity between 60.21 and 150.81 mg Trolox/g, depending also on the extraction

time, respectively on the concentration of the ethanolic solution.

Table 4. Antioxidant activity of raw materials, stevia powder and finished products

Sample	Antioxidant activity (mg Trolox/g)
Kiwi pulp	9.68±0.31
Orange pulp	7.05±0.14
Stevia powder	112.75±2.28
Kiwi jelly (KJ)	8.98±0.28
Orange jelly (OJ)	6.86±0.11

Nutritional profile of finished products

The proximate composition and energy value for the two varieties of jellies (KJ and OJ) are presented in Table 5.

Table 5. Proximate composition and energy value of KJ and OJ

	KJ	OJ
Protein (g/100 g)	0.97	0.65
Total fat (g/100 g)	0.42	0.18
-saturated fat (g/100 g)	0.02	0.02
Total carbohydrates (g/100 g)	20.19	16.93
-sugar (g/100 g)	8.35	7.89
-dietary fiber (g/100 g)	8,58	6.03
Energy value (kcal/100 g)	53.82	41.76

Both finished products had a low energy value (53.82 kcal/100 g for KJ and 41.76 kcal/100 g for OJ), a very low content of lipids (0.42 g total fat/100 g for KJ, respectively 0.18 g total fat for OJ) and proteins (0.97 g/100 g for KJ, respectively 0.65 g/100 g for OJ), in their proximate composition predominating carbohydrates (20.19 g/100 g in KJ and 16.93 g/100 g in OJ). Sugars (8.35 g/100 g în KJ, respectively 7.89 g/100 g in OJ) are in a much smaller quantity (and naturally derived from fruit) compared to classic jellies with added sugar (54-58%). Instead, there is a significant dietary fiber content in both products (8.58 g/100 g in KJ, respectively 6.03 g/100 g in OJ).

Sensory analysis

The results of the sensory analysis, presented in Figure 1, show that both types of jellies were well appreciated by panelists, the orange jelly variant (OJ) having an overall average of 4.6 which places the product in the class with a high level of acceptability (HA), and kiwi jelly (KJ) was rated with an overall average of 4.4 which places it in the class of acceptable products (A). The two products had the same average score in terms of appearance and texture (4.4 and 4.3 respectively) but in terms of color, taste, smell and aroma, OJ was better appreciated than KJ

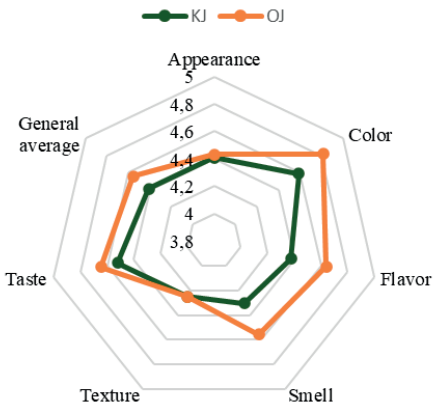

Figure 1. Global values of the sensory evaluation of KJ and OJ by using a 5-point hedonic scale

CONCLUSIONS

Concentrated fruit products, including jellies, are a category of canned products loved by consumers of all ages. As conventional jellies contain significant amounts of added sugar, which is a drawback, as it can cause certain health problems for consumers, it has become necessary to find ways to obtain these products using other types of natural sweeteners that eliminate the disadvantages of sugar, but which to ensure, however, a sensory quality well accepted for finished products and also, if possible, add health benefits. The present paper aims to prepare two varieties of fruit jellies, one of kiwi and the second of oranges, sweetened with powder from dried leaves of *Stevia rebaudiana* Bertoni and using agar-agar as gelling agent.

The products obtained, as they were minimally thermally processed, were distinguished by a vitamin C content very close to that of raw materials, a level of total polyphenols even higher than that of raw materials and a good antioxidant activity. To the high values of total

polyphenols content and of antioxidant activity registered for kiwi jelly respectively for orange jelly, stevia powder used as a sweetener also brought an important contribution. Although orange jelly was noted for its almost double total polyphenol content compared to kiwi jelly because the latter contained a higher amount of vitamin C, the antioxidant activity of kiwi jelly was higher.

Nutritional profile analysis of the two varieties of jellies showed that both were low in calories, low in sugar compared to classic jellies or other sweet products, and can bring a fairly good intake of dietary fiber.

In terms of sensory analysis, both products were well appreciated by panelists, but orange jelly was slightly better evaluated in terms of color, taste, smell and aroma than kiwi jelly, thus obtaining a score that places it in the class of highly acceptable products, while kiwi jelly was included in the class of acceptable products.

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INFLUENCE OF QUANTITIES OF RAW MATERIALS AND MATURATION TIME ON THE SENSORY QUALITY OF DRIED BABIC SAUSAGES

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Abstract

This paper aimed to evaluate the differences between six experimental batches of babic sausages from a sensory point of view. The six samples have been differentiated by the ratio of meat raw materials introduced in the composition (mutton, beef, fat) and by the maturing time (20 days, 40 days). The products were manufactured in the Processing Microsection of the University of Life Sciences Iasi and the sensory evaluation was carried out with the help of 8 tasters, in three repetitions, in the Sensory Analysis Laboratory of the Faculty of Agriculture Iasi. The sensory attributes evaluated were appearance, colour, aroma, texture and taste of the experimental lots. The ageing time had major influences from a sensory point of view on the intensity of aroma, salty taste and texture attributes. In terms of the quantities of raw materials, lots L1 and L3 showed the smallest differences, with lot L2 standing out due to its higher fat and beef content.

Key words*: comparison, meat products, sensory analysis.*

INTRODUCTION

Sensory evaluation of food products, used in the past to accept or reject a product from consumption, involves investigating, studying, explaining and interpreting responses given by evaluating subjects using the primary senses (visual, olfactory, gustatory, tactile and auditory) on food products (Ventanas et al., 2020; Ruiz-Capillas et al., 2021). Sensory analysis is defined as an interdisciplinary science that accumulates information and methods adapted from fields such as physiology, psychology, statistics, linguistics, food science, nutrition, chemistry, medicine, sociology, and many others (Chambers, 2019).

Directions in sensory analysis refer to three categories of methods: discriminative methods (duo-trio tests, triangle tests, pairing tests), descriptive methods (descriptive analysis: aroma profile, quantitative descriptive analysis) and affective methods (acceptance, preference, hedonic tests - 9-point scale). In recent years, there has been a selection of sensory analysis methods, thus the main methods used are discriminative and descriptive (Lawless & Heymann, 2010; Stone et al., 2020).

Descriptive sensory analysis is considered a basic method in characterizing food products in terms of perceived attributes and their intensity by the group of evaluators (Drake & Civille, 2003; Suwonsichon, 2019). To carry out sensory analysis of a food product in order to obtain accurate and relevant data, it is necessary to develop descriptive terminology specific to the evaluated product (Chambers, 2019). In descriptive sensory analysis, vocabulary (descriptive terminology) is the communication pathway between different stakeholders, such as evaluation panel members, manufacturers, marketers and suppliers, who have different views on sensory attributes due to different perceptions, backgrounds and cultures (Suwonsichon, 2019).

While descriptive methods of sensory analysis are used to identify and quantify sensory aspects of evaluated products, acceptability and preference tests are tests applied to consumers to identify how well the product fits into consumer preferences (Drake & Civille, 2003).

Sausages are processed meat products that can be obtained in many varieties, depending on the specificity of each area and the availability of raw materials (Carballo, 2021; Artamonova et al., 2021). Babic and ghiudem sausages are two

traditional varieties of dried raw sausages. Babic sausage is a product native to the Buzau area, Romania, traditionally made from a mixture of minced beef and pork in equal proportions, salt, sweet and hot paprika. Today, however, recipes differ depending on the producer and may also include other ingredients such as pepper, thyme and garlic.

The paper aimed to evaluate the sensory changes in babic products following technological interventions on the proportions of raw materials introduced in the batches (beef/sheep meat/fat) and on the maturation (drying) period of the products (20 days, 40 days).

MATERIALS AND METHODS

Preparation of samples

The research was based on the preparation of technological sheets and the formation of experimental batches of babic sausages manufactured in the Processing Microsection of the University of Life Sciences Iasi. For experimental batch 1 (L_1), the proportions of raw materials used were: 40% mutton, 40% beef and 20% fat; for experimental batch 2 (L_2), the proportions of raw materials used were: 20% mutton, 50% beef and 30% fat, and for experimental batch 3 (L_3), the proportions of raw materials used were: 60% mutton, 30% beef and 10% fat.

Two sub batches were formed from the three batches, each of which was subjected to different ripening periods; thus batches L_{12}, L_{22} and L_{32} were matured for 20 days and batches L_{14}, L_{24} and L_{34} were matured for 40 days. All the manufactured samples contained salt (2%), sweet paprika (0.7%), hot paprika (0.5%), thyme (0.5%) and juniper (0.3%).

The raw meat materials (beef, mutton and fat) were purchased from two local slaughterhouses on different days when the production of the batches in question took place. To obtain the products, the raw materials were ground in a meat grinder using a plate of 6 mm of diameter; salt and spices were added and the mixture obtained was homogenised so that the ingredients were evenly distributed in the product mass. The sausage mixture was stuffed using the filling machine, in thin natural membranes which were desalted and moistened. Once obtained, the products have undergone heat treatment, after the following smoking program: preheating: 1 hour at 21-23°C, smoking: 30 minutes at 20-22°C (the smoking stage was carried out in three steps, for 30 minutes each). After smoking, the products were pressed to shape for 48-72 hours (at a temperature of 0-6°C) and smoked again for 2,5 hours at 15-17°C. The batches were dried and matured at 14-15°C and 70-75% humidity for 20 and 40 days, respectively.

Sensory analysis

The sensory analysis session involved five steps to obtain the most accurate and relevant results for the proposed study. In the first stage, the development and drafting of the evaluation questionnaire, the terms, implicitly the attributes to be followed in the evaluation of the samples, were selected and defined with reference to the literature (Perez-Cacho et al., 2005; Braghieri et al., 2009). Therefore, 15 specific attributes for appearance, aroma, texture and taste were selected (Marangon & Moura, 2011; Braghieri et al., 2016). The second stage involved the selection and training of the panelists, the group of evaluators was composed of 8 tasters (5 women and 3 men, between 22 and 24 years of age) who evaluated 3 samples in a session, thus the 6 samples were evaluated in 6 sessions (3 repetitions for each batch).

The sensory analysis session took place between 10-12 a.m., after breakfast, in the Sensory Analysis Laboratory within the University of Life Sciences Iași, a laboratory equipped with individual booths. The test preparation phase consisted of slicing the products using a slicer, placing them on plates and coding by random three-digit numbers.

Samples were served at 16-18°C, water and unsalted biscuits were provided for all members of the evaluation team to clean the oral cavity between samples. A 9-point rating scale was used for the actual evaluation of the samples, with 1 representing the minimum score (very low intensity) and 9 being the maximum score (high intensity); if no score was assigned, it means that the respective descriptor was not identified and the score is 0 (Coloretti et al., 2014; Ruiz et al., 2014; Teixeira et al., 2021).

The collected results were statistically analysed and the significance of the differences between the means obtained for the samples from the six

experimental batches (L_{12}, L_{22}, L_{32}, L_{14}, L_{24} and L_{34}) was calculated with T test with two variables (2-tailed) using the Data Analysis function of the Excel program.

RESULTS AND DISCUSSIONS

On the 9-point rating scale, the appearance of the six samples was analysed in terms of colour uniformity, colour intensity and fat/lean distribution. Ripening time positively influenced the colour intensity of the evaluated samples as well as colour uniformity but to a smaller extent. Hence, insignificant differences ($p>0.05$) were observed between batches, except for batch 2 which obtained lower average scores, being at the inferior limit (L_{22} obtaining 7.33±0.32 points). Diversification of the quantities of raw materials introduced resulted in statistically significant differences ($p<0.01$) between lots L_{12}, L_{22} and L_{32}.

Fat/lean distribution is an attribute that shows the uniformity of fat distribution in the meat over the slice surface (Perez-Cacho et al., 2005). Table 1 shows results according to which the higher amount of fat introduced in batch 2 had an impact on the scores given by the evaluators, this batch obtaining the lowest means for this attribute, 7.12±0.38 (L_{22}) points and 7.08±0.43 points (L_{24}) respectively. Therefore, distinctly significant differences ($p<0.01$) were observed between batches with different raw material ratios.

Table 1. Sensory appearance of Babic dry sausages as influenced by maturation time and raw materials ratio

Attributes		Exp. batch		n	$\bar{X} \pm s_{\bar{x}}$	V%	Difference interpretation - T-Test (2-tailed)			
							Maturation time		Raw materials ratio	
APPEARANCE	Colour intensity	L_1	L_{12}	24	7.95±0.56	9.43	L12-L14	t=-1.42;	L12-L22	t=3.25; p=0.0022**
			L_{14}		8.20±0.17	5.05		p=0.16ns	L12-L32	t=-0.189; p=0.85 ns
		L_2	L_{22}		7.33±0.32	7.70	L22-L24	t=-2.40;	L22-L32	t=-3.39; p=0.001**
			L_{24}		7.87±0.90	12.02		p=0.124 ns	L14-L24	t=1.57; p=0.124 ns
		L_3	L_{32}		8±0.61	9.75	L32-L34	t=0.54;	L14-L34	t=1.81; p=0.07 ns
			L_{34}		7.91±0.64	10.13		p=0.55 ns	L24-L34	t=-0.14; p=0.88 ns
	Colour uniformity	L_1	L_{12}	24	8.20±0.43	8.02	L12-L14	t=-0.706;	L12-L22	t=3.14; p=0.002**
			L_{14}		8.33±0.32	6.78		p=0.48 ns	L12-L32	t=2.007; p=0.051 ns
		L_2	L_{22}		7.58±0.51	9.46	L22-L24	t=-3.69;	L22-L32	t=-0.6; p=0.34 ns
			L_{24}		8.20±0.17	5.05		p=0.0007**	L14-L24	t=0.874; p=0.387 ns
		L_3	L_{32}		7.79±0.61	10.00	L32-L34	t=-0.961;	L14-L34	t=1.78; p=0.082 ns
			L_{34}		8±0.52	9.03		p=0.341 ns	L24-L34	t=1.22; p=0.228 ns
	Fat/lean distribution	L_1	L_{12}	24	7.87±0.64	10.13	L12-L14	t=-0.549;	L12-L22	t=3.65; p=0.0007**
			L_{14}		8±0.61	9.75		p=0.58 ns	L12-L32	t=-2.01; p=0.051 ns
		L_2	L_{22}		7.12±0.38	8.59	L22 -L24	t=0.227;	L22-L32	t=-7.29; p=6E-09**
			L_{24}		7.08±0.43	9.23		p=0.82 ns	L14-L24	t=4.41; p=6E-05**
		L_3	L_{32}		8.25±0.20	5.36	L32 -L34	t=0.257;	L14-L34	t=-1; p=0.322 ns
			L_{34}		8.20±0.43	8.02		p=0.79 ns	L24-L34	t=-5.94; p=4E-07**

n - no. of evaluations per sample; T- test (2-tailed) - for each analysed character, comparative on experimental batches: ns. insignificant differences ($p>0.05$); *significant differences ($p<0.05$);**distinct significant differences $p<0.01$).

The flavour of the products is determined mainly by added spices, microbial metabolism of lipids, proteins and carbohydrates, and lipid oxidation (Sunesen et al., 2001). Therefore, the flavour of the experimental batches was evaluated in terms of intensity, spice flavour, smoke flavour and rancid flavour, the results of which are shown in Table 2. The ripening time had a positive influence on the total flavour intensity, with significant differences ($p<0.05$) between batches. The intensity of added spice flavour and the smoke flavour was influenced by both time and raw material ratio, with significant ($p<0.05$) and distinctly significant ($p<0.01$) differences between batches. As reported by other authors, the higher the percentage of fat in the meat, the products require a higher amount of seasoning, as fat attenuates the intensity of the seasoning (Braghieri et al., 2016). Concerning the rancid flavour, both maturation time and raw materials introduced had a significant influence. The experimental batches with the highest fat percentages and longer ripening time (L_2 and L_3) received the highest mean scores. Hence, the differences observed between batches L_2 and L_3 were insignificant ($p>0.05$), and batch L_1 stood out with distinctly significant differences ($p<0.01$) compared to the other two.

Table 2. Sensory flavor profile of Babic dry sausages as influenced by maturation time and raw materials ratio

Attributes		Exp. batch		n	$\bar{X} \pm s_{\bar{x}}$	V%	Difference interpretation - T-Test (2-tailed)			
							Maturation time		Raw materials ratio	
AROMA	Aroma intensity	L_1	L_{12}	24	6.12±0.46	11.09	L12-L14	t=-3.47; p=0.001**	L12-L22	t=1.16; p=0.248 ns
			L_{14}		6.70±0.21	6.92			L12-L32	t=-0.17; p=0.481 ns
		L_2	L_{22}		5.87±0.63	13.57	L22-L24	t=-0.95; p=0.034*	L22-L32	t=-1.91; p=0.062 ns
			L_{24}		6.08±0.51	11.79			L14-L24	t=3.58; p=0.346 ns
		L_3	L_{32}		6.25±0.28	8.50	L32-L34	t=-2.23; p=0.03*	L14-L34	t=0.89; p=0.376 ns
			L_{34}		6.58±0.253	7.65			L24-L34	t=-2.79; p=0.007**
	Spices aroma	L_1	L_{12}	24	4.41±0.254	11.40	L12-L14	t=-5.34; p=3E-06**	L12-L22	t=1.50; p=0.138 ns
			L_{14}		5.29±0.389	11.79			L12-L32	t=2.35; p=0.023*
		L_2	L_{22}		4.16±0.406	15.28	L22-L24	t=-4.02; p=0.0002**	L22-L32	t=0.28; p=0.778 ns
			L_{24}		4.79±0.172	8.65			L14-L24	t=3.26; p=0.002**
		L_3	L_{32}		4.12±0.114	8.19	L32-L34	t=-8.67; p=8E-11**	L14-L34	t=0.77; p=0.441 ns
			L_{34}		5.16±0.231	9.32			L24-L34	t=-2.89; p=0.005**
	Smoke aroma	L_1	L_{12}	24	2.62±0.245	18.83	L12-L14	t=-5.75; p=7E-07**	L12-L22	t=3.86; p=0.0004**
			L_{14}		3.45±0.259	14.71			L12-L32	t=8.05; p=2E-10**
		L_2	L_{22}		2.04±0.303	26.94	L22-L24	t=-3.30; p=0.008**	L22-L32	t=3.81; p=0.0004**
			L_{24}		2.58±0.341	22.59			L14-L24	t=5.53; p=2E-06**
		L_3	L_{32}		1.45±0.259	34.90	L32-L34	t=-6.80; p=2E-08**	L14-L34	t=6.80; p=2E-08**
			L_{34}		2.45±0.259	20.70			L24-L34	t=0.79; p=0.433 ns
	Rancid aroma	L_1	L_{12}	24	0.83±0.319	67.76	L12-L14	t=-3.30; p=0.0019*	L12-L22	t=-4.51; p=5E-05**
			L_{14}		1.33±0.232	36.12			L12-L32	t=-2.73; p=0.009**
		L_2	L_{22}		1.62±0.418	39.81	L22-L24	t=-2.65; p=0.0109*	L22-L32	t=0.93; p=0.355 ns
			L_{24}		2.16±0.58	35.14			L14-L24	t=-4.53; p=5E-05**
		L_3	L_{32}		1.41±0.775	62.16	L32-L34	t=-1.95; p=0.057ns	L14-L34	t=-3.00; p=0.004**
			L_{34}		1.87±0.548	39.51			L24-L34	t=1.34; p=0.185 ns

n - no. of evaluations per sample; T- test (2-tailed) - for each analysed character, comparative on experimental batches: ns. insignificant differences (p>0.05); *significant differences (p<0.05);**distinct significant differences p<0.01).

The texture of the six products obtained was sensory evaluated using the attributes stiffness (hardness), tenderness, elasticity and fat consistency (oiliness), as shown in Table 3. Rødbotten et al. (2004) defined stiffness as the force required to bite into a sample. The stiffness or hardness of the batches scored mean values ranging from 2.16±0.492 (L34) to 3.12±0.375 (L12). The samples were evaluated on a 9-point scale, with 1 representing very low hardness and 9 representing very high hardness. Ripening time did not significantly influence hardness, except for lot 2, with distinctly significant differences ($p<0.01$) between lots L22 and L24. The ratio of raw materials significantly influenced the evaluation and scoring, with differences between batches being significant ($p<0.05$) and distinctly significant ($p<0.01$), as according to the results obtained by Gadiyaram & Kannan (2004) sausages made from beef showed higher hardness compared to those made from mixed meat.

Differences in tenderness between batches were evident and significant, with this attribute being influenced by both maturation time ($p<0.05$; tenderness increased with maturation time) and the ratio of raw materials introduced ($p<0.01$), with the order of mean scores for the three batches in terms of tenderness being L3, L1 and L2.

Elasticity was defined as the speed with which the product returns to its original shape after the action of a deforming force has ceased (Braghieri et al., 2009; Marangoni & Moura, 2011), and fat consistency (oiliness/fatness) is an attribute referring to the perception of the amount of fat released during mastication (Perez-Cacho et al., 2005). Ripening time influenced elasticity and fatness perception, products with a higher ripening period showed lower elasticity, and fatness sensation was more intense after 40 days of ripening. Regarding the ratio of raw materials introduced, the batches showed distinctly significant differences ($p<0.01$) in elasticity, and batch L1 showed distinctly significant differences from the other two batches ($p<0.01$) in fat sensation.

Table 3. Sensory texture of Babic dry sausages as influenced by maturation time and raw materials ratio

Attributes		Exp. batch		n	$\bar{X} \pm s_{\bar{x}}$	V%	Difference interpretation - T-Test (2-tailed)			
							Maturation time		Maturation time	
TEXTURE	Hardness / Stiffness	L_1	L_{12}	24	3.12±0.375	19.596	L12-L14	t=1.41; p=0.164^{ns}	L12-L22	t=1.28; p=0.0204*
			L_{14}		2.87±0.375	21.300			L12-L32	t=4.97; p=1E-05**
		L_2	L_{22}		2.91±0.253	17.267	L22-L24	t=3.75; p=0.0005**	L22-L32	t=4.10; p=0.0002**
			L_{24}		2.37±0.244	20.823			L14-L24	t=3.11; p=0.003**
		L_3	L_{32}		2.33±0.231	20.638	L32-L34	t=0.959; p=0.343 ^{ns}	L14-L34	t=3.72; p=0.0005**
			L_{34}		2.16±0.492	32.398			L24-L34	t=1.18; p=0.0241*
	Tenderness	L_1	L_{12}	24	5.62±0.331	10.236	L12-L14	t=-2.73, p=0.0087**	L12-L22	t=2.87; p=0.006**
			L_{14}		6.08±0.340	9.593			L12-L32	t=-4.41; p=6E-05**
		L_2	L_{22}		5.20±0.172	7.965	L22-L24	t=-2.09; p=0.042*	L22-L32	t=-8.52; p=6E-11**
			L_{24}		5.54±0.432	11.874			L14-L24	t=3.01; p=0.004**
		L_3	L_{32}		6.29±0.215	7.380	L32-L34	t=-2.08; p=0.0426*	L14-L34	t=-3.17; p=0.002**
			L_{34}		6.58±0.253	7.650			L24-L34	t=-6.15; p=2E-07**
	Elasticity	L_1	L_{12}	24	2.87±0.114	25.889	L12-L14	t=-2.208; p=0.034*	L12-L22	t=-4.56; p=5E-05**
			L_{14}		2.54±0.432	11.751			L12-L32	t=2.42; p=0.0019**
		L_2	L_{22}		3.29±0.215	14.105	L22-L24	t=2.84; p=0.006**	L22-L32	t=7.38; p=5E-09**
			L_{24}		2.83±0.405	22.483			L14-L24	t=0.28; p=0.0077**
		L_3	L_{32}		2.08±0.427	31.385	L32-L34	t=0.64; p=0.522 ^{ns}	L14-L34	t=10.09; p=4E-13**
			L_{34}		1.96±0.476	35.247			L24-L34	t=6.48; p=2E-07**
	Oiliness/ fatness	L_1	L_{12}	24	3.25±0.282	16.357	L12-L14	t=-2.84; p=0.006**	L12-L22	t=-3.18; p=0.002**
			L_{14}		3.66±0.231	13.133			L12-L32	t=-3.54; p=0.0009**
		L_2	L_{22}		3.70±0.215	12.521	L22-L24	t=-1.15; p=0.256 ^{ns}	L22-L32	t=-0.31; p=0.751 ^{ns}
			L_{24}		3.87±0.288	13.850			L14-L24	t=-1.41; p=0.163 ^{ns}
		L_3	L_{32}		3.75±0.195	11.795	L32-L34	t=-0.62; p=0.535 ^{ns}	L14-L34	t=-1.19; p=0.236 ^{ns}
			L_{34}		3.83±0.231	12.562			L24-L34	t=0.28; p=0.778 ^{ns}

n - no. of evaluations per sample; T- test (2-tailed) - for each analysed character, comparative on experimental batches: ns. insignificant differences (p>0.05); *significant differences (p<0.05);**distinct significant differences p<0.01).

The taste was assessed in terms of four basic tastes: salty, sour, bitter and sweet, which are familiar and easily identified by tasters. Table 4 presents the mean scores obtained for each experimental batch. These data were analysed using Student's t-test to highlight differences between batches generated by time and raw material quantity.

The specificity of the raw materials used and also of the maturation time applied for each experimental batch were found on the sensory properties of the analyzed batches. The salty taste was the most intense of the four, with mean scores ranging from 1.83±0.318 (L32) to 2.62±0.505 (L12). Ripening time influenced the perception of salty taste (p<0.05), being more intense after the 40 days of ripening. Sour, bitter and sweet tastes, although showing an increase in mean scores, differences between batches were not significant (p>0.05). The different amounts of raw materials introduced in the experimental batches caused small changes in taste, the differences being insignificant (p>0.05).

In relation to the raw materials used and also to the ripening time, the bitter taste and the salty taste were identified as having the lowest intensities, from 0.83±1.536 (L1-L12) for the bitter taste and 0.75±0.717 (L2-L22). However, for both, the bitter taste and the sweet taste, the differences identified between the experimental groups were generally insignificant (p> 0.05).

Table 4. Sensory taste evaluation of Babic dry sausages as influenced by maturation time and raw materials ratio

Attributes		Exp. batch		n	$\bar{X} \pm s_{\bar{x}}$	V%	Difference interpretation - T-Test (2-tailed)			
							Maturation time		Maturation time	
TASTE	Salty	L_1	L_{12}	24	2.41±0.340	27.083	L12-L14	t=1.109; p=0.027*	L12-L22	t=2.76; p=0.086 ns
			L_{14}		2.62±0.505	24.149			L12-L32	t=4.27; p=0.051 ns
		L_2	L_{22}		2.04±0.563	36.764	L22-L24	t=-2.04; p=0.046*	L22-L32	t=1.08; p=0.283 ns
			L_{24}		2.45±0.432	26.766			L14-L24	t=-0.23; p=0.817 ns
		L_3	L_{32}		1.83±0.318	30.800	L32-L34	t=-2.45; p=0.019*	L14-L34	t=0.18; p=0.852 ns
			L_{34}		2.37±0.853	38.894			L24-L34	t=0.36; p=0.720 ns
	Acid	L_1	L_{12}	24	0.87±0.375	69.985	L12-L14	t=-1.14; p=0.164 ns	L12-L22	t=-2.016; p=0.05 ns
			L_{14}		1.12±0.375	54.433			L12-L32	t=-1.21; p=0.230 ns
		L_2	L_{22}		1.29±0.650	62.435	L22-L24	t=-0.36; p=0.715 ns	L22-L32	t=0.47; p=0.637 ns
			L_{24}		1.37±0.592	55.976			L14-L24	t=-1.25; p=0.219 ns
		L_3	L_{32}		1.16±1.01	86.333	L32-L34	t=-0.88; p=0.384 ns	L14-L34	t=-1.45; p=0.150 ns
			L_{34}		1.29±0.331	41.875			L24-L34	t=-0.55; p=0.580 ns
	Bitter	L_1	L_{12}	24	0.83±1.536	148.73	L12-L14	t= -0.2; p=0.806 ns	L12-L22	t=-0.67; p=0.501 ns
			L_{14}		0.91±1.210	120.01			L12-L32	t=-1.03; p=0.308 ns
		L_2	L_{22}		1.08±1.731	121.48	L22-L24	t=-0.47; p=0.638 ns	L22-L32	t=-0.14; p=0.889 ns
			L_{24}		1.25±1.239	89.05			L14-L24	t=-1.04; p=0.302 ns
		L_3	L_{32}		1.12±0.375	54.43	L32-L34	t=0.65; p=0.515 ns	L14-L34	t=-0.13; p=0.895 ns
			L_{34}		0.95±1.172	112.97			L24-L34	t=0.92; p=0.362 ns
	Sweet	L_1	L_{12}	24	0.91±1.384	128.34	L12-L14	t=-0.48; p=0.631 ns	L12-L22	t=0.56; p=0.576 ns
			L_{14}		1.08±1.471	111.96			L12-L32	t=-0.11 p=0.910 ns
		L_2	L_{22}		0.75±0.717	112.93	L22-L24	t=-1.16; p=0.250 ns	L22-L32	t=-0.63; p=0.529 ns
			L_{24}		1.12±1.766	118.14			L14-L24	t=-0.11; p=0.910 ns
		L_3	L_{32}		0.95±1.867	142.61	L32-L34	t=-0.79; p=0.432 ns	L14-L34	t=-0.51; p=0.606 ns
			L_{34}		1.29±2.38	119.67			L24-L34	t=-0.40; p=0.690 ns

n - no. of evaluations per sample; T- test (2-tailed) - for each analysed character, comparative on experimental batches: ns. insignificant differences (p>0.05); *significant differences (p<0.05);**distinct significant differences p<0.01).

CONCLUSIONS

The descriptive sensory analysis of the products allowed a description and comparison of the six experimental batches obtained in the Meat Processing Microsection of the University of Life Sciences in Iasi. According to the Student test, the ripening time influenced the sensory quality, especially the attributes of aroma and texture, as well as the intensity of the salty taste. The overall aroma intensity of the products, as well as the perception of the spicy and smoky aroma, showed distinctly significant differences (p<0.01), the batches matured for 40 days were appreciated with higher average scores by the evaluators.

The ratio of the raw materials introduced in the technological sheets of the six experimental batches was the factor that showed the most evident differences between the samples. Texture attributes showed the greatest differences, with the batches showing distinctly significant differences in stiffness, tenderness and elasticity. The assessors scored the samples from batches 1 and 3 as showing insignificant differences in appearance (intensity, colour uniformity, meat/fat distribution). Although the percentage of fat is different in the two experimental batches, L_3 contains a higher amount of mutton, a raw material with a higher fat content compared to beef.

After the diversification of the technological sheets in terms of the quantities of raw materials introduced in the three experimental batches, we found that the L_1 and L_3 samples were assessed with higher average scores compared to L_2, possibly due to the lower quantity of fat, which influenced the evaluation of the analysed attributes.

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STUDY OF BEHAVIOR OF SCHOOL CHILDREN ON MILK CONSUMPTION IN SCHOOL PROGRAM

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Abstract

The paper is based on the study of various reports and different type of articles regarding the milks in school programs. In order to determine the Romanian consumers' perception regarding the milk consume, a questionnaire was prepared which was completed during a school year by scholar from Romania. We want diversity in the milk assortments served in the Milk and Croissant school program. One-third of respondents suggested that milk be replaced with yogurt or fruit yogurt and various dairy based on products. The concern of young people for a healthy diet should be made from infancy and this is reflected in the answers provided.

Key words*: milk, school program, students.*

INTRODUCTION

Globally, most consumers still have a strong positive opinion about the goodness of milk, they understand its nutritional value, but they believe that the diversity and benefits of this product do not keep up well enough with their modern lifestyle and expectations.

The influences of globalisation on eating habits have led to an increase in the variety of diets by including new foods, ingredients, and preparations. At the same time, the growing influence of ethical, health or sustainability values in attitudes and preferences has led to substantial changes in individual consumption behavior and this is reflected in the diet of consumers (Allen et al., 2020). These values are extremely important for the European consumer, as reported by recent Eurobarometer data (80% of Europeans buy sustainable products (European Commission, 2019). The evolution of consumer preferences determines the inclusion of new foods in the diet and, unfortunately, the exclusion of others.

Foods consist of a large number of different nutrients that are contained in a complex structure. The nature of the food structure and the nutrients therein (i.e., the food matrix) will determine the nutrient digestion and absorption, thereby altering the overall nutritional properties of the food. Thus, the food matrix may exhibit a different relation with health indicators compared to single nutrients studied in isolation (Thorning et al., 2017).

Dairy products have been an important part of the human diet for about 8,000 years and are part of the official nutritional recommendations of many countries around the world. They provide a package of key nutrients that are difficult to obtain in diets with limited or no dairy products, such as vegan or restrictive dairy diets. Indeed, dairy products are rich in calcium, protein, potassium and phosphorus. They contribute about 52-65% of the reference dietary intake (DRI) of calcium and 20-28% of the protein requirement, depending on the age of the consumer (Smit et al., 1999; Feskanich et al., 2003; Skinner at al., 2011).

Milk has always been considered a fundamental component of human nutrition; it is healthy, beneficial, and fortifying for all age groups. Cow's milk is included in one of the seven basic food groups developed for providing high biological value proteins, calcium, and phosphorus, as well as being a relevant source of bioactive components (e.g., immunoglobulins, conjugated linoleic acid, lactoferrin, etc.) with beneficial effects on human health. Even considering the negative effects on human health from excessive milk consumption, the

essentiality of milk in human nutrition in a balanced diet has been demonstrated in several researches. However, this product is no longer part of the consumers' dietary plan, especially in some countries of the world (Houg et al., 2007; Gomez-Cortez et al., 2018; Liang et al., 2018; Merlino et al., 2022).

Growth and development are the most important indicators of adequate and balanced nutrition. The determination of age-appropriate growth is possible with the help of body weight and height measurements assessed according to the age and sex of children.

The first sign of inadequate protein and calorie intake in children is the growth and development retardation. Children, who cannot get sufficient and balanced nutrition, are prone to diseases and become often sick. They may have problems of absenteeism and low school success rates (Tüfekci, 2019).

School milk programme represent an important vehicle for the promotion of milk. Such programme are currently seeing a resurgence of interest and are enjoying a renaissance more imaginative and appealing way (Șerban et al., 2021).

MATERIALS AND METHODS

A hybrid (online and writable) survey was conducted, reaching 340 Romanian children consumers (N = 340) between September 2021 and March 2022. The questionnaire was made available by Google Form and print. Data regarding the consumer's perception on milk consumption has been collected by filling a questionnaire with 25 questions.

The study carried out is in fact a statistical survey, which was based on the questionnaire survey, the questions referring both to the preferences regarding the milk product consumed in "Milk and Croissant" school program and their opinion on the improvement of the program and the possible milk replacements in the program.

The aspects of the research methodology were: establishing the topic of this research, determining the objectives that were pursued by the survey method, determining the respondent population and its sample size, determining the question models used and printing the questionnaire, postcoding all answers received, processing the results obtained and their interpretation, the drafting of the final report and its presentation.

Data collected via questionnaires was graphic processed and presented based on each characteristic investigated and it will be detailed for each item.

RESULTS AND DISCUSSIONS

School milk programs are common in many countries around the world for a good reason. Nutritionists and social policy experts believe that encouraging healthy eating should be the main goal of this general and universal program. The benefits of school milk are many, so milk and dairy products are considered the main sources of nutrients needed for the harmonious development of children (Bulletin of the IDF N° 505/2020).

The main objective of the article is to identify the cause of the decrease in milk consumption among children and the cause of the rejection of milk consumption in the Milk and Croissant Program. In this sense, students from several schools in Bucharest, aged between 8-14, participated in the survey. The sample is balanced in terms of gender 45.6% girls and 54.4% boys (Table 1).

Table 1. Socio-demographic profiles of scholar milk consumers (n = 340)

Variable	%	N
Age		
8-10	7.4	250
10-12	19.1	65
12-14	73.5	25
Gender		
Female	45.6	155
Male	54.4	185
Education		
Primary school	10.3	35
High school	89.7	305
Place of residence		
Urban	86.8	295
Rural	13.2	45

The students participating in the survey come from family backgrounds. The study involved children from families with 4 members (52.9%), 3 members (25%) and 5 members (8.8%), respectively families with at least 2 children, most cases. The behavior of milk consumption in the family is derived from the question "What is the most common time you drink milk?".

Where 73.5% of respondents consume milk for breakfast and 25% in the evening.
Frequency of milk consumption among students, 44.1% of respondents stated that they consume milk 2-3 times a week and 38.2% every day. Data processing shows that approximately 15% consume milk occasionally or never (Figure 1).

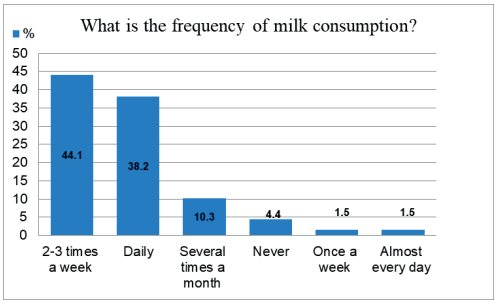

Figure 1. Frequency of milk consumption

The most consumed milk is cow's milk (95.6%), milk from other species is in the order of preferences goat's milk (5.9%), the rest at values below 1.5% (Ilie et al., 2021; Kempen et al., 2017). The preference for cow's milk is also justified in terms of greater shelf availability and a much more affordable price. In terms of goat's milk, it is the second most popular in the list of respondents' preferences, being preferred due to its health benefits and its lower allergenicity, due to the lack of beta-lactoglobulin protein.
The reason they buy milk is that it is good for the body (67.2%), followed by taste (51.6%), useful for the diet (17.2%), and 14.1% of respondents appreciate milk as an indispensable food for the body.
It was looked at whether the students have the necessary information on the benefits of consuming milk on the body. Thus, when asked about the positive effects of milk consumption on the body, approximately 45.6% of respondents said that milk is important for bones, 42.6% said that milk gives a good feeling to the body and an energizing 22.1%.
Milk is one of the most complex foods, with benefits for both the skeletal system and the brain. However, milk consumption in Romania is 4.4 times lower than the average milk consumption in Western Europe and 2.4 times lower than the average in Eastern Europe. Although various programs have been set up at national level to encourage milk consumption, the number of milk consumers is declining. Reduced milk consumption affects health in the country and is also an economic problem.
Fluid milk consumption among children has declined for decades. Adequate consumption of milk and dairy products, especially during childhood, has beneficial health outcomes for growth, development, and reduced risk of osteoporosis, hypertension, obesity, and cancer during adulthood. Satisfaction with milk flavor, perceived health benefits derived from milk, and habit are primary drivers of lifelong milk consumption. Child preferences and attitudes for milk may differ from those of adults, and as such, understanding and fulfilling the needs of children is crucial to reverse the decline in milk consumption (Sipple et al., 2020).
To the question, which refers to the students' opinion about the milk received through the "Milk and Croissant" school program. Most students mentioned that the milk received in the program is not always as tasty and often creates digestive problems. It was also mentioned that the individual milk packaging has no straw or has difficulty consuming.
School meals make liquid milk accessible to millions of children every day; however, school lunch regulations and procurement systems sometimes make it difficult to provide new or value-added dairy products in these programs.
Milk and Croissant school programme, part of the European School Milk Scheme, aims at providing healthy food choice to all Romanian pre-school to secondary school students (ages 5-6 to 14-15) by offering them in each school day, at lunch time, a small bottle of milk/yoghurt and a croissant/bagel.
Since the introduction of the *Milk and Croissant Program* of 1995, the government has provided subsidized milk to schools to ensure adequate nutrition of Romanian schoolchildren. The program allows schools to offer this nutritious beverage to low-income students in place of other drinks that may have been at their reach instead, such as sodas or fruit juice.
But there’s an important drawback to consider. Because many health organizations now recommend lower fat milks for kids due to the obesity epidemic, schools are limited to serving only fat-free or low-fat milk.

Whole milk is creamier, making it tastier and more generally accepted by younger kids. Therefore, the milk being offered may be harder to be accepted by kids and more likely to be wasted and thrown out (Sipple et al., 2020).
The purpose of the study is to find out the students' opinion regarding the milk received through the *Milk and Croissant school program.* Most of the students mentioned that the milk received in the program "does not taste", "does not attract me", "is very watery" etc. and most often creates digestive problems. They have no straw or have difficulty consuming.
The fact that children consume less milk is a problem with more girls. There is several other factors are contributing to the decline in childhood milk consumption: (1) parents are opting for milk alternatives; (2) increased consumption of juice and carbonated soft drinks; (3) lactose intolerance or other allergy-based.
When asked about the milk replacement suggestions in the *Milk and Croissant school program*, the most frequently mentioned was the replacement with apple juice, fruit yogurt, milk-based soft drinks, cocoa milk or a greater diversity of products offered. The students also mention that most of the time they receive a simple dough bun, respectively a piece of bread that is not attractive to the studied age segment (Figure 2).

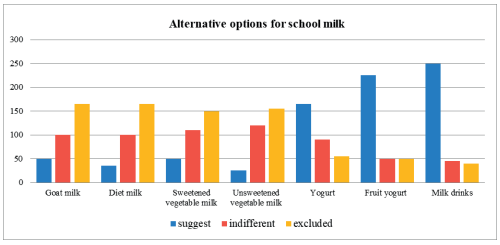

Figure 2. Alternative options for school milk

Lactose intolerance is a clinical syndrome that manifests with characteristic signs and symptoms upon consuming food substances containing lactose, a disaccharide. Normally upon lactose consumption, it is hydrolyzed into glucose and galactose by the lactase enzyme, which is found in the small intestinal brush border. Deficiency of lactase due to primary or secondary causes results in clinical symptoms. This activity describes the pathophysiology of lactose intolerance and highlights the role of the interprofessional team in its management (Vesa et al., 2000; Malik et al., 2022).
In the first years of school, the incidence of people with lactose intolerance is higher. Thus, of those interviewed, over 41.2% mentioned that they know of a colleague who has intolerance to lactose or beta-lactoglobulin. It is important to take into account this aspect and to be concerned with a distribution, in parallel with milk, of analogous drinks with no allergens.
Ask "What type of alternative milk product are you consuming?" The answers were varied and highlight that many students know and consume these alternatives to milk (Figure 3). Milk alternatives can include beverages made from plants, such as soy, oat, rice, coconut, cashew, and almond. The graph shows that the best known alternative to milk, by students, is coconut milk, followed by almond milk.

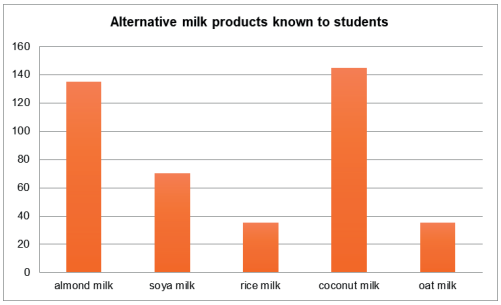

Figure 3 Alternative milk products known to students

Extremely important is the justification provided by each respondent to the question about the benefits to the body through the consumption of milk alternatives.
Many types of innovative food drinks from plant sources are promoted as an alternative to cow's milk, but there are many technological impediments, either to processing or to preservation. Most of these milk alternatives do not have a nutritional balance compared to cow's milk, however they contain functionally active components with health-promoting properties that attract health-conscious consumers. In the case of legume-based milk alternatives, sensory acceptability is a major limiting factor for its widespread popularity (Sethi et al., 2016; Vanga et al., 2018).

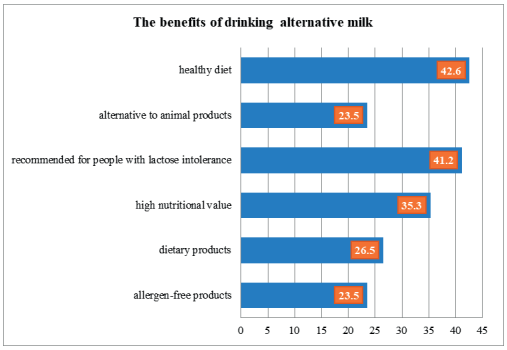

Figure 4. The benefits of drinking alternative milk for health

About 42.6% of respondents believe that they have a beneficial effect on health and about 41.2% know that these products are intended for people with lactose intolerance (Figure 4).

We can say that the flow of information, from all information channels, regarding food allergies and intolerances, has been extremely well assimilated by children. This leads us to believe that if we use the same model of information, we can increase awareness of the positive effects of milk and dairy products on the body and increase consumption among young people.

Both promoting and providing healthy food in schools has been shown to improve food choices and may have an influence on long-term food behavior and health. This ability to influence food choice is known as choice architecture and has been used in a number of school settings to influence healthy food behaviors, such as increasing the consumption of plant based foods (Marsh et al., 2018; Ensaff et al., 2015).

CONCLUSIONS

In conclusion, the study found that students prefer to drink less milk than ever before, and opinions about milk as a healthy choice change, with vegetable milk growing rapidly in popularity.

One way to revitalize the consumption of milk and dairy products in all markets is to delight consumers about the benefits of milk. It is also important to create new products and develop communication campaigns to show that milk is beneficial, tasty, even a treat and important for all of us.

This is the first study to explore the determinants of milk consumption in the *Milk and Croissant school program*, in a sample of boys and girls between the ages of 6 and 14, and thus makes a significant contribution to the literature. Complete their decision-making process regarding increasing milk consumption in this age segment.

These aspects can be used in planning the next steps for promoting food consumption through milk school program. A better understanding of young people's opinion about the milk offered in the milk school program is important for the milk and dairy products sector, the Romanian and European regulatory system. Future dietary assessments and recommendations should also carefully consider evidence of the effects of whole foods and similar plant products, as well as evidence of the effects of individual nutrients.

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STUDY ON THE INCIDENCE OF GLUTEN INTOLERANCE ASSOCIATED DISEASES WITH CONSUMPTION OF AGLUTENIC FOODS

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Abstract

Celiac disease (CD) is an immune condition that patients have to deal for lifelong. It affects almost 1% of the general population and determines lifestyle and social changes due to diet control and restrictions. Gluten ingestion in patients with CD determines bloating, abdominal pain, constipation, nausea and vomiting conducting to anaemia, osteoporosis, neurological problems, herpetiform dermatitis can occur later in life. The incidence of CD increased especially for women (17.4 cases of 100,000 for one year); for men, the incidence is 7.8 per 100,000 people in one year. More and more children are also diagnosed with celiac disease, 21.3 per 100,000 person-years compared to 12.9 per 100,000 in adults. Wheat allergy, gluten sensitivity and celiac disease represent medical conditions that are correlated with cereal consumption and become of increased interest for consumers in order to have an appropriate diet or a preventive one. Gluten free diet (GFD) become more popular among consumers and its popularity is increasing, health practitioners started to question if there is a real health benefit for everyone or just a medical nutrition therapy for those in need.

Key words*: allergy, celiac disease, gluten allergy, gluten sensitivity, gluten free diet.*

INTRODUCTION

Wheat is the most cultivated and consumed cereals of all times; it was the first cultivated one and it determined the evolution of humankind. Some of the cultivars have been modified in order to meet new challenges in agriculture, but it seems that this has been altered the level of gliadin, the protein fraction that is responsible for gluten sensitivity diseases group.

Celiac disease is the result of both environmental (gluten) and genetic factors (HLA and non-HLA genes), and the distribution of these two components can probably be used to identify the areas of the world at risk for gluten intolerance (Cataldo et al., 2007).

MATERIALS AND METHODS

For this research we have analysed Scopus Elsevier database and SpringerLink Journals by specific key words like “celiac disease”, “gluten related diseases”, “symptoms”, “effect on social life”, “incidence”, “diagnosis and treatment”, “clinical studies”. We have also used the statistics found on statistica.com and other official information of different states. We analysed and compared data from 2009 to 2020 in order to have an overview on the evolution.

RESULTS AND DISCUSSIONS

Gluten, the responsible factor

Gluten represents the main wheat, rye and barley storage proteins known as prolamins that have an important role in bakery. Gluten is essential for dough formation, elasticity, dough gas retention capacity, porosity and bread quality in the end. Biochemically, gluten is a mixture of gliadins (alcohol soluble) and glutenin (alcohol insoluble), proteins rich in glutamine and proline. The high level of proline determines a certain resistance of these proteins to the proteolytic activity of gastric and pancreatic enzymes; they are also resistant to the small intestinal brush border membrane enzymes. Due to this enzymatic resistance the formation of long gliadin peptides in the gastrointestinal tract is occurring and this induces a detrimental immune response in patients with celiac disease. The most studied peptide is known as the “33mer” and it is considered the main initiator of the inflammatory response to gluten; it was found in all food grains that are toxic to celiac

disease patients and absent in other nontoxic cereals. Shan et al. (2002) found that this peptide can be hydrolyzed *in vitro* by a *Flavobacterium meningosepticum* prolyl endopeptidase. They observed that in vivo study using the rat intestinal perfusion model, enzyme can break down the "33mer" with the diminishing of its toxic effects and also the decrease of the T cell stimulatory potential with important positive effect on detoxifying gluten in celiac disease patients by peptidase therapy; this would suggest a strategy for a therapy with oral peptidase for celiac disease patients.

Furthermore, the effect of undigested peptides reaches the gut, become available as good nurture for intestinal bacterial gluten metabolism which can change the gut microbiota (Caminero et al., 2016).

Genetic factors

There are two factors that might concur celiac disease development: ingestion of gluten and genetic predisposition. The latter is evidenced by the fact that the prevalence of celiac disease among first degree relatives of patients is 8%, higher than that of general population (Singh P. et al, 2015). Regarding the genetic factors that might determine a risk for celiac disease, there were identified HLA- DQ haplotypes HLADQ2 and HLA- DQ8 with the highest risk, contributing with 25-40% of the genetic risk. Almost 40% of the North American and European populations also carry these haplotypes but they do not develop coeliac disease, which can conclude that HLA- DQ2 or HLA-DQ8 is necessary but not sufficient for coeliac disease to develop (Lindfors et al., 2019).

Environmental factors

The main environmental factor responsible for celiac disease is dietary gluten. Despite the fact that all humans are exposed to gluten, only some of them develop celiac disease, which means that there must be another factor that is involved in the mechanism of celiac disease development. Microorganisms effect was investigated (Forsberg et al., 2004; Ou et al., 2009; Wacklin et al., 2014) as being linked to celiac disease. Unbalanced gut microbiota, dysbiosis, increased levels of *Prevotella, Clostridium* and *Actinomyces,* increased prevalence of viruses, including rotavirus, bacteria have been found in celiac disease patient's gut.

Other environmental factors might be the changes in the quantity and quality of ingested gluten, infant feeding patterns, the spectrum of intestinal infections, gut microbiota colonization, etc. (Lionetti et al., 2015). Smoking might be also considered involved in the inflammatory bowel disease and as being responsible for modulation of celiac disease (Snook et al., 1996).

In Sweden, a study indicated that the disease risk was substantially lower in infants introducing small amount of gluten when still breast-fed (Ivarsson et al., 2013). The protective role of breastfeeding was also suggested by Akobeng et al. (2006) and the increased risk of getting celiac disease by introducing gluten in weaned children before 4-month-old or after 6 months (Norris et al., 2005). These data were challenged by Stordal et al. (2013) in an epidemiological study performed in Norway on mother and child cohort study (on 107000 children); they found an increased risk of developing celiac disease in those children consuming gluten after 6 months and higher risk in children breastfed after 12 months of age. Development of tolerance may be facilitated by timely introduction of gluten, but factors involved in loss of tolerance needs further studies. The results are unexpected because they show a low protection of breastfeeding for celiac disease, an increased risk associated to longer breastfeeding period and also the reduced risk of introduction of gluten before the age of 4 years old. Meanwhile, there are also two big national cohort studies that have underlined the influence of antibiotic use in the first year of life and the risk of developing celiac disease.

Two national cohorts (in Norway and Denmark) have highlighted the influence of antibiotics use in the first year of life on risk increasing for developing celiac disease, including a dose-gradient effect (Dydenborg et al., 2019). Bach JF. (2018) and Kondrashova et al. (2008) also found that the excessive hygiene and reduced exposure to different types of microbes in early life could overactive the immune response in later life and could be a factor for increasing the celiac disease risk.

Taken together, the development of coeliac disease requires a complex interplay between

the host, dietary gluten and other environmental factors that is currently far from being fully understood and that future research is necessary for a better understanding of this disease etiology (Lindfors et al., 2019).

Among the problematic disorders related to gluten, about 6% may be non-coeliac gluten sensitivity, 10% may be wheat allergy, and only 1% is celiac disease (Figure 1) (Gasbarrini et al., 2014).

Other related diseases

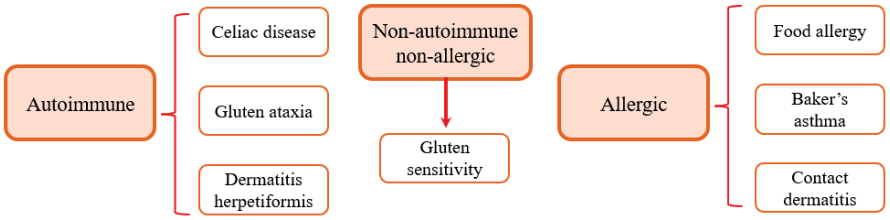

Figure 1. Classification of gluten related disorders

Wheat allergy. The main food allergies are milk, eggs, fish, crustacean shellfish, peanuts, tree nuts, soybeans, and wheat. Almost 5% people may have a true food allergy but only about 0.1% has a documented wheat allergy. Similar to celiac disease, wheat allergy is an immune-mediated reaction to the proteins found in wheat products, but it differs because is an IgE-mediated reaction to the water and salt-insoluble gliadins, particularly ω-5 gliadin.
The symptoms of wheat allergy affect the mouth, nose, eyes, and throat (swelling, itching, and irritation); the skin (rash, hives, swelling); respiratory tract (wheezing, difficulty breathing, anaphylaxis); and gastrointestinal tract (cramps, nausea, emesis, gas, bloating, diarrhoea, and abdominal pain) (Pietzak et al., 2012).

Researches have shown that about 0.3% of children under the age of 5 in Europe are allergic to wheat; 0.1% of all Europeans suffer from this allergy. In people over the age of 18, it can lead to a number of life-threatening factors.

Gluten sensitivity is a condition that imply the improvement of the symptoms when gluten is removed from diet. The patients have neither celiac disease nor wheat allergy, but it is important to eliminate those two diagnostics before labelling a patient as "gluten sensitive."
In Table 1 there is a comparison of symptoms for gluten sensitivity non celiac disease, celiac disease and wheat allergy.

Table 1. Comparison of symptoms for different gluten related diseases

Symptoms	Gluten sensitivity non celiac disease	Celiac disease	Wheat allergy
Gut manifestation	Diarrhea, abdominal pain, bloating, constipation, nausea and vomiting	Diarrhea, abdominal pain, bloating, constipation, nausea and vomiting	Diarrhea, abdominal pain
Other manifestations	Headaches, blurred mind, fatigue, eczema, inflammation of muscle tissue, numbness, mood swings	Anemia, osteoporosis, neurological problems, lymphoma, pubertal delay dermatitis herpetiformis	Rushes, asthma, caught, angioedema, nose flow, eczema
Duration of symptoms	Hours - days	Hours - days	
Intestinal membrane morphology	Intraepithelial lymphocytosis of the small intestine	Atrophy of the intestinal villi	
Diagnosis	Decision tree	Serological testing followed by small bowel biopsy	Allergy test, presence of IgE antibodies to wheat protein
Management	Gluten free diet, probiotic Aspergillus niger prolylendoprotease (AN-PEP).	Gluten free diet	Gluten free diet, subcutaneous epinephrine for acute episodes

Prevalence in the world

It was observed a global increasing of celiac disease incidence: in US, the prevalence of celiac disease raised from 0.2% in 1075 up to 1% in 2000 (Catassi et al., 2010); in Scotland, in 20 years, the incidence of celiac disease raised 6.4 folds in children (White et al., 2013). The reason for this increasing of the reported celiac disease is still unclear, but must be related to the environmental factors.

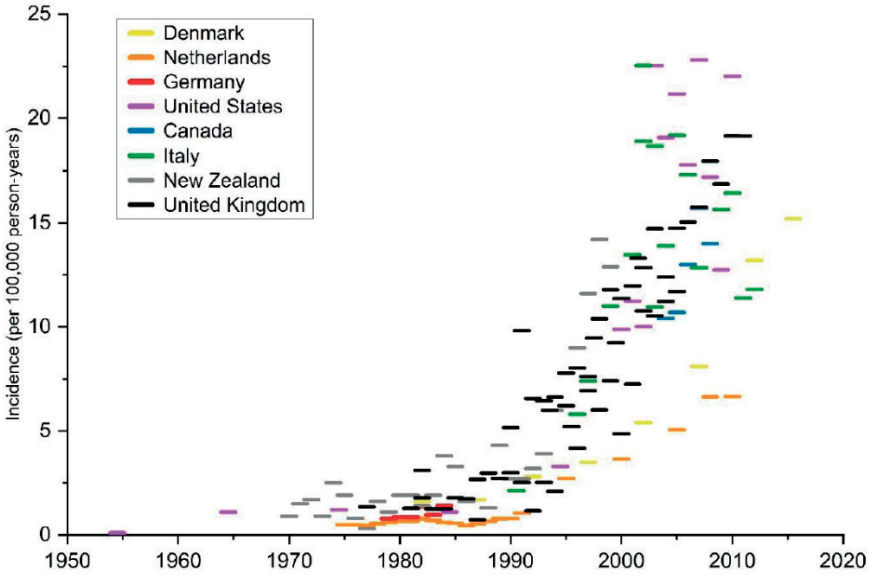

Figure 2. Evolution of the overall incidence of celiac disease in the world (King J.A et al, 2020)

In Africa, the prevalence of celiac disease in children is 5.6%, a tenfold higher than in most European countries; the most exposed are black-eyed, black-haired African population originated from Western Sahara maybe because of the genetic factors considering an increased level of consanguinity in this region and population (Lionetti et al., 2015). The incidence was estimated at 0.8% in Libya, 0.6% in Tunisia and 0.5% in Egypt (Cataldo et al., 2007).

In Middle East, celiac disease incidence is 0.8% in Iran and 0.6% in Turkey (Lionetti et al. 2015). The frequency in India shows a difference between the north and the south part due to wheat-rice shift in diets (Catassi et al., 2014).

King et al. (2020) shown that in the 21st century, the female incidence for celiac disease was 17.4 per 100000 cases compared to 7.8 in males. Children have a bigger prevalence of celiac disease (20.1 in 100000 cases) compared to adults (13.2 in 100000 cases). Diagnosis of celiac disease increased annually up to 8.4%.

Fasano et al (2003) have shown that the incidence of celiac disease is 1 in 33 average healthy people, 1 in 56 with related symptoms, 1 of 22 with first degree relatives, 1 in 236 prevalence for African- Hispanic and Asian-Americans. It was also found that only 35% of newly diagnosed patients had chronic diarrhoea, which contrary the fact that this symptom is mandatory for the celiac disease.

By early diagnosis of the celiac disease the risk of developing certain complication is decreasing. By screening the blood tests of all at risk subjects the diagnosis rate rose 40 times (Catassi et al., 2007).

The average time for a symptomatic person to be diagnosed with celiac disease is four years, which determines an increased risk for developing neurological problems, autoimmune disorders and even cancers (Green et al., 2001).

Gluten free diets: necessity versus fad

Patients dealing with celiac disease must have a lifelong gluten-free diet in order to avoid the harmful peptide from gluten, which basically means avoiding any food product containing wheat, rye, barley. Considering that there are a

wide range of wheat containing foods, it is very difficult to avoid gluten.

Many researches were done in the last years attempting to evaluate how much celiac patients could enjoy a safe, nutritious, tasty and affordable diet. There are some controversial issues that have emerged: the high cost of a gluten free diet, the widespread availability and variety of gluten free products, the possible lower nutritional value of this type of diet and also gluten free as a fad.

The nutritional quality of a gluten free diet is reported to be have higher content of fat, saturated fat, sugar, salt and lower content of proteins, fiber and vitamins. In the blood and urine of celiac patients with gluten free diet, was also observed higher concentrations of heavy metals, especially arsenic and mercury. Overall, a gluten free diet was associated with potential health risks like deficiency of micronutrients, coronary artery disease, hyperlipidaemia and hyperglycaemia (Gorgitano et al., 2019).

Even if the popularity of gluten-free dieting has increased considerably among the general population during recent years, owing to the above-mentioned reasons, the promotion of a gluten-free diet among people without coeliac disease should not be encouraged.

In the context of a continuously increasing market of gluten free products, patients living with celiac disease experience the so called "double-edged sword" symptom. They are grateful for more palatable and available gluten free products, but they are also experimenting the misunderstandings about the severity of the illness as a result of many noncoeliac consumers having gluten free diets (King et al., 2018).

Under EU legislation (Commission Regulation (EU) No. 828/2014), food operators are only allowed to use the following claims regarding the absence or reduced presence of gluten in food: 'gluten-free' and 'very low gluten'; this is providing they meet the relevant compositional requirements (Table 2).

Under certain conditions these claims may be voluntarily accompanied by specified statements (Table 3).

Table 2. Specifications and criteria for gluten-free and very low gluten claims

Claim	Specification	Additional criteria
"Gluten free"	No more than 20mg/kg of gluten as sold to the final consumer	
"Very low gluten"	No more than 20mg/kg of gluten as sold to the final consumer	Must consist or contain one more ingredient made from wheat, rye, barley, oats or their crossbred varieties which have been specially processed to reduce gluten content

Table 3. Voluntarily statements and criteria for gluten free products labelling

Optional statements	Specifications	Additional criteria
"Suitable for people intolerant to gluten"	Used only when accompanying a "gluten free" or "very low gluten" claim	
"Suitable for coeliacs"		
"Specially formulated for people intolerant to gluten"	Used only when accompanying a "gluten free" or "very low gluten" claim	The food is specially produced, prepared and processed to: a) reduce the gluten content of one or more gluten containing ingredients; b) substitute the gluten-containing ingredients with other ingredients naturally free of gluten.
"Specially formulated for coeliacs"		

The most universally acknowledged symbol associated with gluten-free products is a crossed ear of wheat (Grabowicz et al, 2019). It is a common practice for gluten-free foods producers in Europe to label their products with this symbol (Figure 3).

Figure 3. Gluten free symbol for package labelling

Gluten free products consumption in Western societies has become very popular among the consumers not only those with different health conditions that need free gluten diet, but also those oriented to healthy eating and living.
In Figure 4 it can be observed that the number of Americans on a gluten free diet without celiac disease increased from 44% in 2009-2010 up to 72% in 2013-2014.
It can also be observed that the undiagnosed celiac disease decreased from 51% in 2009-2010 to 12% in 2013-2014. The level of gluten free diet among Americans increased from 1.3 to 2.4 in 4 years taken as reference.

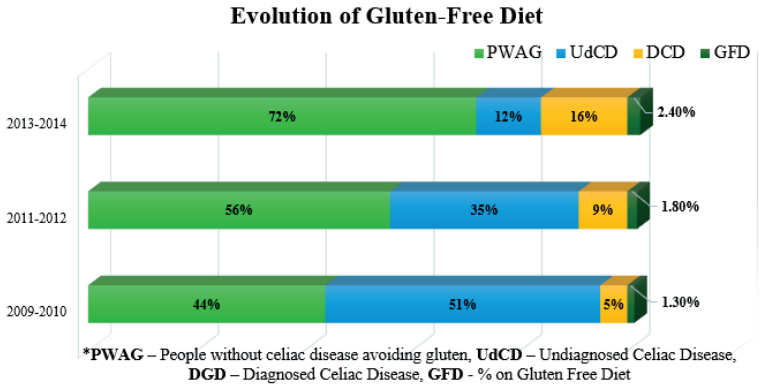

Figure 4. The rise of the gluten free diet (statista.com)

Gluten free foods consumption for non-celiac disease patients had increased among general population (Silvester et al., 2015). This might be due a behavioural cause by perceiving these products as being more healthy and more appropriate for a balanced way of living and eating (Faulkner et al., 2014). This is also known as "halo effect" which states that as a perception level, the products labelled as healthier, low-fat, low carbo, vegan can mislead the consumers about the total nutritional value of food.
57% of consumers eating gluten free foods are doing this because of medical reasons (Dunn et al., 2014), while the rest of 43% are preferring this type of products as part of a special lifestyle and positive image of the products.

The US Department of Agriculture stated that the gluten-free industries revenues reached $1.7 Billion by 2010. In line with this trend, the gluten free product market size was in 2020 up to 5.6 billion USD, from which 1.77 billion representing only US gluten free products market (https://www.statista.com).
There are more than 2,000 gluten free food items available in the United States, and consumers are more likely to find these foods in regular grocery stores. From 2004-2005, sales of gluten-free foods increased by 77.8 million dollars (a growth of 14.6%).

CONCLUSIONS

Celiac disease become a public health issue, with an increased prevalence worldwide in the last years. It still remains significantly underdiagnosed, especially in developing countries but important changes have been made in developing the decisional tree for diagnosis and differentiation among other gluten related

disease like non-celiac gluten sensitivity, wheat allergy, irritable bowel syndrome.

Non-celiac gluten sensitivity is a clinical feature under investigation: it is probably a heterogeneous group that overlaps with irritable bowel syndrome and celiac disease. More research is needed to establish the risk factors, the biomarkers, the definitive diagnostic criteria before patients can really benefit from results.

Continuous gluten ingestion is the major cause of persistent celiac disease, due to its influence on gut membrane healthy and functioning. This may determine serios health threat because of neurological problems, anaemia and osteoporosis that might occur due to intestinal absorption problems.

There is no treatment for celiac disease, but adopting a gluten free diet for lifelong for celiac disease patients.

Multiple potential new therapies are being studied at it can lead to paradigm shifts in the way gluten intolerance, celiac disease and wheat allergy are managed.

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STUDIES REGARDING MARKET TRENDS GLUTEN-FREE ORGANIC PRODUCTS

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Abstract

A gluten-free lifestyle has become one of the most popular diet trends. For this reason, consumers, food manufactory and healthcare professionals are being influenced by the growing popularity of the gluten-free diet. Next-generation food manufacturers have understood the scale of the problems related to gluten intolerance and especially the potential of the market for organic gluten-free products. This translates into creating a new market with potential, that of green gluten-free products. Interest in this type of food is constantly growing, due to its health benefits, nutritional and sensory characteristics, as well as the demand for quality food produced in a sustainable way. The paper presents the impact that the new trend has on the bakery industry and on the health of the final consumer.

Key words*: bakery, food, gluten free.*

INTRODUCTION

Celiac disease is known as an autoimmune condition and inflammatory disease triggered by the presence of gluten in the upper small intestine (Jingwen et al., 2020), which untreated or incorrectly treated can have important health consequences such as osteoporosis, infertility, repeated miscarriages, anemia through iron deficiency and certain forms of cancer. Symptoms in people with celiac disease include malnutrition, diarrhea, stunted growth, anemia, and fatigue (Jingwen et al., 2020). Food allergy is a costly, potentially life-threatening condition (Gupta et al., 2019) if a gluten-free diet and healthy lifestyle are not strictly followed. According to the non-profit organization Beyond Celiac, 1 in 133 people are affected by celiac disease, 83% of patients being undiagnosed or misdiagnosed (Bolst Global.org). On the opposite end of the spectrum are those who suffer from intolerance to gluten. In this case, symptoms similar to celiac disease are observed, but without the autoimmune damage to the small intestine.

The reported prevalence of celiac disease worldwide is continuously increasing, partly attributed to the development of clinical test detection techniques and improved diagnostic capacity, mainly in Australia, Europe and North America (Singh et al., 2018; Lebwohl et al., 2015). A meta-analysis found that the global incidence of celiac disease is increasing "significantly". Singh et al., 2018 noted that "a real increase in the incidence of celiac disease is occurring beyond diagnostic improvements, most likely due to environmental factors". It also found that the global prevalence of celiac disease would be 1.4% (Singh et al., 2018). Celiac disease is a condition that affects people of all races, sexes and ages.

Unlike people who have other autoimmune diseases, such as type 1 diabetes, rheumatoid arthritis or multiple sclerosis, patients with celiac disease are lucky enough to know what caused their body to react against itself: *gluten*.

There are no medical or surgical treatments for celiac disease; it can only be controlled by a strict, lifelong gluten-free diet.

The food industry is evolving at a fast pace, driven by a constant change in eating habits and consumer preferences. In recent years, there has been a growing trend in demand and consumption for gluten-free products. This is due to the increase in the number of people with metabolic problems, the awareness of the various benefits brought to health, so that in the panoply of customers we find gluten-intolerant consumers and gluten-tolerant consumers who adopt a healthy lifestyle.

Gluten-free products have a wide range of advantages: they help prevent and treat health problems; they are extremely useful for people suffering from diabetes, autoimmune problems and digestive disorders. Moreover, these foods can be low in fat and are therefore recommended for people who want to lose weight or reduce their fat intake. Food is essential to personal health, as well as to the health of the planet given that current patterns of food production and consumption have considerable environmental impacts (Rockström et al., 2009). Conversely, disasters such as the COVID-19 pandemic can disrupt our food system (Galanakis CM., 2020) and change our relationship with food (Janssen et al., 2021).

MATERIALS AND METHODS

On the market of gluten-free products, several consumption trends were identified that determine an upward evolution of the consumption of gluten-free products. Market trends are present, organic gluten-free products are presented in the following: (a) food as "Medicine"; (b) satisfaction through food choices; (c) increased intolerance or consumption tendency; (d) choosing plant-based foods; (e) methods of obtaining gluten-free products.

RESULTS AND DISCUSSIONS

Food as "Medicine"

In recent years, thanks to the sanitary measures imposed on the population and social distancing, the average consumer has turned his attention to products obtained from natural ingredients, without the addition of additives, healthy products. Health has the first place in consumers' preferences, and thus the foods consumed are vectors through which nutritious and beneficial substances are brought into the body. We know food is important in the prevention and during the treatment of some diseases, and for this reason it is important to pay attention to the products consumed (Lerner et al., 2021).

Consumer preference for natural and organic foods is continuously increasing due to increasing health awareness among consumers. In addition, increasing disposable income, improved living standards, increased health spending and widespread promotion of organic foods due to their benefits such as their chemical-free nature complement the adoption of products without artificial ingredients.

Consumer interest in and concern about gut health, the benefits of dietary fiber consumption, protein, immunity boosters and anti-inflammatory ingredients are spearheading what's next in dietary preferences.

Healthy products that are easily accepted at the moment, and that are needed, are "sugar-free", "low-fat", "cholesterol-free" products, allergen-free products, plant-based alternatives to dairy products, meat products and so on. These products are more expensive compared to conventional products and have a shorter shelf life.

In the case of food allergies and related diseases, the only effective treatment may be to control what we eat. Dietary and nutritional changes can have an immediate and profound impact on the health and well-being of people with food allergies. And just as important in our society, these people should be able to eat and enjoy meals with their families and eat healthy during social events. Our culture is one that is based on food and social events. Eating and enjoying our food is a choice, but it's also a way of life.

Satisfaction through food choices

These consumers find satisfaction through consistent, health-conscious eating choices. Clean-label solutions, plant-based protein, gluten-free options and healthy swaps form the foundation of this trend.

When it comes to specific ingredients, pseudocereals and plant seeds are critical. Their versatility allows them to naturally fit across a variety of applications and dishes, forward clean-label attributes. Organic, whole grains, pulses and specialty blends that support diets like gluten-free, paleo and more are also popular.

Finding ways to boost the immune system is top of mind for most families these days. People want food and drinks containing vitamins and minerals that will not only help them stay healthy but also prevent any future illnesses.

As people prioritize convenience and spending time with their families and as evidence increase singly cautions against a gluten-free diet for those who don't need it, food retail stores may

start to make a shift in product demand and to inform more.

The results of the studies make by *Mengesha* in 2021, showed that perception is significantly affected when the information concerns safety and nutritional characteristics, whereas the opposite occurs regarding the product flavour. Furthermore, findings revealed that, while the willingness to buy increases after providing positive information, the willingness to try does not. Indeed, willingness to try depends upon further stimuli other than information, suggesting a deeper analysis of the food profile, and the values underlying it, of the population of interest.

Increased intolerance or consumption tendency

Consumers' habits have changed considerably in recent years, many consumers are looking for a healthier, nutritious diet, beneficial to the body, but only these elements count in the continuous growth of the segment of gluten-free products, or are there also gluten-free products. What we can say with certainty is that there has been a considerable increase in the number of diagnoses and the general awareness of consumers suffering from gluten intolerance, but there is no very precise demarcation between those who fall into a spectrum. Many people follow a gluten-free diet as part of a healthy lifestyle.

Allergen-free products know an upward trend, valid worldwide, in the current period. The increase in the number of people with intolerance or allergies has determined a caution on the part of consumers in choosing products. Gluten-free products remain most popular in Europe, North America and Canada, while the dairy-free category is led by North America. In the East, demand for gluten-free, trans-free and caffeine-free products is growing in Southeast Asia (www.pewresearch.org).

Nowadays, a gluten-free lifestyle has become one of the most popular diet trends. One in five people reduce or eliminate gluten, a protein found in wheat, barley or rye, from their diet, according to a 2015 Gallup poll. Avoiding gluten is essential for people with celiac disease. Rothburn et al. (2022) assess the differences between gluten free and gluten containing products with regards to the implications this might have on oral (and wider) health, emphasises the 'health halo' phenomena, where foods for special diets are regarded by consumers as healthy when in fact they are not. Gluten-free manufacturers are using a 'health halo'; promoting foods using emotive words to market their products as 'good for you', despite high sugar and energy contents.

A lot of the individuals who choose a gluten-free diet do so for a number of reasons, these include: reducing inflammation and bloating; the belief that gluten-free products are healthier; seeking less artificial, and more natural, ingredients; perceived weight loss purposes; to improve sportive performance; improving skin conditions and for stabilising your mood, especially with ADHD.

This shows that choose over gluten free foods is down to the perceived benefits of it. There are certainly some real benefits to consuming a gluten-free diet, but there are also disadvantages too.

Either way, the gluten-free trend isn’t stopping any time soon, which makes it a great trend to harness.

Choosing plant-based foods

Consumers prefer to choose foods that are healthier and easier to digest, and this can be seen through an increase in flexitarians (those who prefer plant-based meals with the occasional inclusion of meat) and a greater demand for natural, organic, simple and less processed in both developed and developing markets.

The new generation of consumers prefers to choose healthy foods, with a clean label, obtained from basic ingredients. While members of previous generations might have defined "healthy" as low in fat or high in fiber, millennials might define health from a healthier perspective. They want products that are natural, organic and sustainable; and is also interested in how their food was sourced or grown, and sustainability is a priority when buying food. So of particular interest here are the "millennial food sophisticates" who are looking for foods that are minimally processed and more natural or organic. Millennials are constantly getting new information about food from social networks and friends, because they rely heavily on websites, bloggers, and social media fitness professionals for health information. Anytime they try a snack based solely on an online rating,

review, or social media post. Perhaps this has led to an increase in the number of products labelled as vegetarian by 25% and vegan by 257% (A Global Middle Class Is More Promise than Reality | Pew Research Center; FReSH).

Plant-based food sales have surged in the past years and will only increase as products improve. And vegans and vegetarians aren't the only ones propelling the plant-based food trend. Not too long ago, the vast majority of non-meat alternatives had fillers made from wheat. Now, these companies are looking at going gluten-free because a lot of people who live gluten-free also end up going dairy-free.

This is because consumers who are recently diagnosed with celiac disease have villi that have been damaged in their small intestine, and that makes it more difficult to digest often-inflammatory food groups such as dairy as they try to heal.

Methods of obtaining gluten-free products

Conventional alternative flours are used for two different reasons: first, to reduce or eliminate the use of wheat for economic reasons in underdeveloped regions or countries, and second, to modify the nutritional characteristics of a product by enriching it with proteins, vitamins or minerals, especially for celiac disease patients.

Consumers appear to be wary of accepting new food technologies due to perceived risks and lack of benefits. In general, the "information deficit" approach has not overcome aversion to new technologies applied to food concepts (Cox et al., 2007).

The formulation of gluten-free bakery products is still a challenge for baking technologies.

Gluten is an essential structure-building protein, contributing to the appearance, crumb structure, and consumer acceptability of many baked goods. Therefore, the biggest challenge for food scientists and bakers in the field of gluten-free products is probably the production of high-quality gluten-free bread (Arendt et al., 2008). According to a report by Arendt et al. (2011) it is mentioned that most gluten-free products are of inferior quality and very often, they are crumbly and very dry.

Replacing gluten functionality has been a challenge for food technology. The absence of gluten leads to poor cohesion and elastic dough, which results in a crumbly texture, poor color and low specific volume in bread. Therefore, in recent years, numerous studies have been attempted to improve the physical properties of gluten-free foods, especially baked and fermented foods, by using the interaction of numerous ingredients and additives that could mimic the properties of gluten (Šmídová et al., 2022; Houben et al., 2012).

Ingredients proposed for obtaining gluten free baked foods include the utilization of different naturally gluten-free flours (amaranth, almond, rice, maize, sorghum, soy, buckwheat) and starches (maize, potato, cassava, rice), dairy ingredients (caseinate, skim milk powder, dry milk, whey), gums and hydrocolloids (guar gums, xanthan gums, alginate, agar, carrageenan, hydroxypropyl methylcellulose, carboxymethyl cellulose), emulsifiers (DATEM, SSL, lecithins), non-gluten proteins from milk, eggs, legumes and pulses, enzymes (cyclodextrin glycosyltranferases, transglutaminase, proteases, glucose oxidase, laccase), and non-starch polysaccharides (inulin, galactooligosaccharides) etc. (Šmídová et al., 2022). Generally, the combinations of ingredients and the optimization of the breadmaking process have resolved the technological problems, yielding gluten free products that met the consumer's expectations concerning texture and appearance of the fresh bread (Matos et al., 2015). Baked products produced using the allergen-free flours thus have a moistness, springiness, rise, texture, and flavor comparable to products containing major food allergens. Baked products according to the disclosure include, but are not limited to, cookies (e.g., chocolate chunk/chip cookies), cakes (e.g., cupcakes), muffins, pancakes, waffles, pizza crust, pie crust, and bread products.

The flours, whole flours, bran products, proteins of vegetables, oilseeds, and other minor edible seeds can be used effectively for nutritional improvement of bakery products. Attempts have been made to enrich bakery products with nutritionally-rich ingredients for their diversification (Peres et al., 2011; Onyango et al., 2011; Rai et al., 2016; Ziobro et al., 2016; Shevkani et al., 2014; Dragomir et al., 2019).

An important aspect of the market is the correct education of the population regarding foods that do not contain gluten. These foods have

physical-chemical and technological characteristics different from those of conventional products that you must take into account when purchasing these products.

Consumer trust in food suppliers is a feature that plays an important role in the modern food marketing system. Consumers' food choices are increasingly influenced by cues of trust, as other levers of trust and direct relationships with producers have disappeared within the globalized food system (www.sgsgroup.ro). Labels and information signalling are thus important search attributes. While trust cues cannot be accurately assessed by consumers, the expectations they generate have an effect on consumer perceived quality and sensory experiences (Fernqvist et al., 2014).

The factors that influence the evolution of trends in the market of gluten free products Are presented in following. An aspect that must be taken into account is the concern for nature, so several concepts have appeared that must be known and taken into account when developing a new product. These concepts are the foundation stone for the following trends that will dictate the gluten free food market and beyond.

Food's carbon footprint, or footprint

Is the greenhouse gas emissions produced by growing, rearing, farming, processing, transporting, storing, cooking and disposing of the food you eat. The unit of measure used is carbon dioxide equivalents (CO2e) emitted through the food supply chain per year. Some organic companies are already able to claim net zero (carbon) (University of Michigan, 2021).

Eco-convenient gluten free food

The lure of convenience remains strong for many consumers. Some green brands recognize the opportunity of eco-convenience.

Convenience is a major driver of food consumption in cities, as time is a limited resource. As people become more urbanized, their food selection is increasingly driven by what is quick, available and ready to eat. Therefore, highly convenient digital shopping plays an important role, as it makes food accessible anywhere and anytime. Overall, digital platforms have the potential to change consumption behaviors by increasingly providing consumers with consistent and high-quality food, consumption data and speed of delivery. Also, interest in products with ecological or gluten-free characteristics and certifications has increased in recent decades (Ricci et al., 2018).

Fueled by increasingly capable mobile technologies, consumer choice is also heavily influenced by social media. While technologies enable increased transparency and affordability of food in urbanized areas, the rise of low-price discounters and price transparency can enable access to food in low-income neighbourhoods and communities.

Wholefood campaign it

Is a reaction to worries over the growing prevalence of ultra-processed foods has promoted a discussion about food integrity, and the complex nutrient matrix of whole foods. For people with gluten intolerance, the danger of consuming ultra-processed foods in excess is extremely high, especially the limited number of ingredients and production costs.

Fair food

The fair trade movement has achieved huge progress in advancing the cause of sustainable and equitable trade. 'Fair' is also one of the four key principles of organic, so expect to see the concept of 'fairness' taking a more central role in organic messaging. The climate crisis is shining a light on the issue of food justice, and demand for fair food is growing quickly.

Traditional food and social eating

In times of uncertainty, consumers gravitate to both familiarity and comfort. Consumers are not only demanding nostalgic foods but also infusing craftsmanship and artisanal approaches into their diets to redefine "comfort."

Sustainable solutions - personal and planetary health is evolving

Consumers want to both eat well and do good - seeking products that nourish their bodies and also actively contribute to a better food system for all. The perfect crossover is ingredients that push toward better well-being and "well-doing" spanning everything from plant-forward initiatives to regenerative agriculture.

Clean label

The level of consumer awareness has increased regarding the clean label on products used daily. This is a key factor in the global food market. Beyond clean label and removing unwanted ingredients, food formulators face the complexity of meeting consumer demand for

ingredients that are perceived as natural. Many brands now use extracts and fermentation products for flavoring. In addition to being natural, the use of these products also offers advantages such as full traceability and transparency of the supply chain. Consumers demand transparency from food producers, being increasingly interested in knowing how a product's ingredients were produced, processed and transported, as well as how natural resources are renewed.

Label transparency is becoming more and more important, and more and more buyers are looking for a certain type of certification. And having a gluten-free certification can help smaller companies stand out when they are looking for more regional and national distribution channels.

Crossed Grain, the European standard for gluten-free product licensing has exceptional recognition and trust among people with celiac disease and gluten intolerance. It is a complex certification that checks and audits all stages of the production, packaging and distribution of gluten-free food, and imposes one of the most rigorous standards in the food industry (www.sgsgroup.ro). Crossed Grain certified products are strictly audited and analysed to demonstrate that they have a maximum gluten content of 20 mg/kg or parts per million (ppm). Laboratory analyses and certification audits look at ingredients as well as production processes, storage, packaging and distribution processes (Reg EU 828/2014).

Gluten-Free diet: The Downsides

While the gluten-free diet "is lifesaving for those with celiac," for those without medical indications, a gluten-free diet is a very healthy diet, because it's low in fiber and often enriched in fat and calories.

It also puts people at risk of nutritional deficiencies, because wheat flour is of then fortified with folic acid, B vitamins, and iron. Rice flour, a mainstay of gluten-free foods, is not.

A danger is represented by parents who provide their children with a gluten-free diet without a medical reason. If the child does not have celiac disease or an allergy (to wheat), there is no reason to follow a gluten-free diet". A gluten-free diet without a medical necessity does not lead to a healthy lifestyle, on the contrary it would Such a dietary pattern could increase fat and calorie intake, contributing to nutritional deficiencies, especially micronutrients, and sometimes mask celiac symptoms, which could delay proper diagnosis of the condition.

Likewise, "prophylactic" elimination of gluten from the diet of first-degree relatives of people with gluten sensitivity has not been shown to be effective in healthy individuals. The consumption of free from dairy and free from gluten foods is not only limited to nutritional needs but is also gaining importance, due to issues related to intolerance or allergies. Gluten-free food helps to reduce gluten content from the body.

CONCLUSIONS

Our consumer behavior has become much more sophisticated, and the factors that influence consumption trends for gluten free products have become extremely diverse.

The guidelines drawn by the EU policies regarding the increase in the consumption of organic products, responsible consumption, sustainability and ethics in the food chain are already adopted by the average consumer. The increase in disposable income, the improvement in the standard of living, the increase in health expenses and the widespread promotion of organic food, due to its benefits, has determined that the consumer's preference for natural and organic gluten free food is constantly growing. This is also due to the increase in awareness among consumers of maintaining the health of the body through food. Online shopping has taken this aspect to another level. A strong evolutionary trend can be observed in terms of sustainable, equitable and collaborative consumption - involving all actors in the food chain from "fork to fork".

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RESEARCH ON OBTAINING ORGANIC GLUTEN-FREE COOKIES WITH AMARANTH FLOUR AND PUMPKIN PULP

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Abstract

Pumpkin pulp (Curcubita maxima), from organic farming, is an extremely versatile product that can be a basic ingredient in obtaining gluten-free cookies. In this paper, a cookie was obtained using amaranth flour and pumpkin pulp in two forms: fresh and baked. Following the analyses, the aim was to obtain a product with appropriate characteristics. Dough with amaranth flour and pumpkin pulp is characterized by a good ability to bind the dough and retains its shape when pouring. In the sensory analysis of the gluten-free cookies with organic baked pumpkin obtained in the product testing phase, the taste was better appreciated. From the analysis of the average values it is observed that the products obtained with the basic ingredient prepared in advance (baked pumpkin) showed a more intense perception on the attributes of smell, taste and aftertaste.

Key words*: amaranth, cookie, gluten-free, pumpkin pulp*

INTRODUCTION

The incidence of metabolic diseases among the active population has an upward trend, which requires the diversification of the assortment range of organic gluten-free products. The development of gluten-free products with organic pumpkin pulp meets the requirements of the consumer segment with gluten intolerance.

Proteins improve the nutritional value of gluten-free products. The choice of flour and possibly another source of protein affects the rheological properties of the dough and the water binding in the dough. Proteins interact with starch and lipids and together contribute to the stability of the dough and the structure of the product. They also give the impression of full product flavour. Proteins can be of plant origin (legumes, soya, gluten-free cereals, rapeseed, canola, sunflower, potato), animal origin (whey, egg, casein, caseinate) or microorganism-, algae-, seaweed- and insect-based (Skendi et al., 2021; Dragomir et al., 2019).

Pseudocereals, often used as substitutes for wheat, have a nutritional value that is closely related to their protein content and quality, and usually higher compared to cereal grains. This is especially the case with amaranth, which has the highest protein content, 13.1–21.0%, among pseudocereals (Alvarez-Jubete et al., 2010; Bhat et al., 2015; Venskutonis & Kraujalis, 2013).

Amaranth's name comes from the Greek for "immortal" or "everlasting." Grain amaranth species include *Amaranthus caudatus, A. cruentus* and *A. hypochondriacus*.

Considered a pseudocereal unrelated to wheat or other true grains, amaranth delivers high-quality protein -- roughly 13.1–21.0% by weight and contains plenty of other healthful nutrients. Amaranth flour is obtained from amaranth seeds (*Amaranthus*) with a protein content almost 2 times higher than wheat (up to 19%). High levels of the amino acid lysine help your body to properly absorb calcium from the

digestive tract. It is a naturally gluten-free flour, versatile and with a moreish earthy flavor.

Generally, the protein content depends on variety and environmental conditions. In a study by Tömösközi et al. (2009) eight samples of *A. hypochondriacus* and *A. caudatus* lines, grown in Hungary and Austria, showed a range of protein content of between 14.23% and 17.40%. Amaranth proteins consist of about 40% albumins, 20% globulins, 25%–30% glutelins, and only 2%–3% prolamins (Venskutonis & Kraujalis, 2013). A lower proportion of 0.48%–0.79% prolamins was found by Muchova et al. (2000). For some amino acids the overview showed considerable variations, especially for lysine (3.3–9.2 mg/g protein) and leucine (3.6–7.9 mg/g protein).

Pumpkin (*Cucurbita maxima*) is an ingredient used in Romanian gastronomy, only in certain periods of the year, respectively in the winter season, and for this reason we propose the integration in products with added value and functional role. Organic pumpkin can be profitably used in a variety of value-added foods. Pumpkin is rich in β-carotene, carbohydrates, dietary fiber, vitamins (A, C, B1, B2 etc.), potassium, calcium, magnesium and iron, etc., with special sensory properties agreed by the Romanian consumer, with a low caloric content.

Pumpkin pulp has very good stability over time, in terms of color, influencing the color and favoring of the finished product.

MATERIALS AND METHODS

The objective of this study was the production of innovative functional cookies enriched with ecological pumpkin pulp and monitoring the impact of these enrichments on the nutritional, physicochemical and consumer acceptance.

Methods of sample preparation and physicochemical analyses it was carried out at the laboratories of Research Center for Studies of Food Quality, University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania.

Ingredients

The experimental variants of gluten-free cookies were developed from the amaranth flour (AF) in mixture with ecological pumpkin pulp (*Curcubita maxima*): raw and baked.

The physicochemical characteristics and sensorial properties of the cookies were evaluated in a pursuit to identify an innovative pastry ingredient, easy to find, accessible; with high nutritional value and potential function that could be exploited by the food industry.

Pumpkin is a local raw material and a more economically advantageous ingredient.

The utilization it in production will allow ensuring the food safety of consumers suffering from celiac disease, the use of cheap and accessible local raw materials, the reduction of the cost of gluten-free snacks, and the widening of the assortment of gluten-free pastries.

Konjac flour - glucomannan is a beneficial, soluble and fermentable dietary fiber, gluten-free, derived from the rhizomes of the *Amorphophalli Konjac* plant.

Konjac Glucomannan bio is an essential prebiotic for health that increases the feeling of satiety.

Preparation of product

Organic gluten-free cookies were developed in the Bakery Pilot Station within the Faculty of Animal Productions Engineering and Management -USAMV Bucharest, recipes and technologies for the manufacture of gluten-free cookies to which was added in the recipe raw pumpkin pulp and baked pumpkin pulp (Table 1).

Table 1 Codes used for samples analysis

Code	Sample
A0	Organic cookie with amaranth flour
A1	Organic cookie with amaranth flour and raw pumpkin pulp
A2	Organic cookie with amaranth flour baked pumpkin pulp

Sensory Evaluation

A first sensory analysis was carried out using a semi-structured hedonic scale to evaluate the acceptability of the cakes made with the selected percentage of substitution. A total of 30 potential consumers evaluated biscuit color, texture, taste and overall acceptability according to a box-scale (1 - 5) anchored in the following steps: “dislike very much”, “indifferent” and “like very much”. Samples

were presented to the evaluators randomly arranged and coded with three digit numbers.
Once the formula it was optimised, a sensory analysis was carried out where the panelists were asked to evaluate color, texture, taste, and overall acceptability of control and the selected amaranth flour and pumpkin pulp (fresh or baked) cookie formulations.

Elemental analysis C, H, N and determination of protein content

The analysis of the total N, H, C content was carried out using the Dumas method, using the Elemental Analyzer EA 3000. This method involves the total combustion of the sample in atmosphere of oxygen. The gases produced are reduced with copper to H_2O, N_2, CO_2 and SO_2 and quantified using a universal detector. From the ground sample, was weighed of 2–3 mg, enter in a tin crucible and subjected to combustion at 950°C. The amount of protein was calculated by multiplying the total nitrogen content by a factor of 6.25.

Total polyphenol content (TPC)

Depending on the type of sample, 1 g of material is mortared in the presence of quartz sand and 10 ml of 70% methanolic solution in water. The extracted sample is left overnight in the dark at room temperature. The next day, shake for 60 minutes to favor the extraction, centrifuge for 5 minutes at 5000 rpm and 4°C, after this transfer the supernatant to another bottle. Over the remaining sediment, add another 10 ml of 70% methanolic solution, shake and centrifuge. Repeat procedure. Finally, the 3 supernatants are combined, the final volume of the extract being 30 ml.
For the quantitative determination of the total content of polyphenols, the Folin-Ciocâlteu method following a protocol adapted from George et al. (2005).

Antioxidant activity

The antioxidant activity of the samples is determined based on the DPPH test, using the stable free radical 2,2-diphenyl-1-picrylhydrazyl – DPPH, after the method described by (Bujor et al., 2016).
To determine the antioxidant activity, a volume of 200 µl of the extract solution obtained for polyphenols is used, and 2 ml of DPPH solution (0.2 M) in methanol is added. Stir magnetically in the dark for 30 minutes.
After incubation, the absorbance is measured at a wavelength of 515 nm.

Determination of the dry matter (DM)

Determination of the dry matter (DM) was achieved by weighing 1 g of the sample, and then dries at 105°C, in an oven until a constant weight.
The determination of the dry matter content (DM) was carried out by the gravimetric method, through the removal of water by evaporation and weighing, according to the European Pharmacopoeia, edition 7, the results being expressed in percentages.

Determination of the content of carotenoid pigments by UV-VIS

The carotenoids pigments content was quantified after petroleum ether extraction method. In a mortar with pestle, 1 g of the sample was mortared in the presence of quartz sand, and washed quantitatively several times with petroleum ether. The ethereal extract was vacuum filtered and transferred quantitatively into a 50 ml volumetric flask.
It was dosed spectrophotometrically against a petroleum ether blank at wavelengths 452 and 472 nm, using the Specord 210 Plus UV/VIS spectrophotometer.
Results were calculated according to the formulas proposed by Rodriguez-Amaya et al., (2001) and Pelissarii et al. (2016).
The results obtained and presented are the average of three independent values and are expressed as mean ± standard deviation (SD).
Statistical analysis of obtained data was performed using Microsoft Excel for standard deviation; represent the average of three replicates with independent sample preparation.

RESULTS AND DISCUSSIONS

The objective of this study was the production of innovative functional cookies enriched with organic pumpkin pulp and monitoring the impact of these enrichments on the nutritional, physicochemical, texture characteristics and consumer acceptance. The gluten-free cookies

were developed from the amaranth flour (AF) in mixture with organic pumpkin pulp.
The experimental variants (gluten-free cookies) were obtained by different methods of preparing pumpkin pulp: raw or baked.
The physicochemical characteristics and sensorial properties of the cookies were evaluated in a pursuit to identify an innovative pastry ingredient, easy to find, accessible; with high nutritional value and potential function that could be exploited by the food industry.
Organic cookies with amaranth flour and pulp pumpkin is was developed in the Bakery Pilot Station within the Faculty of Animal Productions Engineering and Management - USAMV Bucharest.
Obtaining gluten-free pastries is a major technological challenge, as gluten is essential in strengthening the structure of products, being essential in obtaining high quality pastries. As a result, in the first stage, tests were performed on the influence of amaranth flour on a fat-based cookie dough, in which konjac flour was used as a thickener and emulsifier to improve the texture of the dough and to maintain stability in time.

Preparation of product

It was obtained 3 samples of each product from amaranth flour, respectively a control sample that lacks pumpkin, a sample with the addition of freshly grated pumpkin and a sample with the addition of baked pumpkin in the form of paste (Tabel 2).

Table 2 Recipe used for samples analysis

Code	Sample
A0	62% amaranth flour, 16% butter, 10% unrefined brown sugar, 8% egg, 2% baking powder, 2% konjac flour.
A1	31% amaranth flour, 31% pumpkin pulp fresh, 16% butter, 10% unrefined brown sugar, 8% egg, 2% baking powder, 2% konjac flour.
A2	31% amaranth flour, 31% pumpkin pulp baked, 16% butter, 10% unrefined brown sugar, 8% egg, 2% baking powder, 2% konjac flour.

All products were purchased from retail specialty stores with organic products, except for organic pumpkin, which was purchased from the ecological farms. Organic pumpkin pulp was obtained in the local organic farming. The product was obtained in the Bakery Pilot Station of the Faculty of Animal Productions Engineering and Management, from USAMV Bucharest, within the ECODONELA project support.

Amaranth flour is a great option in baking and works well with both sweet and salty flavors. It has an intense nutty taste, a complex and very dense aroma, difficult to work with. It is suitable for mixtures containing brown sugar or maple syrup. Due to their distinctive taste, crumbly texture and sandy feel, they are used sparingly.
Dough made from AF is characterized by a good ability to bind the dough and retains its shape when pouring. In the samples to be analyzed, where fresh or baked pumpkin is added, a higher humidity of the dough is observed due to the addition of pumpkin.
For the elaboration of the cookie recipes we started from the premise of valuing the pumpkin pulp in new assortments of cookies. The pumpkin was purchased in the harvest season (October) and the pulp was prepared as follows:

- Raw - grated pumpkin stern forming small particles;
- Heat treated - the pulp was cut into cubes and heat treated at 200°C/40 minutes in the convection oven and then mashed.

Repeated tests have shown that the high humidity of the raw or cooked pumpkin pulp requires the addition of substances that bind excess water and form a homogeneous mixture. Konjac flour –glucomannan - was used for this purpose.
Pumpkin pulp was not used in the preparation of the control sample.

The technological process of obtaining the recipe

The technological process of obtaining fat-based cookies gluten-free was obtained as follows: butter and sugar are mixed until a creamy consistency is obtained, the egg and other dry ingredients are added, and finally the pumpkin pulp is added by mixing at low speed, so as not to lose volume. The dough obtained is left to rest at 1-4℃ for 30 minutes. The dough is modelling. Bake the cookies at 180°C/20-25 minutes.
Organic pumpkin pulp, used in the study, is: fresh grated pumpkin pulp and baked pumpkin pulp.
All samples had the same technological regime.
The analysis of the behavior of the dough during preparation revealed that the dough

obtained from amaranth flour - is characterized by a good ability to bind the dough and retains its shape when pouring. In the samples to be analysed, where fresh or baked pumpkin is added, a higher humidity of the dough is observed due to the addition of pumpkin.

Sensory characteristics:

Cookies with AF stand out for their high palatability, thanks to the final earthy note, combined with the sweet taste of pumpkin pulp. The pieces are light brown, crispy on the outside and dense on the inside, with a slight tingling sensation at the end. The brown color of the cookies is due to the use of brown sugar and pumpkin pulp in the recipe. The taste is sweet, aromatic and towards the end with a touch of earth, a taste given by the presence of amaranth flour.

It is recommended to store in hermetically sealed packages and keep at room temperature, without large fluctuations in humidity.

The analysis of the behavior of the dough during preparation revealed that the dough obtained from amaranth flour - is characterized by a good ability to bind the dough and keeps its shape when pouring. In the samples to be analyzed, where fresh or baked pumpkin is added, a higher humidity of the dough is observed due to the addition of pumpkin (Figure 1).

Achieving sensory acceptability has been another big challenge when developing gluten-free bakery products. Gluten-free bakery products usually possess distinct appearance, color, texture, aroma, and taste in contrast to the products made of wheat flour. Color of gluten-free bakery products tends to be darker due to a complex formulation. Specific volume of gluten-free bakery products such as cakes are generally smaller with harder texture compared with the products made of wheat. Taste of glute-free bakery products is in high variability, depending on the gluten-free flours and formulations (Jingwen Xu et al., 2020).

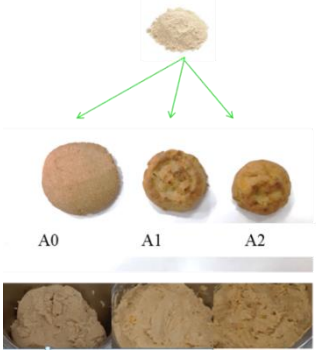

Figure 1. Cookies with amaranth flour and pumpkin pulp before baking (up) and after baking (down) (Original photo)

Table 3 Sensory analysis of gluten free organic cookies with amaranth flour and pumpkin pulp

Sample	Exterior appearance $\bar{X} \pm S_{\bar{X}}$	Section appearance $\bar{X} \pm S_{\bar{X}}$	Form $\bar{X} \pm S_{\bar{X}}$	Color $\bar{X} \pm S_{\bar{X}}$	Taste $\bar{X} \pm S_{\bar{X}}$	Flavor $\bar{X} \pm S_{\bar{X}}$	Texture $\bar{X} \pm S_{\bar{X}}$
A_0	3,979±0,153	3,766±0,137	3,702±0,182	3,681±0,167	3,299±0,206	3,191±0,221	3,128±0,174
A_1	3,594±0,205	3,656±0,188	3,688±0,217	3,594±0,228	3,062±0,233	3,375±0,245	3,250±0,229
A_2	3,800±0,166	3,633±0,159	3,550±0,183	3,283±0,154	2,700±0,178	2,876±0,192	3.267±0,166

Consumer acceptance

The sensorial evaluation of product was carried out in order to observe the impact of organic pumpkin pulp incorporation, on its sensory characteristics.

Consumer acceptance for cookies with amaranth flour and pumpkin pulp it was realized with a 5-point Hedonic evaluation scale, on a panel group by 30 persons. Objective it was to observe the impact of organic pumpkin pulp (raw and baked) incorporation, on its sensory characteristics. Sensory tests were performed taking into account: appearance, taste, color, flavor and smell, texture (Table 3).

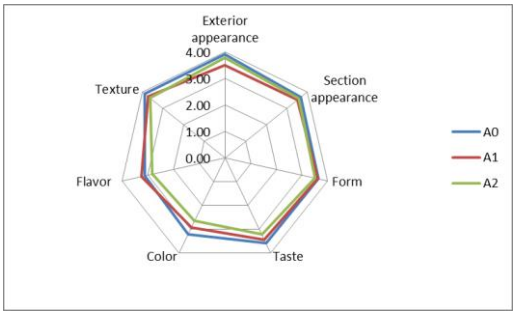

Figure 2. Consumer acceptability scores on a 5-point Hedonic scale for cookies with AF and pumpkin pulp

Following analysis, consumer acceptance was very good in all age segments, the new product being to the liking of consumers and consumer acceptability.

The score obtained for each attribute was processed, and the average values are presented in Figure 2.
The most appreciated attributes were those related to the appearance of the product (outer appearance, section appearance, shape) for all 3 samples analyzed.
In the case of sample A1, the value 3.594±0.205 lower than that of the other samples is due to the increased humidity of the biscuit and the oily sensation.

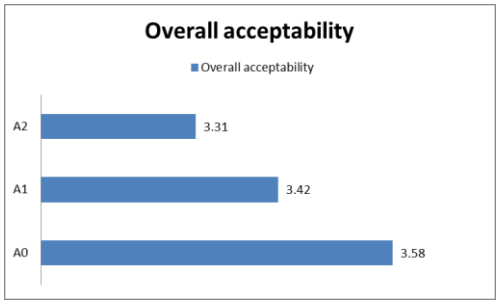

Figure 3. Overall acceptability for cookies with AF and pumpkin pulp

The taste was quite controversial, because many tasters found a pleasant, aromatic taste but did not appreciate the sandy sensation of the core when tasting. It is certain that samples A0 and A1 were the most appreciated products.
The flavor of sample A1 was appreciated the most with a value of 3.375±0.245, which shows that consumers appreciate the combination between the flavor of amaranth flour and fresh pumpkin flesh.
The acceptability of the samples by the consumer showed that the A0 sample has the highest appreciation from the tasters, followed by the A1 sample (Figure 3). The least appreciated is sample A2 with ripe pumpkin pulp, and this is due to a harder texture, a more intense color and a specific taste.

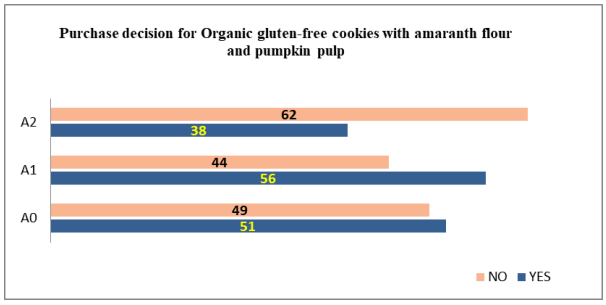

Figure 4. Purchase decision for organic gluten-free cookies with amaranth flour and pumpkin pulp

Following the analysis of consumers' purchase intention (Figure 4), regarding gluten-free cookies with amaranth flour and *Curcubita maxima* pumpkin pulp, a preference is observed in sample A1, respect for cookies with fresh pumpkin pulp, followed by sample A0, respectively gluten-free cookies bio with amaranth flour without the addition of pumpkin pulp.

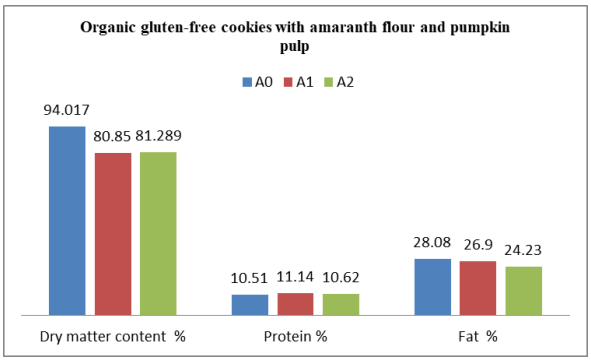

Figure 5. Chemical composition of organic gluten-free cookies with amaranth flour and pumpkin pulp

Following the analysis of the chemical composition, it was found that the dry matter, protein and fat content varied very little. The variation comes from the ingredients used in the recipe (Figure 5).
The dry matter of the samples was consistent with the type of pulp added in the sample. Thus, control sample A0, without the addition of pumpkin, has a dry substance of ≈94%, the addition of pumpkin pulp decreases the percentage of dry substance in accordance with the percentage of pumpkin pulp added in the recipe.
The dry matter (DM) of samples A1 and A2 decreases compared to the control A0 (DM= 93.22 ± 0.68%) for all samples with addition of pumpkin pulp, being between 69.27 ± 2.79% (addition of fresh pumpkin pulp) and 73.46 ± 0.50% (added baked pumpkin pulp).
The increase moisture in products with fresh pumpkin pulp was high because its moisture was also on average 95.45% and for baked pumpkin 91.69%. In conclusion, the state of the pumpkin greatly influences the technological, sensory and stability characteristics of the obtained products.
Carotenoids are susceptible to various degradation and isomerization reactions,

mainly due to carbon-carbon double bonds, causing discoloration and reduction of the biological activities of the relevant food products (Fratianni et al., 2017). In particular, thermal treatments such as cooking pumpkin puree (Provesi et al., 2011), steaming or boiling pumpkin flesh (Ribeiro et al., 2015) and hot air drying of pumpkin slices (Lago-Vanzela, 2013) could induce degradation and structural changes (eg, cis isomerization) of carotenoids. In most studies relevant to pumpkin carotenoids, only provitamin A carotenoids (mainly β-carotene) have been investigated (Ribeiro et al., 2015). The thermal stability of carotenoids without vitamin A activity in pumpkin pulp has generally remained unclear, although some of these carotenoids have been linked to reduced risk of degenerative diseases such as macular degeneration, which may be prevented by macular pigments, including lutein and zeaxanthin (Ziegler et al., 2015).

Pumpkin is a good source of carotenoids (Bergantin et al., 2018) because the color of their pulp varies from yellow to orange, which is associated with different carotenoid compositions (Marek et al., 2008). As precursors of vitamin A, carotenoids are essential components of the human diet and play a significant role in reducing the risk of cancer and stimulating the immune system (Beutner et al., 2001). Carotenoids are effective antioxidants to protect cells from damage caused by free radicals. The intake of carotenoids from pumpkin is an important supplement to the diet of a segment of consumers where vitamin A deficiency is a serious concern.

The recommended daily dose is currently either 2 mg β-carotene (recommended by the DGE, Germany, in addition to 1.0 (0.8) mg retinol equivalents for vitamin A requirements) or 5–6 mg β -carotene (recommended by NCI, USA). The recommended daily intake can only be reached by consuming (100-200 g/day) vegetables and fruits with particularly high carotene content (Müller et al., 1996).

The bioactive substances in pumpkin pulp are from the category of antioxidants, such as alpha-carotene, beta-carotene and beta-cryptoxanthin that support the effect of the body's immune response and improve the activity at the cell level (Ami Ben-Amotzsi et al., 1998). Pumpkin pulp has a high antioxidant activity (containing α-carotene, β-carotene and lutein) beneficial to the consumer with an approximate content of 202 µg/g beta-carotene (Hagos et al., 2022; Javeria et al., 2013). The presence of β-carotene provides the body with the necessary vitamin A.

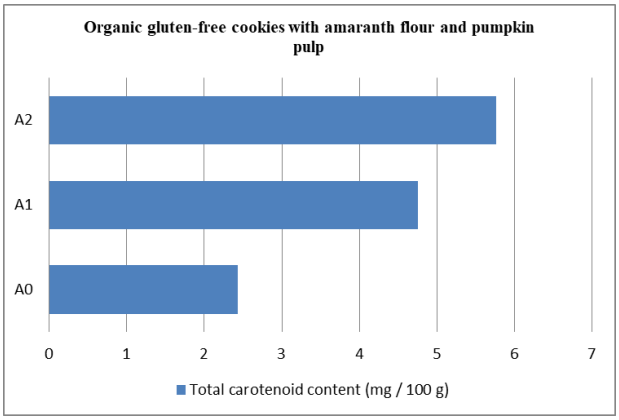

Figure 6. Total carotenoid content for Organic gluten-free cookies with amaranth flour and pumpkin pulp

Determination of total carotenoid content in baked pumpkin (Figure 6) show a total carotene content of 2.388 ± 0.120 mg total carotenes/100 g and 0.776 ± 0.019 mg total carotenes/100 g for fresh pumpkin pulp, determined by the UV-VIS method, which confirms that the pumpkins used, from local organic farms, fall within the limits found in specialized literature. Hagos et al., 2022 found the β-carotene content higher in the pumpkin skin (340-445 µg/g), followed by the pumpkin pulp (317-341 µg/g) by the UV-VIS method, values comparable to those obtained in the determinations ours, respectively 238.8 mg/100 g total carotenes.

Following the results obtained, an increase in the total carotene content was observed in the samples containing baked pumpkin pulp.

The carotenoids contents of samples A1 and A2 increase compared to the control A0 (5.024 ± 0.034 mg / 100 g total carotens) for all samples with addition of pumpkin pulp, being between 6.435 ± 0.161 mg / 100 g total carotens (A1) and 6.629 ± 0.570 mg / 100 g total carotens (A2).

Following the results obtained, an increase in the total carotene content was observed in the samples A2, respectively 6.629 ± 0.570 mg /100 g.

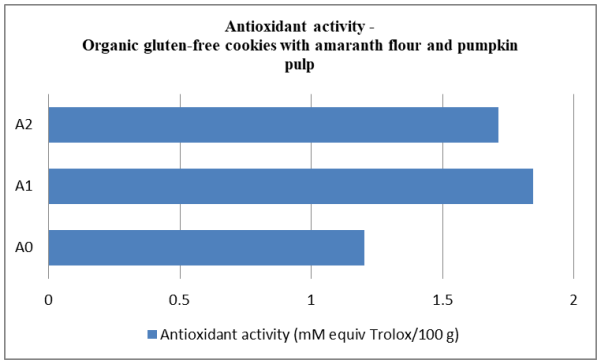

Figure 7. Antioxidant activity for Organic gluten-free cookies with amaranth flour and pumpkin pulp

As expected, the antioxidant activity is higher in organic gluten-free cookies with amaranth flour and fresh pumpkin pulp (Figure 7). The sample without pumpkin pulp has the lowest antioxidant activity.

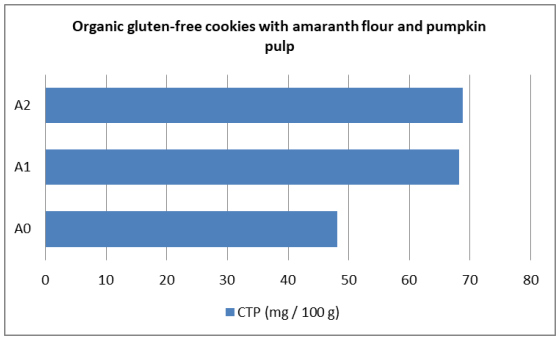

Figure 8. Total content of polyphenolic compounds for organic gluten-free cookies with amaranth flour and pumpkin pulp

The total content of polyphenolic compounds in gluten-free cookies with amaranth flour is observed to be higher in samples with pumpkin pulp (Figure 8), which highlights that *Curcubita maxima* pumpkin pulp, as an ingredient, comes with a high intake of valuable compounds high bioactive.

We can conclude that the addition of pumpkin pulp has a major positive impact on the nutritional value of the products obtained in our study.

CONCLUSIONS

The cookies are rich in nutrients and can be consumed by all individuals to help meet daily needs, especially those of people who have increased needs for these essential nutrients. The obtained results indicated that the developed cookies were not only improved in terms of nutritional value and health benefits, but also had high potential of being accepted by consumers.

In conclusion, each type of product was distinguished by an attribute, and following the sensory evaluation of the samples of gluten-free cookies with organic pumpkin pulp, the formulas used to finally obtain the sensory characteristics desired by the consumer will be reviewed and optimized, obtaining technology. The use of konjac flour has substantially improved the characteristics of the dough, but will especially optimize recipes for gluten-free cookies with the addition of fresh pumpkin pulp.

This work suggested that the amaranth flour and pumpkin pulp should be good potential candidates as gluten-free materials to replace wheat flour in some food products

ACKNOWLEDGEMENTS

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TECHNOLOGICAL ADVANTAGES OF METHODS FOR THE SIMULTANEOUS DETECTION OF SEVERAL CLASSES OF ANTIBIOTIC RESIDUES IN CHICKEN MEAT

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Abstract

Antibiotics are routinely used to treat and prevent infections in humans as well as in animals. Excessive use of antibiotics in animals, especially in poultry, accumulates residues in the meat and organs beyond legal limits. A large number of classes and types of antibiotics often used in the poultry sector raises problems in quantitative detection methodologies. These techniques must be economical, comply with the requirements of the international standard for maximum residue limits detection, and be sensitive, reproducible, and reliable. Given the complexity of the requirements that such a methodology must meet, the study presents the technological advances of modern technology such as High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry (LC-MS), Ultra-High Performance Liquid Chromatography (UHPLC) in the simultaneous detection of several antibiotic residues belonging to different classes. The paper aims to facilitate the simultaneous detection of as many antibiotic residues in chicken meat in the spirit of the One Health concept.

Key words*: antibiotic residues, chicken meat, detection, multiclass.*

INTRODUCTION

The greatest discovery of the last century (Clardy et al., 2009), antibiotics, has become a major public health concern. The reason for this concern is the antimicrobial resistance that humans can develop. Antimicrobial resistance is regarded as a critical One Health issue (Robinson et al., 2016). Antimicrobial resistance involves three distinct elements: man, animal and the environment, elements that are interconnected and cannot exist without each other. The One Health concept agreed that there is a close connection between the three elements and that the approach to antimicrobial resistance must be multidisciplinary (O'Neill, 2015; Holmes et al., 2016). Although antimicrobial resistance is a naturally occurring phenomenon, the excessive and inappropriate use of antibiotics in both human and veterinary medicine amplifies the phenomenon and negatively affects the health systems already severely affected by the Covid 19 crisis. Any intervention to reduce the consumption of antibiotics is beneficial for overcoming the phenomenon of antimicrobial resistance. An effective approach to estimating the occurrence of antimicrobial resistance is to closely monitor sales of antimicrobial substances in veterinary medicine products. The latest report in this regard (EMA, 2021) expresses sales of antimicrobials in veterinary medicinal products (VMPs) in mg/PCU where PCU represents the population correction unit. 31.1% of the overall sales of antimicrobials in 31 countries in 2020, (in mg/PCU), were represented by penicillins, 26.7% by tetracyclines and 9.9% by sulfonamides (9.9%). In Romania, in 2020, 173.7 tons of VMPs were sold for food-producing animals, which means 57, 8 mg/PCU. Tetracyclines, penniclines and sulfonamides had the highest percentage in the sales structure. These antibiotics are on the list of the most common classes of antibiotics prescribed in human medicine (Menkem et al., 2019). Poultry accounted for 15% of the PCU in 31 countries, including Romania. Chicken meat is a relatively inexpensive source of protein, consumed in all

religions and parts of the world. Chicken meat is preferred by children and the elderly, the most vulnerable categories of the population.

Several studies (Al-Ghamdi et al., 2000; Er et al., 2013; Ezenduka, 2019; Baghani et al., 2019; Widjastuti et al., 2022) have reported the presence of many categories of antibiotics in chicken meat.

Taking into account all these aspects, more accurate analysis of several antibiotics in chicken meat is required.

To protect human health and prevent adverse effects from antibiotics in animal products, maximum residue limits (MRLs) of antibiotics and other drugs were established by the European Commission.

Maximum Residues Limits for chicken meat are presented in Table 1.

Table 1. Maximum residue limits (MRL) in chicken meat

Antibiotic	Muscle	Fat*	Liver	Kidney
Amoxicillin	50	50	50	50
Ampicillin	50	50	50	50
Avilamycin	50	100	300	200
Benzylpenicillin	50	50	50	50
Chlortetracycline	100	-	300	600
Cloxacillin	300	300	300	300
Colistin	150	150	150	200
Danofloxacin	100	50	200	200
Dicloxacillin	300	300	300	300
Difloxacin	300	400	1900	600
Doxycycline	100	300	300	600
Enrofloxacin	100	100	200	300
Erythromycin	200	200	200	200
Florfenicol	100	200	2500	750
Flumequine	400	250	800	1000
Kanamycin	100	100	600	2500
Lasalocid	20	100	100	50
Lincomycin	100	50	500	1500
Neomycin	500	500	500	5000
Oxacillin	300	300	300	300
Paromomycin	500	-	1500	1500
Penicillin	25	25	25	25
Sarafloxacin	-	10	100	-
Spectinomycin	300	500	1000	5000
Spiramycin	200	300	400	-
Sulfonamides	100	100	100	100
Tetracycline	100	-	300	600
Thiamphenicol	50	50	50	50
Tiamulin	100	100	1000	-
Tilmicosin	75	75	1000	250
Tylosin	100	100	100	100
Tylvalosin	-	50	50	-

Values expressed in µg/kg

* The fat MRL relates to 'skin and fat in natural proportions'

Source: COMMISSION REGULATION (EU) No 37/2010

The data in Table 1 show that the determinations should be emphasized on muscle, where MRL values are equal or lower than those of kidney, liver, or fat. This paper aims to investigate the advantages of chromatographic techniques and critically discuss their current limitations in the context in which the methods must be validated according to the European Commission Directive 2002/657/EC.

MATERIALS AND METHODS

Information about the simultaneous detection of several classes of antibiotic residues in chicken meat was obtained from a literature search of electronic databases such as Science Direct, Google Scholar, Pub Med, and Scopus. Only articles published in English were included in the study, which may be considered a minor limitation of the present study. The keywords used were: methods, simultaneous detection, antibiotics, and chicken meat. As expected in the databases consulted, a relatively small number of articles discussing the proposed topic were found, respectively 61 articles. Of these articles, only those that were intended for the determination of antibiotic residues in chicken meat samples in one analytical run have been studied. The rest of them were excluded.

RESULTS AND DISCUSSIONS

Confirmatory analysis methods of antibiotics in food matrices are expensive. For the quantitative determination of antibiotics to be performed as economically as possible and as accurately as possible, new methods of confirmation have been successfully developed and validated. The chromatographic systems coupled to different types of detectors and mass spectrometry is the technique that responds best to these requirements. Chromatography is defined as a physical separation method based on different interactions of the specimen compounds with the mobile phase and with the stationary phase as the compounds travel through a support medium. Chromatographic methods also have disadvantages, such as the cost of technical training, laboratory infrastructure, trained personnel, or the cost of equipment implementation.

One method is more economical and can be used for routine analyses in the laboratory when several classes of antibiotics in the same matrix can be detected simultaneously.

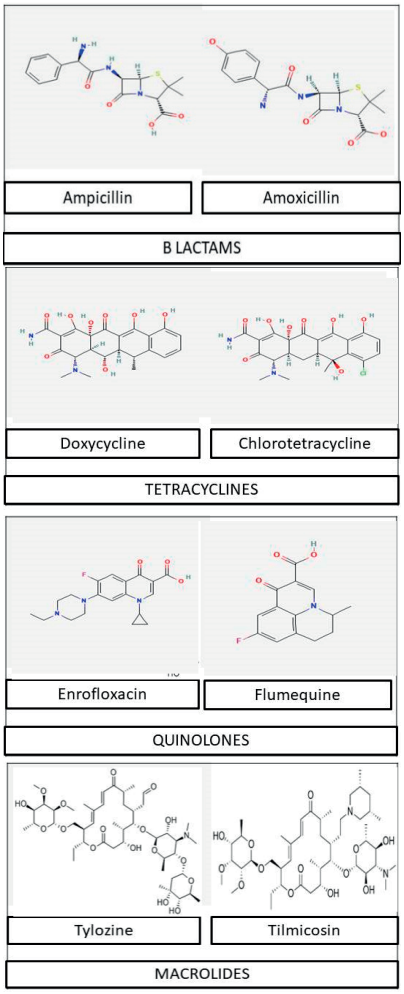

Figure 1. Chemical structure of some common antibiotics used in human and poultry

All these aspects required efforts from specialists in the successful validation of methods for the quantitative determination of antibiotics in chicken meat. The proteins, lipids and other nutrients in the meat make it a complex food matrix. The complexity of this matrix as well as the low level of antibiotic residues leads to the need for pre-determination techniques in themselves, techniques that isolate and concentrate the traces of antibiotics in the meat

When choosing the most efficient method, maximum attention should be paid to the following aspects: chromatographic column and mobile phase selection. Song et al., 2018, used the Kinetex Biphenyl column for the simultaneous determination of eight cyclopolypeptide antibiotics. An Inertsil ODS-3 highly inert and reliable column with high reproducibility and efficiency was used by Hui et al., 2018, for screening 15 sulfonamide residues in different meat types. Using a Waters Symmetry Shield C18 (150 × 4.6 mm^2, 5 µm) column, Mishra et al., in 2020 successfully validated a method of simultaneous and rapid detection of 20 antibiotics in chicken tissues. Neutral alumina, and C18 SPE columns were successfully used to detect 11 quinolone antibiotics in chicken meat by Lu et al., 2019. For the separation of polypeptide antibiotics, the most effective separation effect and the highest response was obtained when was used a Poroshell 120 SB-C18 column (Liu et al., 2019). 30 antibiotics belonging to four different classes were determined by Jammoul & El Darra (2019) using the C18 analytical column (Zorbax 2.1 mm inner diameter I.D × 150 mm length, 3.5 µm particle size). Analyzing the latest data from the literature, eleven analysts were separately using a C8 column (150 mm 3 mm 4 m) in Brazil by Barros et al., 2021. Hypersil BDS-C18 (3 µm, 100 mm × 4 mm) column was used by Lakew et al. (2022) to determine seven classes of antibiotics from chicken meat. The chromatographic column or stationary phase is one of the main parts of the chromatographic system. In modern chromatography, the evolution of the columns was based mainly on the reduction of the internal diameter and the size of the filler particle, which has resulted in an increase in selectivity and an improvement in the resolution of the chromatograms (Snyder et al., 2010).

The mobile phase influences the detection in chromatography, for this reason, its choice is extremely important when determining antibiotic residues by chromatographic methods. The mobile phase can be gas, gel, or liquid but in veterinary drugs determination in one analytical run, the last, (liquid) proved to be most efficient when coupled with mass spectrometry (MS). The most common mobile phases used in the separation of different antibiotics are methanol, acetonitrile, and formic acid. Compare to

the others methanol is cheaper. Lai et al. (2020) showed other advantages of methanol, not only the price.
Methanol increases the solubility of analysts which improves their detection.
In the separation of several classes of antibiotics (quinolones, nitroimidazoles, pleuromutilins, and β-lactams) the roles and efficacy of methanol and acetonitrile have been demonstrated. Acetonitrile provided better peak shape and methanol provided better separations (Wang et al., 2016). When formic acid was added to the mobile phase it was demonstrated that this increases the acidity and improves the ionization efficiency in HPLC–MS/MS.
To improve the ionization efficiency and signal strength in LC-MS / MS, Mishra et al. (2020) used two additives, respectively ammonium formate and ammonium acetate. These additives also generated high chromatographic resolution.
Chromatographic methods that can simultaneously detect several classes of antibiotics are shown in Table 2.

Table 2. Efficient chromatographic methods for the detection of antibiotics in one analytical run

Chromatographic methods		
High-Performance Liquid Chromatography (HPLC)	Liquid Chromatography-Mass Spectrometry (LC-MS)	Ultra-High Performance Liquid Chromatography (UHPLC)

The major challenge during the detection of antibiotics using chromatographic techniques is to detect as many classes as possible.
A comparison of the analytical performance of the methods used for the determination of several classes of antibiotics in real samples is presented in Table 3.

Table 3. Comparison of the analytical performance of the methods used for antibiotics detection

Method	Reference	Antibiotics (n)	Matrix
LC- MS/MS	Yamaguchi et al., 2015	n = 28/multi classes	pork, chicken, beef meat
HPLC-UV	Hui et al., 2018	n = 15/sulfonamides	pork, beef, mutton tissues
HPLC-ELSD	Song et al., 2018	n = 8/cyclopolypeptide	feed
LC-MS	Jammoul & El Darra, 2019	n = 30/multi classes	chicken tissues
HPLC-MS/MS	Liu et al., 2019	n = 4/poplypeptide	infant formula powder
UHPLC-MS	Lu et al., 2019	n = 11/ quinolone	chicken meat, egg
LC-MS/MS	Mishra et al., in 2020	n = 20/multi classes	food, soil
LC-MS/MS	Barros et al., 2021	n = 11/multi classes	chicken meat, liver
LC-UV	Lakew et al., 2022	n = 7/multi classes	chicken tissues

CONCLUSIONS

The chemists and researchers who determine antibiotics in the food matrix are currently facing a situation where there is a requirement to detect more and more compounds at lower and lower concentrations. The challenge is even greater given that degradation products and their metabolites often need to be determined. Chromatographic techniques are commonly used for the detection of antibiotic residues in food samples for a long period.
In the last decade, these methods have been greatly improved by coupling detectors such as detectors, mainly ultraviolet (UV), diode array detector (DAD), and fluorescence detector (FLD) or coupled with mass spectrometry (MS). Among these methods, LC-MS / MS become the gold standard for one-run analysis of antibiotic multi-class in food matrix.

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INFLUENCE OF GARLIC EXTRACT AND PACKAGING METHODS ON THE QUALITY PARAMETERS OF FISH FILLETS STORED AT LOW TEMPERATURES

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Abstract

Fish meat has very high water content, a very low content of connective tissue but has high digestibility. Raw fish is a very perishable food because it has all substances necessary for the development of microorganisms. Fish meat lipids are also very sensitive because they oxidize very quickly during storage. The aim of our research was to examine the influence of garlic extract and packaging methods on the total volatile basic nitrogen (TVB-N), thiobarbituric acid content and pH value in fish fillets during storage at low temperatures. 48 fillets of rainbow trout (Oncorhynchus mykiss) were studied. The fish fillets were randomly assigned to four batches of 12 samples each (C-control and experimental batches E1, E2 and E3) and marinated in a solution with garlic extract in proportion of 0‰ (C), 3‰ (E1), 6‰ (E2) and 9‰ for 12 hours. For each batch, six fillets were individually vacuum packed and six fillets were individually packed in plastic boxes. The samples were stored in the refrigerator at a temperature of 0...+4°C for six days. The studied parameters were determined after marinating, on the 1st, 3rd and 6th day of storage.The raw data obtained was processed by biostatistical methods using the One-Way Anova test, including the Tukey HSD test of the SPPS Statistics application. In the storage period, the amount of TBA increased progressively at each stage of the examination (30 min., 1, 3 and 6 days, respectively), and the amounts in the control samples were significantly higher ($p \leq 0.05$) than those in the E1, E2 and E3 samples. Also, the lowest values after 6 days of refrigeration were recorded in the marinated samples and vaccum packed. The lower values recorded in marinated samples were influnced by the antioxidant effect of the garlic extract used. Regarding the TBA amount in fish and fish products, the admissibility limit recommended for the consumer is 2.0 mg MDA/kg, and in our case the values have been much lower.

Key words: *fish filet, garlic extract, marinating, refrigeration, thiobarbituric acid (TBA).*

INTRODUCTION

Fish meat is an important food source with high biological value, high in protein, minerals, vitamins, unsaturated fatty acids, high digestibility, low cholesterol and superior organoleptic quality (Duan et al., 2010). From a nutritional point of view, fish is one of the most important foods for humans because it has an energetic and plastic role (Francesco et al., 2011).

Fish and fish products are highly perishable, as they have a high content of water and free amino acids and a lower content of connective tissue compared to other types of meat, leading to faster spoilage (Jinadasa, 2014).

Refrigeration and freezing have been used extensively to preserve the sensory and nutritional properties of fish. However, fish meat has a short storage time (Giannini et al., 2007), because it has a high content of unsaturated fatty acids and an important presence of pro-oxidant molecules that lead to rancidity and loss of nutritional quality (Richards and Hultin, 2002).

Lipids contain large amounts of unsaturated fatty acids. Therefore, lipids oxidation is an important problem, which leads to a decrease in meat quality during storage (Secci & Parisi, 2016) and loss of nutritional value (Maqsood et al., 2012). Also, several studies shown the effect of protein oxidation during storage and changes in meat quality (Bertram et al., 2007; Lund et al., 2007; Milijašević et al., 2017). Moreover, the pH value can change the rate of lipid oxidation during storage. Thus, at high pH the TBA value were lower compared to lower pH which accelered lipid oxidation (Balev et al., 2011; Mozuraityte et al., 2016; Wei et al., 2017).

Vacuum packaging is important for the quality of the fish as it extends the shelf life of the fish and to minimize undesirable effects, various antioxidants are used (Taheri et al., 2012; Medina et al., 2009). Also, Navaro-Segura et al. (2020) showed that vacuum-packaging of fish with oregano essential oil vapours had positively effect of shelf life. This technology was applied to sea bream fresh fillets that were kept for 28 days at a temperature of +4°C and the shelf life for refrigerated fillets was established in at least 28 days.

In order to extend the storage time, much attention is directed to antioxidant substances to prevent lipid oxidation. Currently, research activity is focused on replacing synthetic substances with natural antioxidants, due to the possible adverse side effects of synthetic antioxidants and the beneficial effects of natural antioxidants (Sarkardei & Howel, 2008).

In the food industry, some plants are generally used to improve the sensory characteristics of foods (Burt, 2004). Moreover, these plants have substances with antimicrobial and antioxidant activity (Baydar et al., 2004; Frank et al., 2014). According to Griffiths et al. (2002), garlic species are revered as possessing antibacterial and antimicrobial activities and contain potent antioxidants, sulfur, and numerous other phenolic compounds that have attracted great interest in the food industry. These beneficial properties seem to be closely related to the high content of sulfur compounds and flavonoids, due to their activity as antioxidants and anti-carcinogens, their effects on lipid metabolism and the cardiovascular system, their antibiotic effects (Griffiths et al., 2002).

In this work, the antioxidant effect of garlic extract and vacuum packaging on trout fillets during storage at +4°C was studied by determining the physicochemical parameters.

MATERIALS AND METHODS

Twenty-four specimens of trout (Oncorhynchus mykiss) with an average weight of 725.25 ± 16.15 g were purchased during the winter from a local market (Timișoara, Romania), transported in polyethylene bags containing cold water to the Food Processing Technology Laboratory, Faculty of Animal Resources Bioengineering. Then each fish was gutted, skinned, filleted and washed with tap water in the laboratory. The fillets were then randomly divided into four batches of 12 fillets for each treatment. Fish fillets were randomly assigned to one of the four treatments shown below:C - Control batch, garlic extract (0‰); E1 - Experimental batch, garlic extract 3‰; E2 - Experimental batch, garlic extract 6‰; E3 - Experimental batch, garlic extract 9‰.

Preparation of garlic extract: Thirtieth fresh garlic bulbs average sized were obtained from market in Timisoara. They were peeled, washed, chopped and oven dried at a temperature of 40°C until constant weight was reached. 800 mL of methanol was used to soak 100 g of the oven dried garlic for 24 hours. The methanol extracts was decanted and placed in evaporator to concentrate the extract up to 80 mL.

The marinating solutions were obtained from 0 mL, 3 mL, 6 mL and 9 mL of garlic extract and 3.5 g of seea salt added to 1 litter of water. The marinating solutions were stored at a temperature of +4°C before immersed the fish fillets.

For each tratament, 12 fish fillets were immersed in the marinating solution together. The samples were marinated in sealed closed plastic bags and kept in a refrigerator for 12 h at a temperature of +4°C.

After 12 h, the fillets were removed from the solutions and drained on sanitized stainless steel grids for 5 min at room temperature. The fillets were individually wrapped and stored in a refrigerator at 4°C. Twenty four fillets were vacuum packed (six fillets for each batch) and twenty four fillets were packed in plastic boxes (six fillets for each batch).

For the physico-chemical examination, samples were taken from the raw fillets (30 min.) and from the marinated fillets after: 30 min, 1, 3 and 6 days. TVB-N was determined according to the method of Antonacopoulos & Vyncke (1989). For total TVB-N, fish muscle (10 g) was homogenized with 6% perchloric acid (90 mL) for 1 min, filtered through a filter paper, alkalized by NaOH (20%) and distilled. The distillate was titrated with a HCl solution 0.01 N. The TVB-N is an indicator of protein degradation in fish muscle and TVB-N level was expressed as mg N/100 g fish muscle.

Thiobarbituric acid reactive substances (TBARS) were determined according to the method of Buege and Aust, (1978). Thus, 0.5 g

of fish fillet was homogenized in 10 ml of the mixture containing TBA (0.375 g/100 mL), TCA (15 g/100 mL) and HCl (0.25 mol/L). The mixture was heated in the boiling water for 10 min, followed by cooling with tap water. The mixture was centrifuged at 3600 x g for 20 min and the absorbance was measured at 532 nm. The TBARS value was calculated from the standard curve of malonaldehyde and expressed as mg malonaldehyde/kg sample.

pH was measured directly in the fish muscle with an FC 232D glass electrode connected to a Hanna HI99163 portable meat pH meter. The pH was measured at three different places on each fillet. The electrode was also cleaned after each sample and calibrated frequently.

Statistical analysis: The raw data obtained from measurements were processed using methods of biostatistics with Microsoft Excel spreadsheet application (Brudiu, 2010). Significant differences were defined at $P \leq 0.05$. To test the statistical significance of differences between mean values of the characters studied, an analysis of variance with Anova test, included Tukey HSD test from SPSS Statistics, was used.

RESULTS AND DISCUSSIONS

Table 1 showed changes in TVB-N from marinated fish fillets during the whole period of refrigerated storage at a temperature of +4°C.

The total volatile basic nitrogen (TVB-N) is an important indicator of meat resulting from protein and amine degradation. According to Fatih &mental samples (E1, E2 and E3) than in the control samples for which a faster growth was recorded during the six days of storage.

TVB-N values during storage for the control samples ranged from 10.29 mg/100 g after first day of storage up to 53.50 mg/100 g after six days of storage while the values from experimental samples had was between 7.96 mg/100 g up to 35.29 mg/100 g in the same days of examination. Thus, in experimental samples E3 the TVB-N values were lower (7.96-22.76 mg/100 g) than in samples E2 (8.34 and 29.04 mg/100 g) and E1 (8.96-35.29 mg/100 g) because it increased more slowly in samples marinated with high amounts of garlic extract.

Also, to samples in vacuum packaging TVB-N values was lowest, for all batches (from 7.96 up to 33.29 mg/100 g) as compared to the fillets packed in plastic boxes which had the highest values (from 14.96 up to 53.30 mg/100 g).

After applying the statistical test, significant differences ($P \leq 0.05$) were identified depending on the concentration of garlic extract, storage time and packaging method.

The values from this study are similary with results obtained by Muhammet & Sevim, (2007) in freshly caught fish (between 5 to 20 mg/100g).

Table 1. Dynamics of total volatile basic nitrogen (TVB-N mg/100 g)

Storage time		C (n=6)	E1 (n=6)	E2 (n=6)	E3 (n=6)	P value
Vacuum packing						
Initial values		6.25 ± 0.04^{aA}	6.20 ± 0.05^{aA}	6.20 ± 0.06^{aA}	6.22 ± 0.02^{aA}	0.20912
After marinating	**30 min**	6.49 ± 0.09^{aAx}	6.31 ± 0.06^{bAx}	6.27 ± 0.05^{bAx}	6.24 ± 0.02^{bAx}	0.00001
	1 day	10.29 ± 1.35^{aBy}	8.96 ± 1.41^{abBy}	8.34 ± 0.77^{bBy}	7.96 ± 0.61^{bBy}	0.00763
	3 days	14.13 ± 1.12^{aBz}	10.70 ± 1.27^{bBz}	9.12 ± 0.38^{cBz}	8.49 ± 0.05^{cBz}	0.00001
	6 days	33.29 ± 5.56^{aBw}	19.87 ± 4.14^{bBw}	16.12 ± 1.96^{bcBw}	13.16 ± 1.45^{cBw}	0.00001
Plastic boxes						
Initial values		6.25 ± 0.04^{aA}	6.20 ± 0.05^{aA}	6.20 ± 0.06^{aA}	6.22 ± 0.02^{aA}	0.20800
After marinating	**30 min**	6.50 ± 0.09^{aAx}	6.30 ± 0.05^{bAx}	6.28 ± 0.05^{bAx}	6.24 ± 0.02^{bAx}	0.00000
	1 day	17.80 ± 3.25^{aAy}	16.29 ± 3.07^{aAy}	15.13 ± 2.84^{aAy}	14.96 ± 2.47^{aAy}	0.33233
	3 days	30.30 ± 3.49^{aAz}	20.29 ± 2.63^{bAz}	17.88 ± 1.73^{bcAz}	15.59 ± 2.45^{cAz}	0.00001
	6 days	53.30 ± 4.89^{aAw}	35.29 ± 4.52^{bAw}	29.04 ± 1.94^{bcAw}	22.76 ± 3.74^{cAw}	0.00001

Means followed by different superscript lowercase letters in the same row and means followed by different superscript uppercase letters in the same column differ significantly to $P \leq 0.05$ by ANOVA test, included Tukey HSD test; Mean±Standard deviation; a, b, c, d-between batches; A, B-between packaging methods; x, y, z, w-between storage time

Also, Fan et al. (2009); Gulsun et al. (2009) and Frank et al. (2014) showed a gradual increase in TVB-N values in all samples during storage. According by Zhou et al. (2011) the TVB-N of fish is an indicator of the freshness of the raw material. TVB-N values lower than 25 mg/100 g which are considered as the threshold for a good-quality fish product, while high TVB-N values are unacceptable and are associated with organoleptic changes especially unpleasant smell in the fishmeat (Limbo et al., 2009). On the contrary, according by Fan et al. (2009), the upper limit when a fish can be considered spoiled is between 35 to 40 mg/100 g. Also, in fish and fish products, several authors recommend the level of TVB-N up to 25 mg/100 g for high quality, up to 30 mg/ 100 g for good quality, the limit of acceptability up to 35 mg/100 and above 35 mg /100 g spoiled (EU/CE No. 627/2019, Amegovu et al., 2012).

The results of the present study showed the effectiveness of the garlic extract used in the minimum concentration of 3‰ in the marinating solution of fillets, because antioxidants and antimicrobials limited the increased of TVB-N values in the samples treated and protected by vacuum packaging (Table 1). Also, in samples packed in plastic boxes, at a minimum concentration of 6‰ of garlic extract, lipid oxidation was limited after six days to 29.04 mg/100 g.

Thiobarbituric acid (TBA) values were used as an index for lipid oxidation in trout fillets during refrigerated storage (6 days). For fish and fish products they will have a TBA value below 2.0 mg malondialdehyde/kg (MDA/kg), if the TBA value is greater than 2.7 mg MDA/kg the fish meat are poor quality is likely to have a rancid smell and taste (Bonnell, 1994; Baron et al., 2007; Limbo et al., 2009). While in other countries such as Egypt for TBA values in fish and fish products, the permissible limit recommended by EOS (2005) is 4.5 mg MDA/kg.

Table 2 shows the TBA values of trout fillets marinated with garlic extract during storage at refrigeration temperatures. Thus, the data in Table 2 showed a progressive increase in TBA (secondary oxidation product) values with the increase in the storage period under refrigeration conditions from 0.002 mg MDA/kg (initial values) up to 0.902 mg MDA/kg (control samples after six days).

The evolutions of TBA values were very slow in the experimental samples that were treated with garlic extract. Also, the present study shows a significant gradual increase in TBA values for control (from 0.003 up to 0.367, at vacuum-packed and from 0.002 up to 0.902, in plastic boxes-packed) and experimental samples (vacuum-packed E1: 0.002-0.208, E2: 0.002-0.130, E3: 0.003-0.061 and plastic boxes-packed E1: 0.003-0.386, E2: 0.003-0.266, E3 0.003-0.139) for both packaging methods (Table 2).

Table 2. Dynamics of thiobarbituric acid (TBA mg MDA/kg)

Storage time		C (n=6)	E1(n=6)	E2 (n=6)	E3 (n=6)	P value
Vacuum packing						
Initial values		0.003 ± 0.001^{aAx}	0.002 ± 0.001^{aAx}	0.002 ± 0.001^{aAx}	0.003 ± 0.001^{aAx}	0.20001
After marinating	**30 min**	0.019 ± 0.004^{aAx}	0.016 ± 0.003^{abAx}	0.014 ± 0.003^{abAx}	0.010 ± 0.001^{bAx}	0.00791
	1 day	0.110 ± 0.018^{aBy}	0.085 ± 0.018^{abBy}	0.066 ± 0.019^{bBy}	0.032 ± 0.008^{cBy}	0.00001
	3 days	0.205 ± 0.031^{aBz}	0.141 ± 0.030^{bBz}	0.080 ± 0.014^{cBz}	0.044 ± 0.010^{cBz}	0.00001
	6 days	0.367 ± 0.035^{aBw}	0.208 ± 0.053^{bBw}	0.130 ± 0.038^{cBw}	0.061 ± 0.019^{dBw}	0.00001
Plastic boxes						
Initial values		0.002 ± 0.001^{aAx}	0.003 ± 0.001^{aAx}	0.003 ± 0.001^{aAx}	0.003 ± 0.001^{aAx}	0.20800
After marinating	**30 min**	0.020 ± 0.003^{aAx}	0.017 ± 0.004^{aBx}	0.013 ± 0.003^{bAx}	0.011 ± 0.003^{bAx}	0.00000
	1 day	0.302 ± 0.023^{aAy}	0.143 ± 0.017^{bAy}	0.113 ± 0.011^{cAy}	0.074 ± 0.014^{dAy}	0.02333
	3 days	0.652 ± 0.051^{aAz}	0.236 ± 0.023^{bAz}	0.183 ± 0.013^{cAz}	0.094 ± 0.010^{dAz}	0.00001
	6 days	0.902 ± 0.046^{aAw}	0.386 ± 0.034^{bAw}	0.266 ± 0.025^{cAw}	0.139 ± 0.027^{dAw}	0.00001

Means followed by different superscript lowercase letters in the same row and means followed by different superscript uppercase letters in the same column differ significantly to $P \leq 0.05$ by ANOVA test, included Tukey HSD test; Mean±Standard deviation; a, b, c, d-between batches; A, B-between packaging methods; x, y, z, w-between storage time

In this study, the initial values for TBA were very low (0.002-0.003) and after marinating were between 0.010 to 0.020 mg MDA/kg. Thus, our results was lower that the values reported by Orban et al. (2011), Frank et al. (2014) and Hassanin and El-Daly (2013).

Moreover, the E3 samples treated with garlic extract 9‰ showed the lowest amount of TBA (from 0.032 mg MDA/kg after the first day to 0.139 mg MDA/kg after six days), while samples E1 (garlic extract 3‰) had higher TBA values (from 0.085 mg MDA/kg after the first day up to 0.386 mg MDA/kg after six days) and in E2 samples (garlic extract 6‰) were intermediate values. During the storage period, the increase in TBA values was higher for the samples packed in plastic boxes compared to the vacuum-packed which had lowest amount of TBA.

Compared to control samples which had 0.110 - 0.302 mg MDA/kg after first day, 0.205-0.652 mg MDA/kg at three days and 0.367-0.902 mg MDA/kg the end of storage period (six days) in experimental samples the values of TBA have showed the antioxidant activity of garlic extract. This claim is supported by statistical differences revealed between experimental and control samples in all stages of examination.

Thus, for TBA values after applying the statistical test (ANOVA included Tukey HSD test), significant differences ($P \leq 0.05$) were identified depending on the concentration of garlic extract, storage time and packaging method.

Effectiveness of the garlic extract has also been shown by Harris et al. (2001) and Mariutti et al. (2008) which showed the antioxidant properties of garlic due to its compounds (alliin, allicin, ajoene and allylpropyl) that play an important role in inhibited the lipid oxidation.

Vacuum packaging has the role of protecting the oxidation of lipids in food and especially in fish meat, which is highly perishable (Arashisar et al., 2004; Rashidi et al., 2014). Taheri & Motallebi (2012), in their studies showed that vacuum packaging substantially reduce lipids oxidation in frozen fish and fishe products. Also, Giménez et al. (2002) had studied effect of low oxigen content on trout meat in during storage and showed that lower concentrations of TBA in samples vacuum-packing than in fish stored in simple packaging peroxides in simple packaging resulted in an increase in TBA due to the presence of oxygen. Similar results were published by Manju et al. (2007) in baleen fish, Gandotra et al. (2015) in Labeo Rohita, Jezek & Buchtova (2014) in rainbow trout, Gandotra et al. (2016) in common carp.

Table 3 shows pH values in trout fillets according to the concentration of garlic extract, packaging method and storage time.

Table 3. Dynamics of the pH value

Storage time		C (n=6)	E1(n=6)	E2 (n=6)	E3 (n=6)	P value
Vacuum packing						
Initial values		6.032 ± 0.057^{aAx}	6.025 ± 0.110^{aAx}	6.032 ± 0.097^{aAx}	6.023 ± 0.097^{aAx}	0.23001
After marinating	**30 min**	6.173 ± 0.029^{aAy}	6.152 ± 0.042^{Ay}	6.132 ± 0.059^{aAy}	6.128 ± 0.023^{aAy}	0.22477
	1 day	6.232 ± 0.042^{aBz}	6.198 ± 0.030^{aBz}	6.155 ± 0.023^{bBz}	6.153 ± 0.016^{bBz}	0.00016
	3 days	6.281 ± 0.019^{aBw}	6.228 ± 0.024^{aBw}	6.175 ± 0.008^{cBz}	6.162 ± 0.015^{cBw}	0.00001
	6 days	6.316 ± 0.019^{aBw}	6.267 ± 0.040^{bBw}	6.184 ± 0.024^{cBz}	6.167 ± 0.014^{cBw}	0.00001
Plastic boxes						
Initial values		6.032 ± 0.057^{aAx}	6.038 ± 0.102^{aAx}	6.032 ± 0.097^{aAx}	6.045 ± 0.075^{aAx}	0.24573
After marinating	**30 min**	6.177 ± 0.028^{aAy}	6.154 ± 0.036^{aAy}	6.159 ± 0.031^{aAy}	6.127 ± 0.033^{bAy}	0.07436
	1 day	6.308 ± 0.038^{aAz}	6.275 ± 0.027^{aAz}	6.270 ± 0.018^{aAz}	6.202 ± 0.042^{aAz}	0.00016
	3 days	6.402 ± 0.023^{aAw}	6.324 ± 0.018^{bAz}	6.298 ± 0.047^{bAz}	6.243 ± 0.038^{cAz}	0.00001
	6 days	6.532 ± 0.057^{aAw}	6.430 ± 0.037^{bAw}	6.335 ± 0.025^{cAz}	6.286 ± 0.019^{cAz}	0.00001

Means followed by different superscript lowercase letters in the same row and means followed by different superscript uppercase letters in the same column differ significantly to $P \leq 0.05$ by ANOVA test, included Tukey HSD test; Mean±Standar deviation; a, b, c, d-between batches; A, B-between packaging methods; x, y, z, w-between storage time

The pH value of marinated trout fillets increased during storage time, with different dynamics depending of the contraction of garlic extract and the packaging method. Thus, the pH value

ranged between 6.025 and 6.532, with the highest values for the control samples in plastic boxes-packed (from 6.032 up to 6.532) and the lowest values for the vacuum-packed E3 samples (from 6.023 up to 6.167).
Comparaison to raw fillets from day zero, after six days of storage at +4°C the values showed significant increased for all samples. Also, significant differences were recorded between control and experimental samples and between the two packaged methods ($p<0.05$), in all examination stages.

CONCLUSIONS

From the results obtained in this study, it can be concluded that garlic extract had an antioxidant effect on marinated trout fillets. For the physico-chemical indicators studied, the samples marinated with 9‰ garlic extract had the lowest values.
Also, vacuum packaging of samples has been shown to be a suitable way to reduce lipid oxidation in trout fillets and extend shelf life.
The results of this study recommend garlic as a natural herb that could be used to extend the shelf life of fish meat and replace synthetic antioxidant

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RESEARCH ON THE FULTON CONDITION FACTOR, THE HEPATO-SOMATIC INDEX AND THE BIOCHEMICAL COMPOSITION OF CARP (*CYPRINUS CARPIO*) FROM 3 DIFFERENT SOURCES, IN ROMANIA

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Abstract

This study was conducted to evaluate the Fulton condition factor, the hepatosomatic index and the biochemical composition of carp meat (Cyprinus carpio) from aquaculture, reared in earthen ponds (CB), in floating net cages (CV) and in the wild, represented by the Danube River (CD). Significant differences were observed (P<0.05) between the mean values of the Fulton condition factor for fish from the three sources. Regarding the hepatosomatic index and visceral fat, the lowest values were obtained for the CD samples, and the highest in CV. The highest mean value of the protein content (18.55 ± 1.01%) was obtained in the specimens reared earthen ponds (CB), but the values did not differ significantly (P = 0.29) from the CD samples. The differences in lipid content of aquaculture fish meat reared in floating net cages (CV) and in the wild (CD) were statistically significant (P<0.05). In conclusion, fish from aquaculture has a better state of maintenance than that in the wild, fact demonstrated by the values of the Fulton Index. However, wild specimens have a much lower hepatosomatic index and visceral fat values than aquaculture fish.

Key words*: aquaculture, biochemistry, Cyprinus carpio, Fulton, wild.*

INTRODUCTION

In recent years, aquaculture has become a real industry, being one of the great sources of protein with a high biological value. Although fish is considered a healthy food, with beneficial effects on the cardiovascular system, especially due to unsaturated fats such as Omega-3 and Omega-6, in Romania fish consumption is quite low. In the UK, the advice from the authorities is to eat at least two servings of fish a week in order to benefit from its positive health effects (FSA, 2008).

Currently, aquaculture tends to develop very fast, becoming more and more intensive, in order to rapidly increase production, but also to increase profit (Schlag & Ystgaard, 2013). However, the intensification of fish production methods has raised a number of issues for consumers concerned about the consumption of aquaculture fish. As some consumers consider wild fish to be relatively expensive, aquaculture fish may be an alternative for them (Schlag & Ystgaard, 2013).

Rearing fish in floating net cages is widespread in the world, being one of the methods of intensive fish production in tropical areas (Liao et al., 2004). It has the advantage of using existing water resources (rivers, lakes, etc.) (Masser, 2008).

The common carp (*Cyprinus carpio*) is one of the most cultivated and consumed freshwater fish in the world (Böhm et al., 2014). It is a species of fish that easily adapts to different environmental conditions, being reared and marketing since ancient times.

Carp aquaculture in floating net cages has become increasingly popular worldwide (Yee et al., 2012). This involves rearing fish in cages made of netting, with meshes of different sizes, depending on the species cultivated, and open to the surface, placed on a floating frame and which are anchored by a pontoon.

This study aimed to investigate the differences in Fulton condition factor, hepatosomatic index and biochemical composition of wild carp (Danube) and aquaculture carp, reared in earthen ponds and floating net cages.

MATERIALS AND METHODS

The biological material involved in this study was represented by the common carp (*Cyprinus carpio*), caught from 3 different aquatic environments. A number of 10 specimens, weighing between 1.5-1.9 kg and 3 years old, were captured for comparison from: the wild (Danube river - CD) and from an earthen pond (CB) and floating net cages (CV) belonging to the same fish farm.

The floating cages had the size 6 m × 6 m × 3 m, the fish density was 130 kg/m^3. The food administered in the cages was represented by an extruded fodder with a granulation of 6 mm and a protein content of 30%. In the earthen pond, the fish density was 1500 kg/ha, the fish consumed only the food available in the pond (zooplankton and benthos).

The data collected for comparison included the fish mass, determined gravimetrically using a Kern-type scale, the total and standard length, measured using an instrument called "ichthyometer", the height and circumference of the fish - determined using a tailoring meter.

Based on these data, the Fulton condition factor was calculated according to the formula:

$$CF=W*100 / Ls^3,$$

where:

W - fish mass (g);

Ls - fish standard length (cm).

The condition factor reflects the physiological state of the fish and the availability of food (Le Cren, 1951), which may indicate their well-being in the environment (Morado et al., 2017). A higher value of this index shows a better condition of the fish.

The internal organs were then carefully separated and the liver, spleen, heart, and visceral fat were weighed. The hepatosomatic index (HSI) was determined as a percentage of the weight of the liver in relation to body weight. Hepatosomatic index (HSI) and the condition factor (CF) are important in assessing the resources provided by the environmental that are available to the fish (Rizzo & Bazzoli, 2020).

Biochemical analyses for fish meat were performed in duplicate and consisted in the determination of moisture, ash, total protein content, fatty substances and energy value of the meat. All samples were treated under the same conditions. At the beginning of the analysis, the samples were allowed to reach room temperature and were homogenized.

The moisture was determined by drying 5 g of the sample in an oven at 130°C to constant mass. The ash was calculated by calcining the sample in the calcination furnace at 600°C for 4 hours. The Kjeldahl method was used to determine the total nitrogen content of the sample, then multiplied by 6.25 (protein conversion factor). To determine the content of fatty substances, the Soxhlet method of fat extraction with solvents was applied (AOAC, 2004).

The calculation of the energy value is based on the following conversion factors: 4 kcal/g for proteins, respectively 9 kcal/g for lipids, according to Regulation (EU) No 1169/2011.

Data analysis

The data obtained were presented as mean ± standard deviation. One-way analysis (ANOVA) was used to assess the differences between the means, followed by the Bartlet test to verify the homogeneity of the variances and the t-test to compare the means of each parameter determined. The differences were considered significant at values of $P<0.05$.

RESULTS AND DISCUSSIONS

Biometric and bioindicator data, including fish mass and standard length, as well as hepatosomatic index and visceral fat, are presented as mean ± standard deviation in Table 1.

The results showed variations in fish mass for close standard lengths, especially in the case of aquaculture fish. This can be explained by different food availability in the two environments, fish reared in earthen pond having only a small part of the artificial food, being forced to procure natural food existing in the environment (Resen et al., 2017).

The average weight for the specimens collected from the earthen pond, floating net cages and from the natural environment (Danube River) was 1614.23 g, 1889.39 g, respectively 1601.5 g.

Table 1. Biometric data and fish bioindicators from 3 different sources

Fish source		Weight (g)	Standard length (cm)	CF	HIS (%)	Visceral fat (%)
		mean ± sd				
Farmed	Pond (CB)	1,614.23 ± 98.8	35.72 ± 0.71	3.54 ± 0.21	2.02 ± 0.2	1.33 ± 0.06
	Floating net cage (CV)	1,889.39 ± 113.4	36.81 ± 1.88	3.81 ± 0.39	2.15 ± 0.18	1.77 ± 0.04
Wild/Danube River (CD)		1,601.5 ± 22.9	42.92 ± 146	2.04 ± 0.18	1.63 ± 0.04	0.05 ± 0.01

The average standard lengths were 35.72 cm for CB, 36.81 cm for CV and 42.92 cm for CD. Based on these data, the Fulton condition factor (CF) was calculated, an indicator that provides information about the physiological state of the fish (Lima et al., 2002).

The highest value of the Fulton condition factor was recorded for aquaculture fish, caught from floating net cages (CF = 3.81), followed by that of fish reared in the earthen pond (CF = 3.52), while for fish caught from the natural environment (Danube River) the factor had the lowest value (CF = 2.04) (Figure 1).

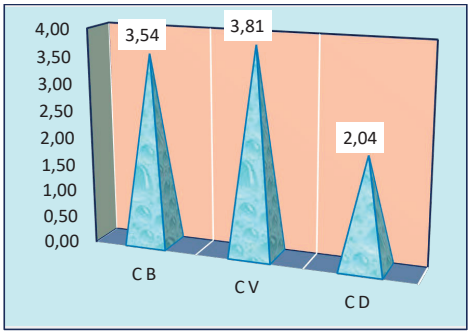

Figure 1. Fulton condition factor for fish from 3 different environments

A Fulton coefficient higher than 1 indicates a good condition of the fish in the studied population (Datta et al., 2013). The fact that the highest value was recorded for specimens reared in floating net cages shows that they had better access to food than other specimens, who were not given additional food, having only food available in the environment in which they lived.

The results of the statistical analysis for the hepatosomatic index (HSI) showed significant differences between the samples studied from three environments ($P < 0.05$). However, the values recorded in CB and CV indicated insignificant differences ($P = 0.23$). Highly significant differences were between CB and CD ($P = 0.0003$), as well as between CV and CD ($P<0.05$). The highest mean value of the hepatosomatic index was recorded in CV, being 6.17% higher than in CB and approximately 32% higher than in CD (Figure 2). The results obtained in this study for HSI were higher than those of Sharma & Ram (2020) who studied the hepatosomatic index in different age groups for common carp.

Gebremichael et al. (2021) obtained a hepatosomatic index value of 2.17% for common carp fed a diet in which fishmeal was 100% replaced by black solider fly meal.

For *Clarias gariepinus*, in 2017, Adesina observed a progressive decrease in the values of the hepatosomatic index with the increase of the level of cooked sunflower seed flour, as replacement for soy flour in feeding diets (from 3.11% to 0.87%).

Regarding the amount of visceral fat of the analysed samples, the statistical tests indicated highly significant differences ($P<0.01$) between the samples captured from the 3 sources. The highest amount of visceral fat was observed in fish reared in floating net cages (CV) (1.77%), while wild fish, from CD, had the lowest amount (0.05%) (Figure 2).

In fish meat, fat plays a very important role, increasing its nutritional value and improving sensory characteristics (Ceballos et al., 2020).

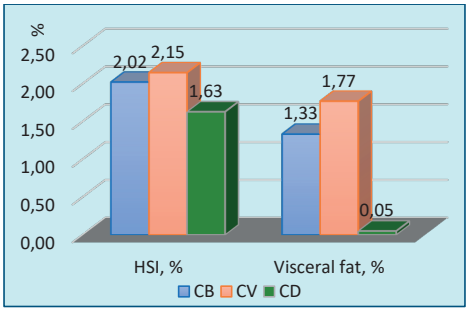

Figure 2. Hepatosomatic index and visceral fat in common carp reared in 3 different sources

However, high fat deposits in the abdominal cavity can have a negative impact on the consumer.

The biochemical composition of fish meat from the 3 sources studied is presented in Table 2.

The results indicated differences in the percentage of protein in the meat of the studied specimens. The highest protein content was recorded in the samples captured from CB (18.55%), followed by those from CD (18.18%), while the lowest percentage was recorded in the CV samples (16.91%) (Figure 3), the results being comparable to those obtained by (Xiu-Ping et al., 2017).

Table 2. Biochemical composition of fish from different sources

Fish source	Proteins (g%)	Lipids (g%)	Moisture (g%)	Ash (g%)	Energy value (kcal/100 g)
CB	18.55 ± 1.01	4.81 ± 1.11	74.93 ± 0.71	1.31 ± 0.21	117.50
CV	16.91 ± 0.38	10.43 ± 0.93	71.40 ± 1.09	0.83 ± 0.03	161.54
CD	18.18 ± 0.08	1.47 ± 0.02	78.81 ± 0.14	1.20 ± 0.03	85.98

Statistical analysis of the data obtained showed that the percentage of meat protein was significantly higher in CB ($P = 0.00074$) compared to CV, but there were no significant differences between CB and CD ($P = 0.29$). Significant differences existed between CV and CD ($P<0.05$).

Protein content is important when it comes to the texture and quality of fish meat. A low-protein meat tends to lose more water during cooking, which affects the texture of the meat. (Afkham et al., 2011).

In terms of moisture, the highest water content was in the CD samples (78.81%), while the CV samples had the lowest amount (71.40%) (Figure 4), the differences recorded being significant ($P<0.05$).

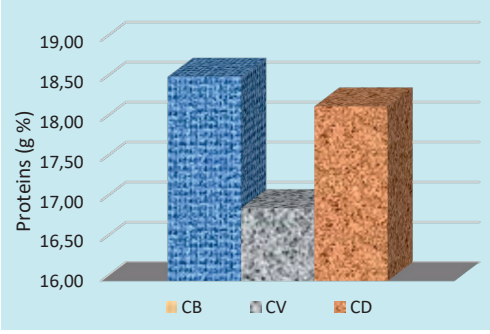

Figure 3. Protein content of *Cyprinus carpio* from 3 sources

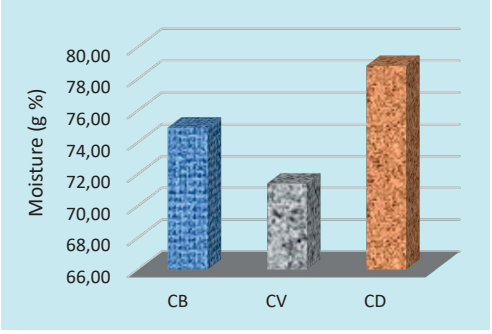

Figure 4. Moiesture content of *Cyprinus carpio* from 3 sources

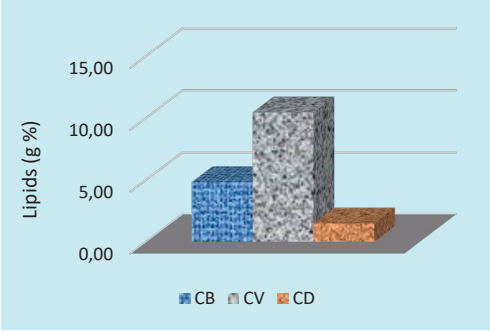

Figure 5. Lipids content of *Cyprinus carpio* from 3 sources

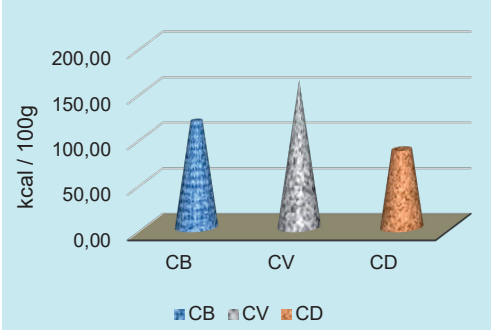

Figure 6. Energy value of *Cyprinus carpio* from 3 sources

It is known that the fat content of meat increases with the accumulation of biomass, this being associated with a decrease in water content. However, the main factor that influences the fat content is diet. (Fauconneau et al., 1995). The carbohydrate content of artificial food increases the fat content of meat.

Analysing the lipid content of meat, a high value is observed in the meat of the CV samples (10.43%), followed by CB (4.81%) and CD (1.47), the differences between all sources being highly significant ($P<0.01$) (Figure 5). The fact that the specimens reared in the floating net cages had access to artificial food, determined the accumulation of lipids in the muscle tissue, unlike the specimens from the wild environment, which had to compete for food.

The results obtained in this study regarding the lipid content are comparable to those obtained by Resen et al. (2017) and Mocanu et al. (2019), for common carp. Similar values were obtained for other freshwater fish (Kamal et al., 2007; Hama & Kamel, 2013).

Due to the high values for lipid and protein content, the energy value of fish in aquaculture was significantly higher (117.5 kcal/100 g in CB, respectively 161.54 kcal/100 g in CV), compared to wild fish (85.98 kcal/100 g) (Figure 6), similar results were obtained by Blazhekovikj & Ahmed (2020) for wild and aquaculture carp in Macedonia.

CONCLUSIONS

In conclusion, this study showed that fish from aquaculture have a better state of maintenance than those in the wild, as evidenced by the value of the higher Fulton index.

However, wild carp have much lower hepatosomatic index and visceral fat values than aquaculture fish, because additional feed leads to the accumulation of fat in tissues and around organs.

Regarding the quality of carp meat, it was influenced by the administration of feed, which led to an increase in lipid content in CV and CB. Higher fat levels have contributed to increase the energy value of fish in aquaculture.

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THE SYNERGISTIC EFFECT OF TECHNOMOS® PREBIOTIC AND BETAPLUS® PROBIOTIC ON THE GROWTH AND BIOCHEMICAL COMPOSITION OF NILE TILAPIA JUVENILES (*OREOCHROMIS NILOTICUS*, LINNAEUS, 1758)

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Abstract

This study aimed to evaluate the effect of additives used in tilapia feed and represented by TechnoMos® prebiotic (Saccharomyces cerevisiae) and BetaPlus® probiotic (Bacillus subtilis and Bacillus licheniformis) on the growth and biochemical composition of biological material. The analysis of the results obtained on the principal growth indicators shows a positive correlation between the applied feeding regime and the biomass gain. In the first experimental stage, there was an upward trend of biomass gain depending on the feeding regime applied, obtaining values between 1439 ± 18.29 g in the control variant (V0) and 1774.61 ± 47.65 g in the experimental variant with synbiotic (V3). In the second experimental stage, the biomass gain obtained varied from 3286 ± 15.05 g in the control variant to 4078.52 ± 69.84 g in the synbiotic variant. Biochemical analysis of tilapia muscle tissue showed an increase in the content of proteins, lipids in biomass of the samples fed with the probiotic Betaplus® correlated with the increase in biomass weight, compared to the control variant. In conclusion, the analysis of growth indicators shows the beneficial effect of symbiotic variant through their synergistic action and the influence of probiotic applied on improving biomass gain and biochemical composition.

Key words*: biochemical composition, growth, prebiotic, probiotic, tilapia.*

INTRODUCTION

The Nile Tilapia ranks second in the world in terms of importance as an aquatic animal due to its high demand, fast growth, and reasonable prices (El Asely et al., 2020a, 2020b). Often a single type of food additive can be used to feed fish, but using a mixture with several strains than one type appears to be more effective (Dawood et al., 2015; Yilmaz et al., 2019; Yılmaz et al., 2020).

Probiotics and prebiotics for fish are commercially available in powder form. They are generally added directly to fish ponds or mixed with feed ingredients. In any case, these approaches can drastically reduce the survival rates of probiotic cells. Incorporation of probiotics and prebiotics into pelleted fish feed could guarantee more than fish would get those functional ingredients with synergic effects. It was reported that the fingerlings fed with mixed probiotic pellets had a higher percentage of weight gain and specific growth rate, while the feed conversion rate was lower than the mixed probiotic fodder (Sivakumar et al., 2020).

The genus *Bacillus* is most studied as a host-associated probiotic for a wide variety of aquatic animals. The introduction of probiotics into aquaculture began with the advent of their use of scientific and sustainable technologies such as recirculating aquaculture systems, zero-water aquaculture production systems, and biofloc technologies.

Bacillus sp. are rich in exogenous enzymes such as lipase, protease, amylase, phytase, chitinases, cellulases, and β-1,3-glucanases, which help break down nutrients and improve digestion (Wang, 2007; Liu et al., 2009; Wu et al., 2012; Soltani et al., 2019).

The mixture of *Bacillus subtilis* and *Bacillus licheniformis* has shown significant benefits in aquaculture, including improved growth

performance, immunomodulation, and survival rate (Abarike et al., 2018).

The application of probiotics in RAS has improved water quality, feeding efficiency, and the growth of various crop organisms (Rurangwa & Verdegen, 2014). Addition of *Bacillus subtilis* to the batch sequencing reactor (SBR) of RAS has increased the efficiency of sludge treatment (Lu et al., 2012). Another study performed in RAS with dietary supplements of *Bacillus subtilis* improved growth performance, immunity, and genetically modified tilapia disease resistance (Zhu et al., 2019).

Prebiotics are used as indigestible food ingredients metabolized by bacteria or probiotics that promote health (Ringo et al., 2016). Prebiotics reduce fish mortality caused by the invasion of pathogens and intensify the biological responses of the host. The use of mannan oligosaccharides (MOS), the insulin that is obtained from the yeast cell wall, *Saccharomyces cerevisiae*, and prebiotics such as fructooligosaccharides (FOS) are immunostimulators that improve the immune response and disease resistance to fish (Carbone & Faggio, 2016).

Synbiotics are products that contain both probiotics and prebiotics (Akrami et al., 2015). The use of symbiotics in aquaculture is considered recent, and studies indicate positive effects on the host, related to enzymatic digestion, production of acetic, lactic, and butyric acids (products of prebiotic fermentation by probiotic bacteria), and activation of the innate immune system. (Huynh et al., 2017; Ringø and Song, 2016).

The positive effect of using two or more feed additives results in three modes, namely: additivity, synergism, or potentiation (Meseguer and Cerezuela, 2011). Thus, the action of the probiotic bacteria have used may be increased by prebiotics due to the contribution of this component to the metabolism and activation of the growth of these bacteria (Akhter et al., 2015).

The present study aims to investigate the synergistic effects of supplementation of a probiotic *(Bacillus subtilis* and *Bacillus licheniformis*) and the prebiotic (yeast extract *Saccharomyces cerevisiae,* MOS, β-1,3-glucans) on growth and biochemical composition muscle tissue of the juvenile tilapia (*Oreochromis niloticus*).

MATERIALS AND METHODS

Experimental design

The study was carried out during an experiment organized in two stages, using the pilot recirculating system within the Department of Foood Science, Food Engineering, Biotechnology, and Aquaculture, University "Dunarea de Jos" of Galați. The experimental recirculating aquaculture system used is provided with 12 growth units (glass growth units with a thickness of 10 mm) with a volume of 0.132 m^3 each (36 × 37.5 × 98 cm), described by Mocanu et al. (2011). Removal of residual solids from the growth units resulting from metabolism has been performed employing a pressure sand filter, while a biological filtration unit was used to control the concentration of ammoniacal nitrogen produced by the culture biomass - trickling filter. The sterilization and disinfection of the water on the essential supply water supply circuit of the growth units was performed on the Tetra Quiet UV-C 35000 sterilization, with a power of 36 W. These water remediation steps is performed using three pumps type *DAB A 80 180 XM* (flow rate: Q = 0.6 - 8.7 m^3/h). To ensure the required dissolved oxygen (DO) has been using a compressor *Fiap Air Active 10000* type with a power of 100 W, pressure 0.042 MPa, which introduces an airflow of 8400 l/h.

The biological material (Nile tilapia) was obtained after reproduction of the mature tilapia, previously grown in the existing recirculating aquaculture system within the department. This biological material was at that time in the juvenile stage and had three months in the first stage and five months in the second experimental stage. Its sorting was performed and a homogeneous batch of fish was retained and distributed randomly in the 12 growth units of the recirculating aquaculture system.

The present study was carried out in two experimental stages, and four experimental variants have been established in triplicate, namely: Control variant (V_0) - without probiotics and prebiotics; Probiotic variant (V_1) - with BetaPlus® probiotics – 1%×BW; Prebiotic variant (V_2) - with TechnoMos® prebiotics – 1%×BW; Synbiotic variant (V_3) - with BetaPlus® probiotics and TechnoMos® prebiotics - 1:1% × BW.

For the first stage was used 960 fish, with an average weight of 1.52 g/fish, and in the second stage 360 fish with an average weight of 73.96 g/fish. During the first experimental stage (40 days), the fish were fed with commercial extruded feed ALLER FUTURA EX, with a protein content of 64% and 12%, the diameter of the granules being between 0.5-1 mm, at a feeding frequency of three meals per day (Table 1). In the second experimental stage (50 days), the fish were fed with ALLER SILVER, with a content of 45% crude protein and 20% lipids, the diameter of the pellets being 2 mm. Feeding intensity was calculated, and feeding frequency was twice daily. The biochemical composition of the feed were shown in Table 1.

Table 1. Biochemical composition of fodder pellets used.

Biochemical composition	ALLER FUTURA EX	ALLER SILVER
Crude protein	64%	45%
Fat	12%	20%
Crude cellulose	0.5%	2%
Crude ash	11%	8.1%
Phosphor	1.5%	1%
NFE	5%	16%
Digestible energy	5035/21 kcal kg^{-1}	5035/21 kcal kg^{-1}
Vitamin A	10000 UI kg^{-1}	10000 UI kg^{-1}
Vitamin D3	1000 UI kg^{-1}	1000 UI kg^{-1}
Vitamin E	400 UI kg^{-1}	200 UI kg^{-1}
Vitamin C	180 UI kg^{-1}	150 UI kg^{-1}
Cystine	0.4%	0.6%
Lysine	2.5%	2.4%
Methionine	0.50%	0.75%

During the experimental period, the daily administered ratio was updated every ten days and, the specific quantities of the two feed additives applied had been calculated, (TechnoMos® prebiotic and BetaPlus® probiotic) and incorporated into feed. TechnoMos® prebiotic is an extract from selected yeast, obtained from *Saccharomyces cerevisiae*, rich in mannan oligosaccharides and beta-glucans (β-1,3-glucans). The probiotic BetaPlus® consists of BioPlus® *2B* and betaine (nitrogenous substance), the concentration being 1×10^{12} CFU/kg feed and betaine - 936 000 mg/kg. BioPlus® *2B* is a 1: 1 mixture of *Bacillus licheniformis* (DSM 5749) and *Bacillus subtilis* (DSM 5750). These prebiotics and probiotics used are supplied by the company Biochem from Lohne, Germany through the Romanian subsidiary Biochem Animal Health and Nutrition affiliated to the one from Lohne and located in Cluj-Napoca, Romania. The prebiotic and probiotic embedding protocol was described by Bocioc E. (2011).

The water quality parameters in the RAS were monitored using the following equipment: the dissolved oxygen concentration was measured with the Hanna HI 98186 oximeter; the pH was measured with the pH meter WTW, model 340; the nitrogen compounds concentrations were measured using the Spectroquant NOVA 400 portable spectrophotometer, using compatible kits from Merck.

Technological indicators assessment

At the end of the each experimental stage, the following technological indicators were calculated:

- Individual Weight Gain (IWG) = Final Weight (Wt)–Initial Weight (W0) (g);
- Weight Gain (W) = Final Weight (Wt) – Initial Weight (W0) (g);
- Relative Weight Gain (RWG %) = (Wt–W0) × 100/Wt;
- Fulton condition factor (K) =$100 \times W/L^3$.

Somatic measurements were made at the end of each experimental stage at all fish. Total length (TL) and body weight (BW) for each variant were used to determine the relationship $W=a \times Lb$, where "a" is the intercept (the initial growth coefficient), and "b" is the allometric coefficient (Ricker, 1975). The coefficient of variation (CV, %) was calculated as the ratio of the standard deviation to the mean of weight to have a measure of fish dispersion.

Biochemical composition of tissue

Proteins were determined with Gerhardt equipment by using the Kjeldahl method, fats were determined by Soxhlet solvent extraction method (petroleum ether) with Raypa extraction equipment, the dry matter was determined by heating at a temperature of 105±2ºC using Sterilizer Esac and ash was evaluated by calcification at temperatures of 550±20°C, in a Nabertherm furnace.

Statistical analysis

Data were analyzed using SPSS 21 for Windows. Results regarding fish growth performance and the biochemical composition of the tissue were expressed by average and standard deviation (Average±SD).

Kolmogorov-Smirnov tests determined the normality of the data used for analysis. Oneway

ANOVA and Duncan's multiple range tests were used to compare the differences between the experimental groups. Significance was determined at $\alpha = 0.05$.

RESULTS AND DISCUSSIONS

Water quality

Oreochromis niloticus (tilapia) is a thermophilic species, the water temperature was the main parameter that had to be kept in the optimal range. In the first experimental stage, the temperature of the water varied between 27.00-28.80°C, with an average value of 27.82±0.48°C, and in the second experimental stage, the temperature remained the same, with a variation between 27.20-28.90°C and an average of 28.34±0.44°C (Figure 1).

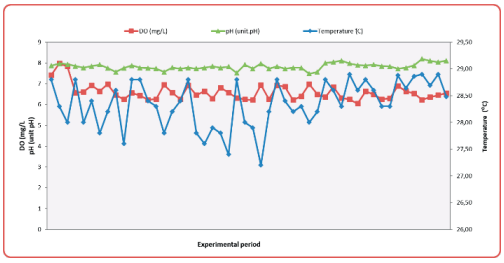


Figure 1. Temperature, dissolved oxygen, and pH during the experimental period

During the experimental period, dissolved oxygen was maintained between 6.25-7.98 mg/L, with a mean value of 7.28±0.48 mg/L in the first stage and a range between 6.05-7.98 mg/L, with a mean of 6.59±0.38 mg/L in the second stage (Figure 1). The pH values recorded during the experimental period varied between 7.45-7.97 pH units, with an average of 7.77±0.12 pH units in the first stage and a range between 7.48-8.20 pH units, with a mean of 7.83±0.15 pH units in the second experimental stage (Figure 1).

Nitrites ($N\text{-}NO_2^-$) recorded during the experimental period remained in the range of 0.03-0.09 mg/L, and nitrates ($N\text{-}NO_3^-$) indicated values between 18.4-21.4 mg/L. Ammonium ion (NH_4^+) values ranged from 0.02-0.06 mg/L. All the registered values of nitrogen compounds have shown in Figure 2.

In the current experimental period, the water quality parameters were kept within the optimal range of *Oreochromis niloticus*, as evidenced by the fact that the water conditioning equipment was able to treat and reuse the water efficiently, achieving a daily water exchange in the proportion of 10-15%.

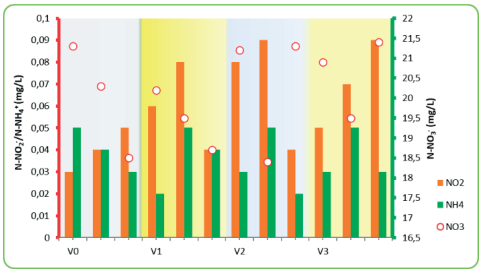


Figure 2. Nitrogen compounds during the experimental period

Water quality indicators during the experimental period had maintained in the optimal range of the species (Nile tilapia), except for nitrate values, which due to the low water exchange showed a minimum exceedance of the optimal interval of the crop species.

Fish growth

The study on the main determination of the synergistic effect of TechnoMos® prebiotic and BetaPlus® probiotic on the growth indicators of juveniles tilapia (*Oreochromis niloticus*) was carried out in two experimental stages. At the beginning of the experimental period, the average individual biomass for the four experimental groups did not show significant differences ($p>0.05$). The distribution of the groups in terms of body mass variability showed normal distribution, an aspect also highlighted by the Kolmogorov-Smirnov test ($p>0.05$). Table 2 summarizes the technological indicators calculated for the first experimental stage.

During the experimental trial, periodically weighing had performed (at an interval of 10 days) to update the administered ratio and, respectively, the quantities of prebiotics and probiotics applied in the feed. In Figure 3 is presented the evolution of fish biomass during the first experimental stage. From Figure 3, it can be noticed the ascending trend of the variants in which the synbiotic was applied, compared to the control variant. There is a more pronounced increase in the last period of the stage, especially in the case of the experimental variant (V_3) where the TechnoMos® prebiotic and the BetaPlus® probiotic were administered in a ratio of 1:1.

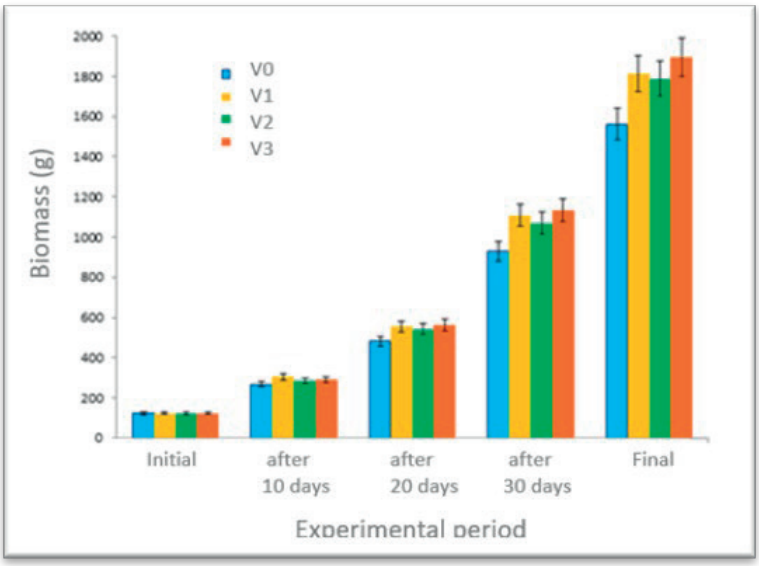


Figure 3. Evolution of biomass during the first experimental stage

Table 2. Technological indicators of the juveniles tilapia in the first experimental stage

Technological indicators	Control (V_0)	Probiotic (V_1)	Prebiotic (V_2)	Synbiotic (V_3)
The initial number of fish	80	80	80	80
The final number of fish	80	80	80	80
Survival rate (%)	100	100	100	100
Initial biomass (g)	121.80±0.32	122.21±0.29	121.70±0.23	122.48±0.62
Initial biomass (kg m^{-3})	0.92±0.00	0.92±0.00	0.93±0.00	0.93±0.00
Final biomass (g)	1561±18.51	1813.26±28.52	1788.58±80.29	1897.09±48.23
Final biomass (kg m^{-3})	11.83±0.14	13.55±0.61	13.74±0.22	14.34±0.37
Weight gain (g)	1439±18.29	1691.05±28.71	1666.88±18.29	1774.61±47.65
Weight gain (kg m-^{3})	10.90±0.14	1691.05±28.71	1666.88±18.29	1774.61±47.65
Initial weight (g $fish^{-1)}$	1.52±0.01	1.53±0.03	1.52±0.02	1.53±0.01
Final weight (g $fish^{-1}$)	19.52±0.23	22.67±0.36	22.36±1.00	23.71±0,.0
Individual weight gain (g)	17.99±0.23	21.14±0.36	20.48±1.00	22.18±0.60
Relative Weight Gain (%)	92.20±0.08	93.19±0.29	93.26±0.12	93.54±0.13

Note: Data are presented as triplicate mean ± SD.

Table 3. Technological indicators of the juveniles tilapia in the second experimental stage

Technological indicators	Control (V_0)	Probiotic (V_1)	Prebiotic (V_2)	Synbiotic (V_3)
The initial number of fish	30	30	30	30
The final number of fish	30	30	30	30
Survival rate (%)	100	100	100	100
Initial biomass (g)	1924±5.93	2320.68±8.66	2221.74±31.34	2408.82±8.71
Initial biomass (kg m^{-3})	14.58±0.04	17.58±0.07	16.83±0.24	18.25±0.07
Final biomass (g)	5210±120.47	6120±208.22	5731.33±198.35	6487.33±62.14
Final biomass (kg m^{-3})	39.47±0.91	46.36±1.58	43.42±1.50	49.15±0.47
Weight gain (g)	3286±115.05	3799.32±205.47	3509.59±181.31	4078.52±69.84
Weight gain (kg m-^{3})	24.89±0.87	28.78±1.56	26.59±1.37	30.90±0.53
Initial weight (g $fish^{-1)}$	64±8.08	77.36±9.5	74.06±10,20	80.29±8.22
Final weight (g $fish^{-1}$)	173.67±4.02	204±6.94	191.04±6,61	216.24±2.07
Individual weight gain (g)	109.52±3.83	126.67±6.85	116.99±6.04	135.95±2.33
Relative Weight Gain (%)	63.05±0,76	62.05±1.26	61.21±1.08	62.87±0,48
Fulton condition factor (K)	1.83±0.03	1.83±0.01	1.83±0.02	1.85±0.02

Note: Data are presented as triplicate mean ± SD.

Some researchers have reported that certain probiotics administered in feed did not have any effect on growth. Thus, the non-viable yeast *Saccharomyces cerevisiae* (Marzouk et al., 2008), *Pseudomonas* spp. (El-Rhman et al., 2009), *Enterococcus faecium* (Biomate SF-20®), *Bacillus subtilis* + *Bacillus licheniformis* (Bioplus 2B®), viable yeasts of *Saccharomyces cerevisiae* under the trade name Levucell SB 20® (Shelby et al., 2006) have shown that these probiotics do not influence the growth of tilapia. Table 3 shows the technological indicators calculated for the second experimental stage by processing the initial and final obtained data.

Although at the end of the first experimental stage, the statistical analysis did not show significant differences between the body mass corresponding to the four experimental variants (ANOVA, $p>0.05$), after this period of maintaining the same experimental conditions, a significant increase of fish in the group with synbiotic (V_3) was noticed. At the beginning of the second experimental stage, the post hoc analysis, Duncan, revealed four subsets of values, corresponding to each experimental variant, and the average individual mass in the four experimental variants was 80.29±8.22 g for the variant with symbiotic, 77.36±9.5 g, for the probiotic variant, 74.06±10.20 g for the prebiotic variant, respectively 64±8.08 g for the control variant. Post-hoc testing had performed after Levene pretesting which confirmed the homogeneity of the variance of values ($p>0.05$).

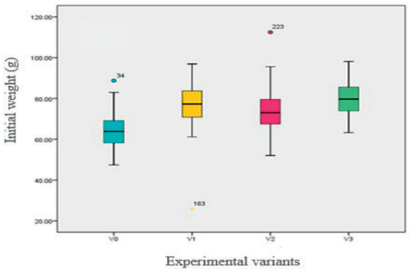


Figure 4. The variation of the initial body masses - boxplot (median, minimum, maximum values and quartiles) in the four experimental variants

Also, at the end of the second experimental stage, the statistical analysis showed significant differences between the four experimental groups (ANOVA, $p<0.05$), the Duncan test highlighting the same four growth subgroups, as at the beginning of the stage. Thus, the highest average body mass was also obtained in variant V_3 (216.24±2.07 g), followed by variant V_1 (204±6.94 g) and V_2 (191.04±6.61 g) while in the control variant (V_0) the body weight was 173.67±4.02 g (Figures 4 and 5).

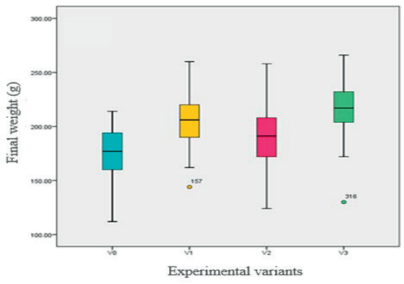


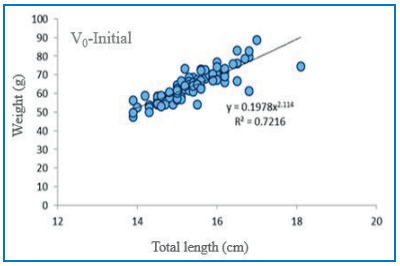
Figure 5. The variation of the final body masses - boxplot (median, minimum, maximum values and quartiles) in the four experimental variants

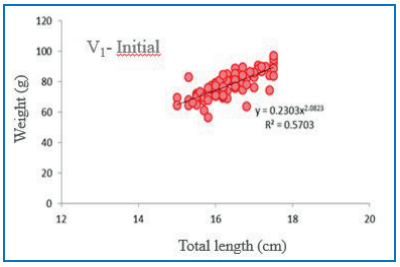
The coefficient of variation calculated for the individual mass of the initial groups registered values between 10.24-13.77%, an aspect that suggests the homogeneity of the lots (Table 4). From the analysis of the coefficient of variation at the end of the experimental stage, it has observed the increase of the homogeneity of the batch in the variant with probiotic, where a coefficient of variation of 10.68% was obtained, while in the control variant, CV increased by approximately 6.90 %, an aspect that underlines the appearance of heterogeneity. The determination of the correlation between total length (cm) and body mass (g) was made based on data obtained from biometrics performed at the beginning and end of the second experimental stage for fish in each variant. Thus, the data obtained from the biometric measurements were processed to determine the growth equations. Growth estimation was performed using power regression: $W = a \times Lt^b$. The correlations between length and body mass for each experimental variant are shown in Figures 6 and 7.

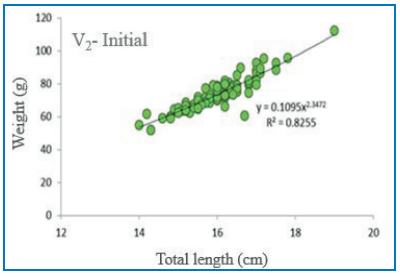
Analysing the values of the coefficient "b" negative allometry was observed in the case of all experimental variants respectively the increase of the body mass was achieved faster than the increase of the total length. A better value of the allometric coefficient was obtained in the synbiotic variant (V_3), where the growth also highlighted higher values.

Table 4. Coefficient of variation (CV %) for the experimental groups at the beginning and the end of the second experimental stage

Experimental variant		V_0	V_1	V_2	V_3
Initial coefficient of variation (%)	Lean body mass	12.55	10.51	13.72	10.17
	Total length	5.04	3.86	5.25	3.83
Final coefficient of variation (%)	Lean body mass	13.45	10.4	13.43	10.57
	Total length	5.34	3.78	5.58	3.92







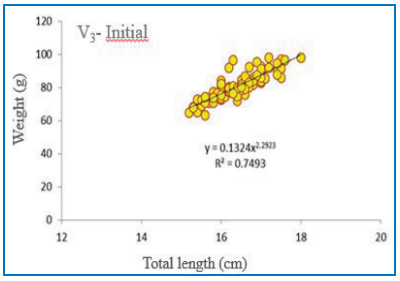
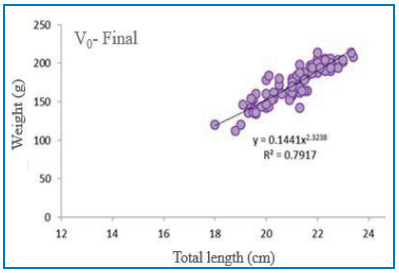
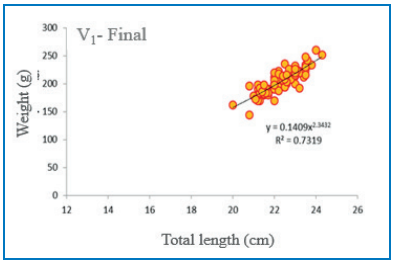
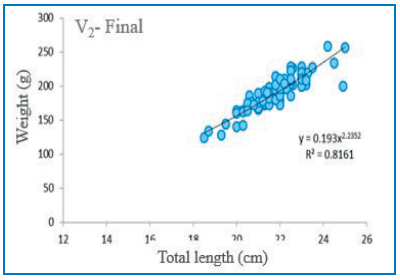


Figure 6. Length-Body mass correlation at the beginning of the second stage in the four experimental variants







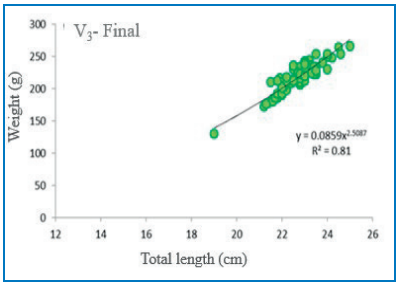


Figure 7. Length-Body mass correlation at the end of the second stage in the four experimental variants

Numerous researchers have reported that feeding tilapia with probiotics has led to improved growth. Tilapia fed on *Saccharomyces cerevisiae* (Lara-Flores et al., 2003, 2010), *Bacillus subtilis* + *Saccharomyces cerevisiae* (Marzouk et al., 2008, Lara-Flores et al., 2003, 2010), *Bacillus subtilus, Lactobacillus plantarum, Bacillus subtilis* + *Lactobacillus plantarum* (Essa et al., 2010), commercial mixtures of probiotics such as Biogen® (Ghazalah et al., 2010; El-Haroun et al., 2006; Mehrim, 2009), and Premalac® (Ghazalah et al., 2010) have been shown to contribute to growth improvement.

The use of both *Bacillus subtilis*, *Bacillus licheniformis*, and the combination showed a significant improvement in tilapia growth. The capacity of bacteria *Bacillus* sp. to contribute to growth improvement is dose-dependent (Elsabagh et al., 2018).

The biochemical composition of muscle tissue

Evaluation of the synergistic effect of Technomos® prebiotic and Betaplus® probiotic in the administered feed on the retention of nutrients in fish meat involved a comparative analysis of the biochemical composition of the muscle tissue of the Nile tilapia at the end of each experimental stage. Following the biochemical analyses performed at the end of the first stage, in the four experimental variants, the statistical analysis did not show significant differences ($p>0.05$) for the moisture, lipids and ash content. Regarding the percentage of protein content, there were significant differences between the variants ($p<0.05$). Thus, the experimental variant with probiotic registered significant differences compared to the control variant ($p<0.05$), respectively, compared to the prebiotic variant $p<0.05$), but without significant differences in the synbiotic variant (V_3).

The results obtained have correlated with data from the literature where similar effects have been reported (Hasan, 2007). The average values of the biochemical parameters of the fish meat obtained at the end of the first experimental stage in the four variants are represented in Figure 8.

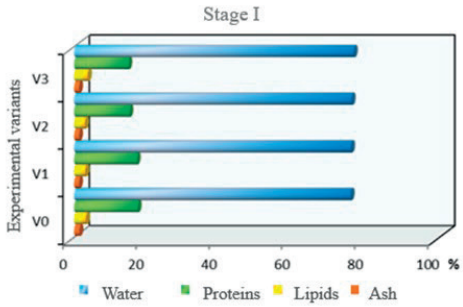


Figure 8. Comparative evolution regarding the biochemical composition of the tilapia muscle at the end of the first experimental stage

The biochemical analyses of the muscle tissue performed at the end of the second stage show significant differences ($p<0.05$) between the experimental variants in terms of the percentage content of water ($p<0.05$), proteins ($p<0.05$) and dry matter ($p<0.05$). The water content (water) of the muscles indicates significant differences in the samples collected from the probiotic variant (74.96%) compared to the control variant (74.9%), respectively with the variant where prebiotic was applied (75.31%) ($p<0.05$), but insignificant with the variant in which synbiotic was administrated (76.11%).

The content of total protein in the muscle tissue of the control variant (V_0) and the probiotic variant (V_1) showed significant differences with as well as between the control and the prebiotic variant (V_2), but no significant differences in the variant with synbiotic (V_3).

The average values obtained for lipids are following the water values from the analysed samples, noting an increase in the case of the probiotic variant (V_1), but without significant differences with the other experimental variants ($p>0.05$). Similar results that indicated the beneficial effects of incorporating oils from different sources in the diet of common carp and are related to the values obtained by Manjappa et al. (2002), in the experiment performed on carp (*Cyprinus carpio*) with an individual weight of 2.13–2.21 g, over 120 days, fed diets in which the concentration of lipids varied. The biochemical parameters of the muscle tissue analysed in the second experimental stage are shown in Figure 9.

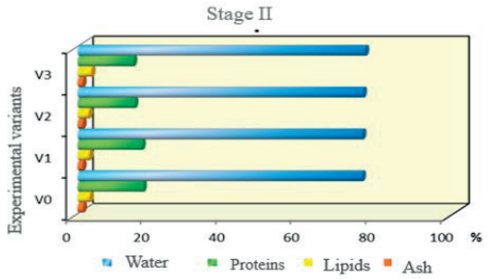


Figure 9. Comparative evolution regarding the biochemical composition of the tilapia muscle at the end of the second experimental stage

The results regarding the biochemical composition showed at higher quality of the muscular tissue in the variant with the probiotic Betaplus®, aspect emphasized by decreasing of the water content respectively increasing of the lipids and proteins content in the body mass compared to the control variant and the other experimental variants. The incorporation of oils in feed diets administered to carp has led to a decrease in water content in favor of increasing the concentration of lipids and proteins in body mass, as weight gain (Mocanu et al., 2020).

CONCLUSIONS

The present study showed that the supplementation of feed administered with synbiotic has a beneficial effect on growth indicators by the synergistic action of the combination of prebiotic and probiotics, simultaneous the influence of the probiotics applied on the weight gain. Biochemical analysis of tilapia muscle tissue showed an increase in the content of proteins, lipids in the body mass of the samples fed with the probiotic Betaplus® correlated with the increase in body mass, compared to the control variant. In conclusion, the probiotic Betaplus® used as a feed additive both simple and combined with the prebiotic Technomos® indicates a synergistic effect on growth and influences the biochemical composition of muscle tissue.

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INNOVATIVE TREATMENT TO COMBAT PHILOPOD CRUSTACEAN (*CYZICUS* SP.) IN FISH NURSERIES

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Abstract

The paper includes the results of experiments on the combat of philopod crustacean (Cyzicus sp.) which cause significant economic losses to the post-embryonic development of common carp and Asian cyprinids (silver carp, bighead carp and grass carp). To combat them, the effectiveness of the insecticide Proteus-OD 110 was tested, correlated with the application of technological procedures for the preparation of the ponds adapted for the growth of fish larvae. The experiments were conducted at S.C.D.P. Nucet, within the Mircea-Vodă experimental base. In order to ensure a profitable harvest, before the stocking of 3-5 days old fish larvae, the ponds were prepared according to the technological procedures. Disinfection and loosening of the bottom of the ponds was achieved by administering quicklime (CaO) in the amount of 200-1000 kg/ha and to increase the productivity of the ponds, manure (5000-10000 kg/ha) and rice bran were administered. In conclusion, in order to obtain optimal results in the period of post-embryonic development and growth in first year of cyprinids, we recommend that the technological instructions be adapted to each type of technology.

Key words*: cyprinids fry, Cyzicus sp., Proteus OD 110 treatment.*

INTRODUCTION

Modern fish farming means improving cultivation practices by adopting various measures such as proper distribution of the supplementary diet, proper application of fertilizers, maintenance of physicochemical factors, proper selection of crop species, disease prevention and other control measures (Sultana et al., 2020).

During larval development of fish, special care must be taken to provide live food at such a high density that it does not require effort on the part of the fish larva to search and capture. In nursery ponds, fish have many enemies and competitors, such as wild fish, frogs, birds and invertebrates from which they should be protected. Aquatic crustacean *Cyzicus* sp. is one of the most common aquatic pests in fish nursery ponds.The adult *Cyzicus* sp. it is about 1 cm long, resembling a shell, with two chitinous valves. They consumes detritus, bacteria and algae, constantly filtering water. The presence in large numbers of adults crustaceans in the nursery ponds produces a marked turbidity of the water which causes losses of up to 100% among fish larvae. Pesticides (organophosphates, commercial insecticides such as Baytex, Dipterex, Dylox, Flibol, Fumadol, Masoten and Sumithion) are used to control aquatic insects and large zooplankton that grow in fish nursery ponds. Pesticides are a mixture of substances that are designed to control or slaughter or control the development of pests (undesirable organisms). These pests usually nematodes, microorganisms, insects and plant pathogens, that compete with human food and are responsible for transmitting diseases and destroying crops (Shefali et al., 2021).

In our country, to combat *Cyzicus* sp. Triclorfon was used (trade names: Clorofos, Onefon) (Oțel, 1989). Experiments over time have concluded that the application of this insecticide causes both complete destruction of zooplankton and serious damage to the liver of fish fry (Boz, 1998).

As an alternative to this we proceeded to test the effect of the insecticide Proteus OD 110 in the control of *Cyzicus* sp. This insecticide has multiple actions: systemic, contact and shock.

The formula is state of the art, oil dispersion (OD). Tiacloprid 100 g/l, Deltamethrin 10 g/l, gives the product an adhesion and a penetration of the systemic component superior to the other forms. Tiacloprid acts on the nervous system of insects, blocking the development of vital activities. Deltamethrin also acts on the nervous system of insects, having at the same time repellent and nutritional inhibitory effect on insects. Both components act by contact and ingestion on pests. The complex mechanism of action gives it a wide range of activities and allows the control of insecticide-resistant forms of other chemical groups.

MATERIALS AND METHODS

The Mircea-Vodă Experimental Base has a total area of 14.3 ha (of which 12 ha of water surface) and consists of 5 earthen ponds (with surfaces of 0.8-4.5 ha) equipped with specific exploitation facilities.

Within the Mircea-Vodă Experimental Base, (which belongs to SCDP Nucet), in the years 2020 and 2021, treatments were carried out to control the philopod crustacean *Cyzicus* sp. with the insecticide PROTEUS OD 110. According to the Safety Data Sheet of the product includes several ecological information, respectively, data on ecotoxic effects in fish, water fleas and algae.

Fish toxicity: LC50 Rainbow trout (*Oncorhynchus mykiss*) 0.386 mg/l, Exposure time: 96 hours

Daphnia toxicity: EC50 water fleas (*Daphnia magna*) 0.0427 mg/l Exposure time: 48 hours

Algae toxicity: IC 50 (*Pseudokirchneriella subcapitata*) 96.7 mg/l; Exposure time: 72 hours.This value refers to the active substance tiacloprid.

Algae toxicity: EC50 (*Pseudokirchneriella subcapitata*) > 9.1 mg/l

Exposure time: 96 hours This value refers to the active substance deltamethrin.

Several tests were performed to determine the optimal doses, lethal doses (LD) of Proteus OD 110 insecticide. For these tests aquariums (50 l) and growth ponds (0.8-4.5 ha) were used. In addition to dose setting, the aim was to determine how to use the product, as well as the effect of the product on the juveniles and fish fry.

Preparation of nursery ponds

The biotechnological works for the preparation of the nursery ponds destined for the larval development of cyprinids aim to ensure optimal environmental conditions by intensifying the natural productivity; ichthyopathological prevention; protection against predators; the possibility of fishing the fish fry completely, quickly and with diminished stress.

Regarding the growth of carp larvae in polyculture with Asian cyprinids, this is done in two distinct stages:

- stage I- postlarval growth up to the juvenile stage (usually done in nursery ponds);
- stage II - rearing the juvenile to the fry stage, usually in the same nursery ponds (Costache et al., 2006).

In the case of the Mircea-Vodă Experimental Base, these stages of growth for cyprinids in summer I are carried out in the same ponds. The synthetic scheme for the preparation and rearing of cyprinids in the first stage included the following technological sequences:

- ponds drying;
- administration of lime with the role of disinfection and loosening of the bottom of the ponds was achieved by administering quicklime (CaO) in the amount of 200-1000 kg/ha and to increase the productivity of the ponds, manure (5000-10000 kg/ha) and rice bran were administered. Due to their availability and low cost, rice bran is used as a fertilizer in ponds to increase production. Dominisac (1974) shows that the use of rice bran acts initially as a pesticide and later as a fertilizer (Rice bran: 400-600 g/m^2 at 5 cm water depth). This is a method that is accepted due to its double effect: control of predators and at the same time, fertilizer of the ponds.
- plowing and disking the bottom of the basins and where the situation requires must apply herbicides on the bottoms of dryed ponds and over the banks.
- flooding the ponds with technological water.
- 6-10 days before the fish larvae stocking, water samples are collected for chemical analysis to determine the content of biogenic substances for the administration of fertilizers. The composition of zooplankton is determined quantitatively and qualitatively. The availability of phytoplankton has a synergistic effect in

raising the growth performance and the survival rate of farmed fish in semi-intensive systems.
- if it is necessary, 9-10 days before the fish larvae stocking, the water is treated for the destruction of zooplankton (especially the crustacean *Cyzicus* sp. adult forms) with the insecticide Proteus OD 110.
- the stocking of 3-5 old fish larvae in nursery ponds should be done in sunny and warm weather, without wind (which can generate waves) and in more protected, shallow shore areas, which provide optimal conditions for theirs growth and development.
- when the amount of natural food (phyto and zooplankton) in the nursery ponds where treatments have been carried out to combat *Cyzicus* sp. has been reduced, the feed ration will be supplemented by the administration of fodder up to 15 kg/ha/day.

Among the most important factors that influence the growth and development in the early stages of fish life and cause significant mortality among the fish population, which negatively affect growth and cause poor health are largely common to both stages of growth:
- inadequate physico-chemical quality of the technological water and the culture ponds;
- availability of specific natural food in nursery ponds;
-presence of aquatic pests (mammals, water birds, predator fish, insects and predatory aquatic invertebrates found naturally in all freshwater bodies;
-cannibalism that occurs among carp juveniles, especially when the larvae and fry have differ in sizes;
-manifestation of extreme meteorological phenomena for long periods (strong winds or sudden temperature variation) especially in shallow water ponds;
-excessive growth of aquatic macro-vegetation and/or phytoplankton which may cause dissolved oxygen depletion especially during the nights and/or after several days of continuous rain and cloudy, causing asphyxiation of fish;
- oxygen supersaturation on hot sunny days can cause gas embolism;
-inherent toxicity due to certain algal blooms;
-diseases caused by ecto- and endoparasites;
-bacterial, fungal and viral infections that cause disease and mortality;
-anomalies and deficiencies of health due to nutritional deficiencies.

The data on the actual preparation of the ponds for growing in the first summer, in order to obtain good production results are included in Table 1.

Water quality was closely monitored during the growing season in the first summer. The main parameters of water quality were determined: temperature (°C), pH (digital pH-meter), transparency (Secchi disk), dissolved oxygen (DO, HQ40d HACH company), abundance and structure of phytoplankton and zooplankton.

In the first stage (up to the age of 30 days) for carp alevins raised in polyculture with Asian cyprinids, was administered artificial feed with a PB content of 32-40%. The ingredients included in the feed are: prestarter type flours, fish meal, powdered milk, brewers yeast, vitamin premixes, rice bran, cereal flours etc.

In the second stage of growth (30-130 days) the fodder has a PB content of 22-25%. The recipe includes traditional ingredients (wheat, corn, sunflower meal, rice bran, soy, etc.).

The main biotechnological works carried out in the years 2020 and 2021 at the Mircea Vodă Experimental Base carried out for polyculture nursery ponds of carp and Asian cyprinids are presented in Table 1.

RESULTS AND DISCUSSIONS

To control *Cyzicus* sp. with the insecticide Proteus OD 110 the therapeutic dose of 0.042 mg / l was established. Exposure time was 96 hours. For the treatment of *Cyzicus* sp. adults from fish ponds the recommended dose is 20 ml Proteus OD 110 insecticide per 10000 m^3.

Table 1. The main biotechnological works carried out in the years 2020 and 2021 at the Mircea Vodă Experimental Base

Year 2020					Year 2021				
EC1	**EC2**	**EC3**	**EC4**	**EC5**	**EC1**	**EC2**	**EC3**	**EC4**	**EC5**
Manure administration 5-10 t/ha					26.04 - Manure administration 5-10 t/ha				
01.05.2020 - administration of lime chloride (kg)					08.05.2020 - administration of lime chloride (kg)				
450	600	460	1000	200	460	600	460	1000	200
02.05.2020 - plowing and disking					11.05.2020 - plowing and disking				
yes	yes	-	yes	-	yes	yes	-	yes	-
08.05.2020 - herbicide ponds and banks					14.05.2021 - herbicide ponds and banks				
12.05.2020 - start water supply to the ponds					15.05.2021 - start water supply to the ponds				
18.05.2020 appearance *Cyzicus* sp.					22.05.2021 appearance *Cyzicus* sp.				
yes	yes	-	yes	-	yes	yes	-	yes	-
Combat treatment *Cyzicus* sp. with PROTEUS OD 110 20.05.2020					Combat treatment *Cyzicus* sp. with PROTEUS OD 110 26.05.2021 EC1 and EC4 28.05.2021 EC2				
29.05.2020 Stocking with koi carp larvae (thousands of larvae)					03.06.2021 Stocking with koi carp larvae (thousands of larvae)				
-	-	100	100	-	100	100	-	100	-
02.06.2020 Nursery ponds filling rates (%)					07.06.2021 Nursery ponds filling rates (%)				
100	100	100	90	75	100	100	100	90	75
14.06.2020 Stocking with *C. carpio* larvae (thousands of larvae)					22.06.2021 Stocking with *C. carpio* larvae (thousands of larvae)				
300	300	200	450	50	300	300	100	400	75
15.06.2020					28.06.2021				
Stocking with *H. molitrix* larvae (thousands of larvae)					Stocking with *H. molitrix* larvae (thousands of larvae)				
100	300	100	100	50	300	300	150	400	75
Stocking with *Ct. idella* larvae (thousands of larvae)					Stocking with *Ct. idella* larvae (thousands of larvae)				
200	250	100	250	50	300	300	300	150	75
From 16.06.2020, started the feeding of the fish					Stocking with *A. nobilis* larvae (thousands of larvae)				
22.06.2020 Stocking with *A. nobilis* larvae (thousands of larvae)					300	300	300	600	75
100	200	300	600	50	From 23.06.2021, started the feeding of the fish				

Distribution of the insecticide is done after dissolving the calculated amounts in cold water. The solution obtained is dispersed by means of a vermorel type spray pump from the boat, at a distance of approximately 2 m from the shores of the ponds. It was not necessary to distribute the solution over the entire surface of the nursery ponds.

Following the application of the Proteus OD 110 treatment in the nursery ponds from Mircea Vodă Experimental Base, the destruction of the philopod crustacean *Cyzicus* sp. resulted. In a very short time, the water was cleared and a significant presence of small zooplankton (rotifers) and green algae was observed, which is the natural food in the first stage of growth for carp larvae and phytoplankton-eating fish.

The natural food formed by zooplankton was the result of fertilization with manure.

After 9-10 days from the application of the treatment, the nursery ponds were stocked with fish larvae (3-5 days old): common carp (*C. carpio*) in polyculture with Asian cyprinids (*Ct. idella, H. molitrix, H. nobilis*) (Table 1).

In nursery ponds after applying the treatment to control *Cyzicus* sp. no clinical manifestations and mortality were recorded for fish alevins. Both in 2020 and in 2021, analyzes of the main physico-chemical parameters of the water and hydrobiological analyzes were performed throughout the growing period (Table 2).

According to the results obtained, the fertilizer doses were established and applied.

The main parameters of water quality for optimal survival and growth are: temperature between 18-28°C, dissolved oxygen 4.0-8.0 mg/l and pH 6.8-8.0 (Table 2).

Table 2. The main parameters of water quality

No.	Physico-chemical parameters	U.M.	Year 2020	Year 2021
1.	Temperature	°C	18-28	19-28
2.	Dissolved oxygen	mg/l	6.2 ± 0.81	6.8 ± 0.93
3.	pH	upH	7.2 ± 0.3	7.21 ± 0.2
4.	Organic substance	mg/l	24.00 ± 1.82	24.2 ± 1.74
5.	Total alkalinity	mg/l	168 ± 36	165 ± 38
6.	NH_4^+ - N (mg/l)	mg/l	0.030 ± 0.006	0.032 ± 0.009
7.	NO_3^- - N (mg/l)	mg/l	0.280 ± 0.072	0.266 ± 0.039
8.	PO_4 - P (mg/l)	mg/l	0.063 ± 0.008	0.064 ± 0.007

It is recommended to maintain water quality parameters, including pH at a level that ensures optimal growth and good survival of the biological material.

The main technological bioindicators of performance obtained within the Mircea-Vodă Experimental Base in 2020 and 2021 for common carp (*C. carpio*) in polyculture with Asian cyprinids (*Ct. idella, H. molitrix, H. nobilis*) are presented in Table 3.

Table 3. The main technological bioindicators of performance obtained within the Mircea-Vodă Experimental Base in 2020 and 2021

Technological bioindicators of performance	*Mircea-Vodă Experimental Base (12 ha)*									
	Carp (C. carpio)		*Grass carp (Ct. idella)*		*Silver carp (H. molitrix)*		*Bighead carp (H. nobilis)*		*Total / Average*	
Years	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
Number of fish larvaes stocked (mil.)	1.5	1.475	0.85	1.175	0.65	1.525	1.25	1.575	4.4	5.45
Percentage by species at stocking (%)	35,3	25.9	20.0	21.6	15.3	26.8	29.4	27.6	100	100
Number of fish fry harvested (thousands)	301	203	214	198	171	276	318	268	1014	923
Average harvest weight (g/ex)	38	42	31	36	29	32	30	34	32	36
Total biomass at harvest (kg)	11435	8539	6480	6513	4995	8829	9529	9118	32400	33000
Survival rate (%)	20	14	25	16	26	18	25	17	23	17

CONCLUSIONS

In conclusion, in order to obtain optimal results in the period of post-embryonic development and growth in first year of cyprinids, we recommend that the technological instructions be adapted to each type of technology.

After the treatment against the phylopods pest (*Cyzicus* sp.) in the cyprinid nursery ponds, a short period of time passed until the appearance of small forms of zooplankton, so the stocking of the fish larvae was made after 9-10 days.

The good growth rate and optimal health were obtained due to the abundance of specific natural food not being affected by the applied treatments.

The survival rate of the fish material (from 3-5 days larvae until autumn at age 0+) in 2020 was 23%, respectively 17% for 2021.

The productions obtained within the Mircea Vodă nursery materialized as follows: in 2020 a production of 2700 kg/ha was obtained and for 2021 the production was 2750 kg/ha.

When additional feeding and fertilizing are done simultaneously, high survival and rapid growth of larvae stocked in the nursery are achieved.

Compliance to the recommendations regarding the application of treatments to control diseases and pests as well as other measures within the fish farm ensures the achievement of good production results.

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ESTIMATION OF GROWTH PARAMETERS AND MORTALITY RATE FOR COMMON CARP AND PRUSSIAN CARP FROM DANUBE DELTA

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Abstract

The purpose of this study was to determine the relationships between total length and weight (L - W), to estimate the growth parameters (von Bertalanffy) L∞, k, and the mortality rates (Z, M, F) for the common carp (Cyprinus carpio, Linneaus, 1758) and Prussian carp (Carassius gibelio, Bloch, 1782). Sampling was carried out monthly, from March to September 2021. The relationship between length - weight (L - W) in the study period for common carp was $W=0.0574\times Lt^{2.6437}$, respectively $W=0.0391\times Lt^{2.7831}$ for Prussian carp. The growth parameters for common carp were L∞=86.10 cm and K = 0.87 per year, respectively L∞= 40.95 cm and K 0.67 per year, for Prussian carp. The total (Z), natural (M) and fishing (F) mortality rates were 1.47, 0.82 and 0.65 per year, for common carp, respectively Z=1.65, M=0.85 and F=0.80 for Prussian carp. Regarding the exploitation rate (E) this was 0.44 for the common carp population, respectively 0.48 for Prussian carp, suggesting that both fish population were not in over-exploited condition.

Key words*: fisheries resource, fresh water fish, Length-Weight relationship, von Bertalanffy's equation.*

INTRODUCTION

In Romania, commercial inland fishing takes place in rivers, ponds, and reservoirs, including the Danube River, the Danube Delta, and Danube Delta Biosphere Reserve (EUROFISH, 2021). Cyprinid species are by far the best-represented species of fish from the Romanian fishery. Between cyprinids species common carp (*Cyprinus carpio*, Linnaeus, 1758) and Prussian carp (*Carassius gibelio*, Bloch, 1782) are the most preferred freshwater fish by Romanian consumers. However, in Romania, the average apparent consumption of fish is still low, being estimated at 7.4 kg per capita in 2019 (EUROFISH, 2021).

According to the data provided by the National Agency for Fisheries and Aquaculture (ANPA), cyprinid catches vary between 2000 and 5000 tons per year, while the production from aquaculture is almost double.

The Danube Delta fisheries have declined in the last years, mainly due to the habitat loss by great floodplain and delta impoundments, an aspect that caused the decline fish stocks (Năstase et al., 2017). In this context, the sustainable exploitation of this commercial fish species requires a detailed study of the population and mortality parameters of these populations.

In this context, some population characteristics of common carp and Prussian carp, such as the length and weight distribution, condition factors, length-weight relationship, and some growth parameters and mortality rates, were investigated.

MATERIALS AND METHODS

Fishing area. Scientific fishing was realized during the year 2021 in the Danube Delta, lake-complex Matiția-Merhei (Figure 1). The Deltaic surface of the Matița- Merhei is 24,420 ha total surface area, from which 20,000 ha is aquatic surface, consisting of a total of 106 lakes.

The lake complex includes Merhei lake (1137.47 ha), Matita (641.83 ha), Trei Iezere (433.5 ha), Bogdaproste (400.19 ha), Balbina (427.35 ha) and some small lakes around 50 - 200 ha: Cioticul, Rădăcinoasele, Poludionca, Poludeanca, Iacub, Roșca, Argintiu, Merheiul mic and other lakes around 50 ha (Romanescu, 1996).

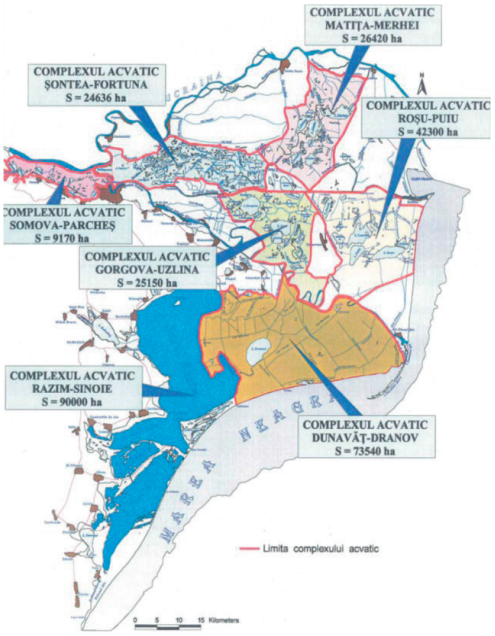

Figure 1. Natural aquatic complexes from the Danube Delta Biosphere Reserve (photo after Năvodaru, 2008)

Data collection. All samples were collected during September- October by gillnets (carp nets have a mesh size of 50-60 mm, and those for Prussian carp 40 mm). The assessment of the stock of common carp and Prussian carp in the analyzed area was based on methods of a random collection of representative samples from commercial catches. Throughout the study, 76 common carp and 106 Prussian carps were investigated on the criterion of including all length classes.

The total length (TL, cm), fork length (FL, cm) and height (h, cm) was measured using an ichtyometer to the nearest 0.1 cm, whereas the weight (W, g) was measured using the electronic weighing scale to the nearest 0.01 g.

The estimation of the growth parameters and mortality. The determination of the length-weight relationship (L - W) for the fishing population was done using the equation:

$W = a \times L^b$, where: W - individual weight (g).

The growth parameters ($L\infty$, k, t_0) were estimated by the length frequency analysis using the ELEFAN model from the FiSAT II program. The estimation of mortality rates was done according to Pauly (1980) and Pauly (1983). Total mortality (Z) was determined using the length converted catch curve analysis (Ricker, 1975) from FiSAT II computer software package. The natural mortality (M) was estimated by the Pauly's empirical formula (Pauly, 1980), using a mean surface temperature (T).

$$\text{Log M} = -0.0066 - 0.279*\text{Log}(L\infty) + 0.6543*\text{Log}(k) + 0.4634*\text{Log}(T),$$

where: $L\infty$ represent the asymptotic length, T is the mean annual temperature of water (12°C) and k refers to the growth rate coefficient of Von Bertalanffy.

Fishing mortality (F) was calculated using the relationship: F=Z-M (Gulland, 1971), where: Z is the total mortality, F is the fishing mortality and M is the natural mortality. The exploitation level (E) was obtained using the relationship: E=F/Z (Gulland, 1971). If the exploitation rate registered values under 0.5, fish stocks are easily exploited and if the E values are between 0.5-1, the stocks are heavily exploited.

Data analysis. The length frequency data was pooled into groups with 3 cm length interval. For data analysis we use Microsoft Excel 2019 and the software package FiSAT II (FAO-ICLARM Stock Assessment Tool).

RESULTS AND DISCUSSIONS

Table 1 presents the mean values of the individual weight, total length, fork length and height of the population of carp and Prussian carp.

Table 1. Mean, minimum and maximum values of weight, total lengths, fork length and height of common carp and Prussian carp population collected from the study area

Fish species		Weight (g)	Total length (cm)	Fork length (cm)	Height (cm)
Cyprinus carpio	Mean±SD	1939±897	50.51±7.49	49.96±7.21	14.6±2.31
	Min.-Max.	1200-6350	40-81	37-71	14-22
Carassius gibelio	Mean±SD	471.1±248.4	28.26±4.94	24.97±3.19	10.6±2.27
	Min.-Max.	200-1300	21.6-41	20.1-34	7.9-16

A total of 76 common carp were sampled, 53.95% females and 46.05% males. The total length of all individuals varied from 40 cm to 81 cm and the mean total length was calculated as 50.51±7.49 cm. Dominant length groups were between 43-46 cm, followed by 49 cm and 54-56 cm, respectively (Figure 2).

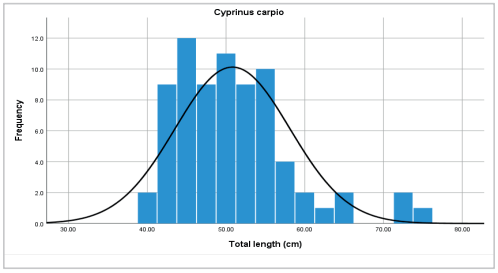

Figure 2. Length-frequency distribution of *Cyprinus carpio*

Regarding the Prussian carp, 106 specimens were sampled 71.69% females and 28.30% males. The total length of all individuals varied from 21.6 cm to 41 cm and the mean total weight was calculated 28.26±4.94 cm (Table 1). Dominant length groups were between 24-25 cm, followed by 23 cm and 28-29 cm respectively (Figure 3).

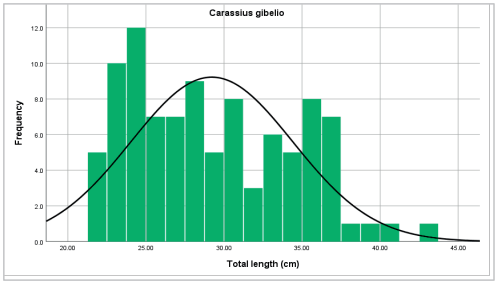

Figure 3. Length-frequency distribution of *Carassius gibelio*

The estimation of the a, b coefficients from the equation $W = a \times L^b$ for the carp and Prussian carp population was determined from the regression between length and mass. The length-weight relationship of the carp population was $W=0.0574\times Lt^{2.64}$ (Figure 4), respectively $W=0.0391\times Lt^{2.78}$ for Prussian carp (Figure 5). The slope values of the length-weight relationship showed that fish body length increased allometrically with total length, both in the case of common carp (b=2.64) and Prussian carp (b=2.78). Usually, the values of these coefficients are variable from one year to another, as a result of the physiological processes of growth, fattening, sexual maturation, reflecting the specific adaptation and creating the unity species-environment (Năvodaru, 2008; Serajuddin et al., 2013). In general, the length increments decrease with increasing fish age, while the weight increase with growing fish weight.

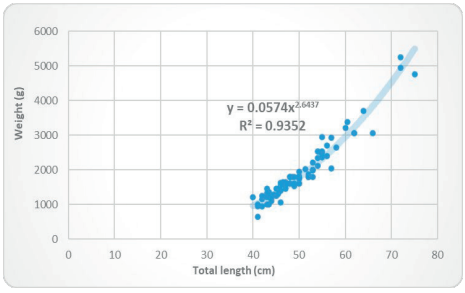

Figure 4. Length-Weight relationship for the common carp population

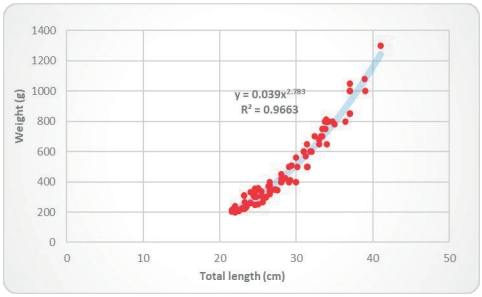

Figure 5. Length-Weight relationship for the Prussian carp population

Growth parameters. For growth parameter estimation, the data set for common carp and Prussian carp was used to calculate Von Bertalanffy growth parameters, which was shown in Table 2. The L∞ and k for common carp were estimated as 86.10 cm and 0.87 $year^{-1}$ from length frequency data using ELEFAN I by FiSAT II software, while for Prussian carp, the L∞ and k for common carp were estimated as 40.95 cm and 0.67 $year^{-1}$.

The mortality and exploitation rates. The total mortality (Z) was estimated through the linearized curved catch method based on the length frequency structure where the Bertalanffy's equation is used to convert lengths in ages.

Table 2. Parameters of the von Bertalanffy growth equation for common carp and Prussian carp population at Matița-Merhei

Species	L_∞	k	t_0	Z	M	F	E
Cyprinus carpio	86.10	0.87	-0.077	1.47	0.82	0.65	0.44
Carassius gibelio	40.95	0.67	-1.37	1.65	0.85	0.80	0.48

Note: L∞ - asymptotic length; k - growth rate coefficient of Von Bertalanffy; Z - total mortality; M - natural mortality; F - fishing mortality; E - exploitation rate.

For common carp, the total mortality was 1.47 per year while the natural mortality (M) was found to be 0.82 per year and the estimated fishing mortality (F) was 0.65 per year. For Prussian carp, the total mortality was 1.65 per year while the natural mortality (M) was found to be 0.85 per year and the estimated fishing mortality (F) was 0.80 per year.

In order to maintain optimal fish stocks, estimating of mortality rates are important to avoid the over-exploitation of fishery resources. In our study, the exploitation ratio for common carp was found to be 0.44, while for Prussian carp was 0.48, suggesting that both fish populations were not in over-exploited condition. The previous studies of Ibănescu et al. (2011) found for common carp from Danube River (km 170-km 196) an asymptotic length of L_∞ -87.15 cm, k - 0.260 year^{-1}, while total mortality (Z) was 0.85 per year, natural mortality (M) was 0.37 per year, and mortality due to fishing (F) was 0.48 per year. The authors state that the population is overexploited by fishing or poaching, reaching an exploitation rate (E) of 0.56. Also, the same author, found for Prussian carp (Danube River, km 170-km 196) an asymptotic length of L_∞ - 39.38 cm, k - 0.63 year^{-1}, while total mortality was 2.29 year^{-1}, natural mortality 0.826 year^{-1}, fishing mortality 1.46 year^{-1} and the exploitation rate 0.63 aspect which demonstrates that the Prussian carp population from the studied sector is overexploited, both by legal and illegal fishing (Ibănescu et al., 2012). In a similar study from 2006, at the same fishing area (Matița-Merhei), Năvodaru et al., 2008, reported for *Carassius gibelio* (total length min-max. between 18÷31.7 cm, and weight min-max between 142-797 g) an asymptotic length of 41.3 c, k - 0.21 year^{-1}, while natural mortality and total mortality was lower compared with our study (M - 0.41 year^{-1} and Z - 1.3 year^{-1}).

CONCLUSIONS

In order to avoid the over-exploitation of fishery resources it is important to estimate the growth and mortality rates of fish stocks. From the results of our study, it can be concluded:

- the length-weight relationship of the studied population showed an allometric growth pattern, fish body length increased with total length;
- the most abundant are the carp fishes with a size range between 43-46 cm and the Prussian carp 24-25 cm;
- the studied population of *Cyprinus carpio* and *Carassius gibelio* from the Matița-Merhei area isn`t in a situation of overexploitation.

However, changes in the population structure and stock size must be monitored continuously and further studies are recommended for this fishing area.

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GROWTH OF BREAM, *ABRAMIS BRAMA* (LINNAEUS, 1758), IN THE ROMANIAN SECTION OF THE DANUBE RIVER

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Abstract

The main aim of the paper is to investigate the condition of the population of this fish species in a sector of the Romanian Danube River. The common bream is an important species in commercial catches, therefore the knowledge of its growth parameters is important for management of the multi - species fishery. The study is based on a sample of 1580 specimens caught with a total biomass of 197.66 kg. The study took place between 2018 and 2019. Our objective was to estimate the growth parameters (von Bertalanffy) L∞, k, t and the mortality rates (Z, M, F) for the bream population (Abramis brama, Linnaeus, 1758) in the Danube. The relationship between length - weight (L-W) in the study period for common bream population is $W = 0.0201 * Lt^{2,9212}$. *Von Bertalanffy growth parameters were found as, asymptotic length L□= 49.35 cm, growth coefficient (K) = 0.37 yr^{-1}. The estimated values of the mortality rates for the studied population are: total mortality (Z) is 1. 51, the natural (M) reaches 0.55. and fishing mortality (F) is .0.96.*

Key words*: growth parameters, mortality rates, von Bertalanffy's equation.*

INTRODUCTION

The commercial inland fisheries are considered a central environmental resource of large rivers, providing a protein source and income for multitudes of people worldwide (Schletterer et al., 2018).

Common bream, *Abramis brama* (L., 1758), is a benthic freshwater species that is distributed throughout Western and Central Europe and Asia (Guettaf et al., 2019). Adults inhabit a wide variety of lakes and large to medium sized rivers. Most abundant in backwaters, lower parts of slow - flowing rivers, brackish estuaries and warm and shallow lakes.

In Romania, it is found all over the Danube, floodplain and in the most rivers and lakes from the hilly area to the plain.

The bream is one of the commercially valuable species of the Romanian inland fishery.

It is one of the most abundant fish species in the commercial catch of 2008-2018, representing 11.16% of these catches (Ibanescu et al., 2020).

For sustainable management of fishery stocks, it is important to know the dynamics of component populations.

The objective of this paper is to know the dynamics of the bream population, from an important sector of the Danube, the bream being the second most caught species from commercial catches in the period 2008-2018.

MATERIALS AND METHODS

Fishing area. The Danubian sector taken into consideration for this study is located between km 170 - Brăila locality and km 197 - Gropeni locality, upstream Brăila municipality (Figure 1). The scientific fishing was realized in 2018-2019. Fish samples were collected from Danube river arms.

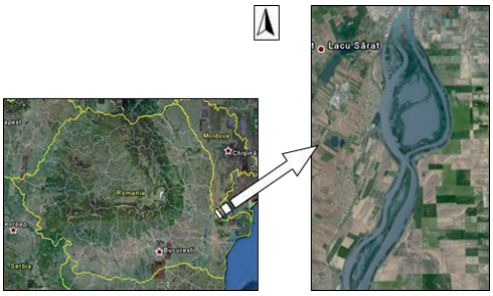

Figure 1. Area study

Data collection. It was collected and sampled a number of 1580 individuals which the total biomass being ~ 198 kg. The main biological parameters registered for each individual were:

total length (Lt ± 1 mm), individual mass (W ± 1 g).

The estimation of the growth parameters and mortality. For data analysis was used software package FiSAT II (FAO - ICLARM Stock Assessment Tool) based on length frequency distribution (Gayanilo F.C. et al., 2003).

The length-weight relationship

The length-weight relationship (LWR) was estimated using the equation, $W = a * L^b$

where: W - total body weight (g), L_t - total length (cm), b and a are the coefficients of the functional regression between W and L

The estimation of the growth parameters

The growth of the fish was assumed to follow the von Bertalanffy growth function (VBGF)

$$L_t = L_\infty \left(1 - e^{-\left[K(t - t_0) \right]} \right)$$

where, L_t = length at age t, L_∞ = the asymptotic length of fish; k = curvature parameter that determines how fast the fish approaching to L_∞; t_0= the theoretical age at which the fish length is 0 (Ricker W.E., 1975; Pauly D.,1983; Sparre P.; et al., 1989).

The mortality

The estimation of mortality rates represent an important component of fisheries management.

The total instantaneous mortality rate (Z) was estimated using length converted catch curve method as implemented in FiSAT II. Natural mortality rate (M) was estimated using Pauly's empirical relationship

$$\ln M = -0.0152 - 0.279 \ln L\infty + 0.6543 \ln k + 0.463 \ln T^0 C$$

(Pauly, 1983; Jones, 1984; Sparre et al., 1989).

M is instantaneous natural mortality, k and L_∞ are growth parameters from VBGF; T^0C - is the annual average temperature.

The average temperature at Braila I judged $T=12^0C$.

The fishing mortality (F) was calculated using the relationship: F = Z – M (Gulland, 1971),

where Z is the total mortality, F the fishing mortality and M is the natural mortality. The exploitation level (E) was obtained using the relationship: E = F/Z (Gulland, 1971), Optimum fishing (F_{opt}) which is directly related to the natural mortality (M) was calculated for the selected fish species using the expression below: F_{opt}=0.4*M (Pauly, 1984).

Length at first capture (L_C50)

The ascending left arm of the length converted catch curve incorporated in FiSAT II tool was used to estimate the probability of length at first capture (Lc50). The probability of capture gives clear idea about the estimate of the real size of the fish in the fishing area that is being caught by specific gear. It is an important tool for fisheries managers in sustainably managing a target fisher, because it helps would be managers determining the minimum mesh size of a fishing fleet (Wehye et al., 2017).

Length at first maturity (Lm)

To estimate the length at first maturity (Lm) for this bream population was used the procedure by Hoggarth et al. (2006).

Length at first maturity (Lm) = L∞ * 2/3.

RESULTS AND DISCUSSIONS

Growth and mortality parameters

The total length ranged from 12 to 48 cm, with a mean of 33.86±0.18 and weight ranged between 34-1500 g with a mean value of 670 ± 8.83.

The equation expressing the length-weight (LWR) relationship for bream population from the studied sector is $W = 0.0201 * Lt^{2.9212}$ (r = 0.97) (Figure 2).

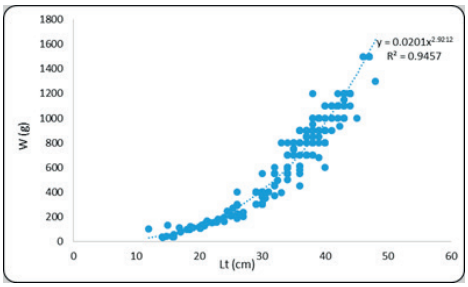

Figure 2. Length-Weight relationship for the bream population

The LWR relationship has a great importance in the ecology and management of the fisheries stocks (Savaş et al., 2011).

The growth character revealed by the "b" coefficient value (2.92) shows us an allometrical

growth of the bream population (that increased weight is slower than the length increase).
For our country, values of the "b" coefficient for this species reported on fishbase are between 2.98 and 3.38 (lakes in the Danube Delta).
The asymptotic length (L∞) and growth coefficient (K) values for the bream were obtained to be 49.35 cm respectively 0.37 y^{-1}.
The index of growth performance (φ – phi prime) were estimated at 2.96.
This value is comparable to that reported by Staras (1992; 1995) and Cernișencu (1992) for the lakes and ponds of the Danube Delta.
The growth performance of a fish population is influenced by the geographical area, the type of aquatic ecosystem in which it lives, the abundance and availability of food. Kakareko (2001) and Stankus (2006) indicated that growth rates of *A. brama* depended on the abundance of benthic organisms.

Mortality coefficiens and current exploitation rate

The total mortality (Z) of *bream* estimated by the length converted catch curve was 1.51 per year while the natural mortality as per Pauly's empirical formula keeping the habitat temperature as 12°C was found to be 0.55 per year and the estimated fishing mortality (F =Z-M) was 0.96 per year (Figure 3).

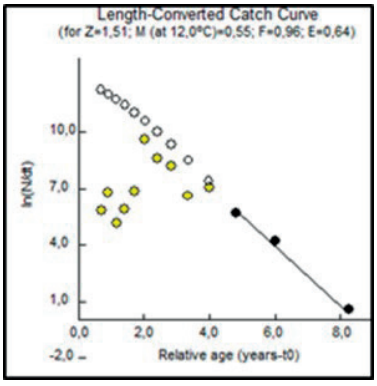

Figure 3. Length converted catch curves of *Abramis brama*

The exploitation ratio (E) of *Abramis brama* was found to be 0.64.
The value of the optimum fishing is Fopt = 0.22, a value that is much exceeded (Table 1).

Table 1. The growth and mortality parameters of bream

Parameters	Value
L∞	49.35
K (yr^{-1})	0.37
φ	2.96
Z (yr^{-1})	1.51
M (yr^{-1})	0.55
F (yr^{-1})	0.96
F opt (yr^{-1})	0.22
E	0,64

Figure 4 shows the length at which 25%, 50%, 75% of the specimens of the studied bream population are vulnerable to fishing gear. L_{50} is considerated the length at first capture (Lc_{50}) and at this length the fish has 50 % chance of being retained by the gear used to capture it (King, 2007).
For the studied bream population these values are: L_{25}=27.06 cm, L_{50}=36.42 cm, L_{75}= 39.40 cm.

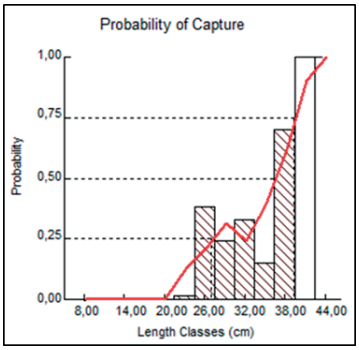

Figure 4. The probability of capture of bream population

The value of the length at the first maturity of the studied bream population is Lm=32.9 cm.

Virtual Population Analysis (VPA)

From virtual population analysis it was found that the maximum fishing mortality of *Abramis brama* occurred at the length range between 26 cm and 38 cm (Figure 5).
As can be seen from the VPA, the highest fishing mortality is in the 26 cm length group.
The abundance of small - sized fishes in the catches could be explained by the indiscriminate use of small mesh sized gears and the non-selectivity of fishing gears mostly deployed within the nursery zone of juvenile fishes (Wehye et al., 2017).

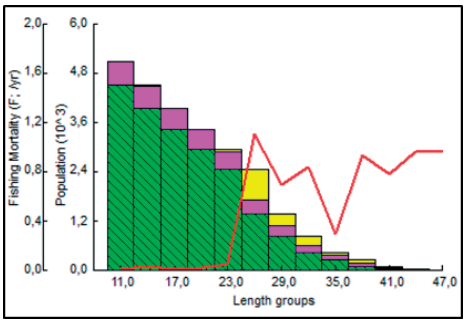

Figure 5. Virtual population analysis of *Abramis brama*

CONCLUSIONS

The length-weight relationship (LWR) for the common bream population in this section of the Danube revealed a strong correlation (r = 0.97) between the two variables, and the exponent (b) value of 2.92 indicating an allometric increase that implies the increase in length faster than weight.

The values of the parameters of the von Bertalanffy linear growth model, and the overall growth performance index (Ö) calculated in this study are comparable to those published for this species in other sectors of the Romanian Danube River.

The fishing mortality (0.96 yr^{-1}) for *Abramis brama* from the current study was greater than the optimum fishing rate (F_{opt}=0.22 yr^{-1}). Also, the estimated current exploitation rate (E) was 0.64, which indicated heavy exploitation (Beverton et al., 1957).

From the probability of capture analysis L_{25}, L_{50} and L_{75} values of *Abramis brama* were found to be 27.06 cm, 36.42 cm and 39.40 cm, respectively.

In this case, the fish becomes susceptible to fishing gear when it reach at length of 36.42 cm and at this length there is a 50% chance of catching it.

Value of the length at the first maturity (L_m) in this study is 32.9 cm.

The high values of the exploitation rate (E) and additionally the high fishing mortality rate (F) comparable to F_{opt} shows that the bream stock is overexploited.

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ANALYSIS ON THE USE OF NEW INGREDIENTS IN TROUT FEED

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Abstract

The fodder for captive-bred trout contains ingredients such as fish oil, fishmeal, blood meal, peas, soybeans, wheat, oats, rapeseed, animal protein. The quality of the ingredients is the basis of healthy, mineralizing, protein and vitaminizing feeds. Excessive use of fishmeal in the market puts pressure on the fisheries sector, which is struggling to meet demand. Algae, crustaceans, aquatic plants, insects and seeds are ingredients that could replace fishmeal by helping the fishing industry as well as sustainability of the aquatic environment. In the process of feeding trout with new, experimental feed, research has highlighted the importance of several factors for the development and growth of fish. The level of protein in the feed directly affects the body weight of the trout and the replacement of fishmeal and fish oil with other types of flour and oils can lead to a change in the taste of trout meat. Feeding and control can be of particular importance in reducing losses and increasing environmental sustainability.

Key words*: environment, feed, fish, innovative, sustainability.*

INTRODUCTION

Due to its organoleptic qualities, trout are among the main species of fish consumed nationally and internationally. Aquaculture trout need fodder that reproduces the food from the natural environment as faithfully as possible from a nutritional point of view. Currently, when obtaining a feed for rainbow trout (*Oncorhynchus mykiss*), one of the main ingredients is fishmeal. Excessive use of fishmeal in fish farming and aquaculture as a protein base for rainbow trout feed and not only has led the European Union to focus on finding new sources of protein ingredients in fish feed in aquaculture.

By identifying new sources of ingredients used in trout feed, the aim is to eliminate all or part of the fishmeal from the feed currently used in intensive farming systems and at the same time protect the environment by reducing the consumption of fish meat for fishmeal. Removing fishmeal from feed and replacing it with other ingredients can reduce the final cost of feed, with an impact on the shelf price, and also reduce the negative impact on the environment.

Therefore, researchers have begun to use different ingredients, both of animal origin (insects, crustaceans, worms) and of vegetable origin (algae, different types of oils, cotton flour, rapeseed, brewer's yeast), to replace the fishmeal.

According to the European Commission (2021), the European Green Pact and the "Farm to Fork" Strategy emphasize the potential of marine food, obtained from aquaculture. This is considered a source of protein for food and feed with a low carbon footprint, which plays an important role in building a sustainable food system. In an effort to ensure a sustainable growth of the sector, global aquaculture is in a constant search for new solutions to reduce dependence on fishmeal and fish oils and to efficiently manage manure in animal and poultry facilities. Insects are a new source of sustainable, high-protein ingredients that can be use to feed farmed fish, especially rainbow trout (A European Green Deal - Farm to Fork strategy, 2019).

Aquaculture creates jobs in fisheries and related fields such as aquaponics, and the agro-tourism sector, thus helping the economic development of the countries involved. Directly or indirectly, aquaculture combats climate change and

mitigates its impact by reducing pollution. Through a long-term strategic approach to aquaculture, it can become sustainable, profitable, and environmentally friendly.

MATERIALS AND METHODS

This paper aims to conduct a bibliographic review of the research on new sources of protein ingredients that can be used in feed intended for aquaculture fish.
The paper can be a basis for studies aimed at replacing conventional feed with alternative feed.

RESULTS AND DISCUSSIONS

The studied bibliographic literature shows that **fishmeal** is one of the classic ingredients used in the trout diet. Administered individually or as an integral part of certain mixtures, it has become, over time, the subject of numerous researches, in order to establish the possibility of replacing it, in certain proportions, with other types of feed. Thus, those of vegetable origin, but also those of animal origin were studied as alternative sources of protein ingredient.

Sources of plant origin

In a long-term comparative study (2 years), published in 2004 by Francesco et al., the diet based on fishmeal is analyzed in relation to that based on a mixture of vegetable protein sources supplemented with free amino acids. These studies were carried out by the Department of Animal Science at the University of Florence, Italy, in collaboration with the Fish Nutrition Laboratory of France. They showed significant changes to the detriment of rainbow trout fed with enriched mixtures, in terms of growth rate, organoleptic characteristics and the level of the chemical composition of the fillet in n-3 polyunsaturated fatty acids and n-3 / n-6 ratio. As regards replacing the rainbow trout diet based on fishmeal in variable proportions with 60 to 330 g/kg^{-1} of protein concentrate from **rapeseed**, the Canadian researchers at the University of Saskatchewan conclude in the same year 2004 that it does not compromise fish farming performance (Thiessen et al., 2004).
Experimental diets based on the partial or total replacement of fishmeal in rainbow trout feed were also the basis of a study published in 2006 by Chinese researchers at the Beijing Academy of Agricultural Sciences.
Cotton flour, extracted as a solvent and supplemented with lysine and methionine, replaced 0; 112.5; 225; 337.5; 450 g/kg^{-1} protein from fishmeal for 8 weeks. Luo et al. (2006) consider that, while the diet that completely excluded fishmeal adversely affects the growth rate of juvenile rainbow trout, there is no significant difference in growth or fish meat taste when replaced by up to 305 g/kg^{-1}.
The use of other types of flour has also been considered by many researchers. Kasiga & Brown (2019) replaced fishmeal with 50, 100 and 150 g of carinata flour (*Brassica carinata* - Ethiopian mustard), and studies have shown that there has been no change in whole body weight or visceral weight.
In order to reduce dependence on fish oils and to avoid problems related to contamination of aquaculture products (used for the production of fishmeal and fish oil) with persistent organic pollutants and heavy metals, the efforts of the farms have been directed towards reducing the percentage of fish oil in fish food, by partially replacing it with vegetable oils. This trend has emerged from a series of studies that have shown that a significant percentage of salmonid feed fish oil can be replaced without affecting the survival, growth and efficiency of feeding. However, vegetable oils have a different fatty acid profile than fish oils. When included in high levels in the diet, they influence the composition of meat fatty acids and reduce the concentration of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFA), such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), well known for their beneficial cardiovascular and cognitive properties (Bélanger-Lamonde et. al., 2018).
Coconut oil has been the subject of research based on experimental diets, replacing herring oil and cod liver oil in rainbow trout food. The research was conducted in 2006, over a period of 231 days, by Italian researchers Ballestrazzi et al., using four diets with increasing coconut oil content, from 0 to 13%, but no significant changes were recorded in carcass features or meat composition.
Flaxseed and sunflower oil have been used since 2006 by Czech researchers in the diet of rainbow trout in the amount of 2% and 5%

individually or 5% mixture of both oils. Although this did not affect the firmness or juiciness of the meat, the major impact was on the intensity and oily taste of the fish (Drobna et al., 2006). Replacing fishmeal and fish oil with rapeseed protein concentrate and a mixture of rapeseed oil and flaxseed oil, led to a significant reduction ($P < 0.05$) in the residual concentration of PCDD/F and DL-PCB in diets and fish fillets according to studies by Sealey et al. (2011).

Algae and microalgae

Algae are a highly valued source of protein, essential amino acids (Fabregas & Herrera, 1985; Becker, 1994) and vitamins (Becker, 2004). They play an important role in human nutrition as well as in animal feed, such as broilers (Marton et al., 1968; Venkataraman et al., 1994) or pigs (Fevrier & Seve, 1976). In aquaculture, algae feeding experiments are related to their use as general sources of protein, due to the complete replacement of fishmeal or as additives in fish feed. The positive effect of the additive used lowers cholesterol and fat levels and improves lipid metabolism in fish. Due to the wide variety of biologically active agents found in spirulina species (*Arthrospira platensis*, *A. fusiformis* and *A. maxima*), they have been the subject of numerous investigations into their use in biotechnology and medical sciences. Spirulina is a rich source of the pigment C-phycocyanin C-PC (Richmond, 1986), with role as an antioxidant (Romay et al., 1998; Bhat & Madyastha, 2000; Pinero et al., 2001), as well as its anticancer properties (Dasgupta et al., 2001).

Different types of algae have been use to determine a stronger coloration of rainbow trout. Microalgae, *Chlorella vulgaris* was used in a study to compare the quantitative effect of the incorporation of food algal biomass (ALG) on rainbow trout muscle pigmentation with that obtained by feeding diets supplemented with a synthetic mixture of canthaxanthin and astaxanthin (MIX) equivalent with the amounts of carotenoids found in dried seaweed (Gouveia et al., 1997). The research was conducted over nine weeks by administering to two batches of fish. One batch was fed *Chlorella vulgaris* microalgae included in the diet, and the other batch was fed synthetic carotenoids (canthaxanthin and astaxanthin). At the end of the research, no significant differences were found between the weights of the batches compared to the total food consumption. The effect on the development of microalgae-fed fish was not as expected, compared to the results obtained in fish fed on a diet containing synthetic carotenoids.

Iranian researchers (Teimouri et al., 2013) following an experiment on 216 rainbow trout, which lasted 10 weeks, concluded that *Arthrospira platensis* could be introduced as an alternative, natural source of carotenoids instead of synthetic astaxanthin in rainbow trout diets. The inclusion of 7.5% *S. platensis* proved to be an adequate food level to ensure pigmentation, as there were no negative effects on fish growth.

Brewer's Yeast

Brewer's yeast is an ingredient, rich source of chromium, and B vitamins, used in the production of beer and bread. It is obtain from *Saccharomyces cerevisiae*, a one-celled fungus. Brewer's yeast can be use as a nutritional supplement in human and animal alimentation.

According Coroian et al. (2019), yeasts seem to provids new sources of protein in fish feed. It also acts as a probiotic. Dry brewer's yeast (1.5%) added to standard feed for rainbow trout has led to better-feed use and strengthened immunity.

Sources of animal origin

Insects and insect larvae are a new source of sustainable and high-protein ingredients that can be use in farmed fish feed, especially rainbow trout.

In 2007, in a 9-week study, American researchers St-Hilaire S. et al. investigated the effects of the partial replacement of fishmeal with diets containing 40% crude protein and 15% fat (67% of dietary protein was derived from fishmeal and all fat was derived from fish oil). Two of the test diets included the black soldier fly, *Hermetia illucens*, and a third test diet involved the use of housefly pupae, *Musca domestica*, which have 70% protein and 16% fat as a 25% substitute for the fishmeal component of the control diet. Studies have shown that with the rise in the price of fishmeal and fish oil, fly prepupae can be an economically advantageous food, but also sustainable for carnivorous fish diets. In areas where there is an aquaculture industry close to intensive farming units, fly larvae could be used to reduce animal waste and

provide a good source of protein and fat for the aquaculture industry. Research in this direction has also been conducted by Sealey et al., in 2011, when four test diets were developed by replacing 25 and 50% of fishmeal with fly prepupae (BSF) or fly prepupae enriched with fish organs (EBSF). The dietary fat was adjusted to about 20% fat, using fish oil and poultry fat. In fish fed using these diets, there was no significant difference in the taste of meat compared to the one of the fish on control diet. There were significant differences in their growth however, the enriched diet offering visible effects clearly superior to the others. The effectiveness of insect meal was tested on the occasion of the study by Rema et. al., in 2019, which assessed the effect of incorporation levels of defatted yellow mealworm protein meal (*Tenebrio molitor*). It was concluded that yellow mealworm protein flour could effectively replace 100% fishmeal in the rainbow trout brood diet, with positive effects on its overall zootechnical performance and raising questions about the potential effects of the diet on immunity and the general health of fish.

To determine the potential of insects as a substitute for fishmeal in aquaculture fish feed, Barroso et al., 2014 examined 16 different species, 5 of them in different stages of development, from the orders *Coleoptera* (4), *Diptera* (7) and *Orthoptera* (5). The resulting conclusion was that insects have an amino acid profile similar to fishmeal, *Diptera* being the group most similar to fishmeal. In addition, insects have been shown to have higher ratios of omega 6 and monounsaturated fats, which is an advantage in growing rainbow trout. Researchers at the University of Turin (Belforti et al.) also investigated in 2015 the effects of including a defatted BSF in rainbow trout feed on performance, somatic indices and certain quality parameters. The studies were performed on 360 fish randomly divided into four batches that were fed diets with different percentages of TM: 0% (TM0), 25% (TM25) and 50% (TM50) as a feeding base. *Tenebrio molitor* larvae flour (TM) has been considered an innovative raw material and seems promising to be used as an alternative food to fishmeal in trout feed (Belforti et al., 2015).

Even more recent studies show that insect meal can be widely considered a promising raw material for aquatic food (Józefiak et al., 2019) and can be used even in the early stages of fish development (Lock et al., 2016), i.e., as a starter food. The main advantages of using insects are their easy production, rapid growth, short reproductive cycle of insects and high efficiency of feed conversion (Katayama et al., 2008). The use of insect meal in fish diets is an ecological method of obtaining a valuable source of protein. Furthermore, the use of combinations of different protein sources in aquatic food has been shown to have a positive effect on fish growth (Coyle et al., 2004; Mundheim et al., 2004; Lim & Yildirim-Aksoy, 2008; Sookying & Davis, 2011).

However, researchers such as Stadtlander et al., 2017, consider that further studies are needed on specific feeding strategies and the establishment of feed formulas. These conclusions were drawn following the use in the diet of rainbow trout of a content of 28% insect flour, mechanically defatted, prepared from black soldier fly larvae, *Hermetia illucens* (HIM).

Complementary studies are considered necessary to limit the negative effects on the lipid fraction of fillets and to avoid decreasing the efficiency of protein utilization in HIM-fed fish, which could lead to decreased production efficiency when applied over an entire production cycle and not only during the 7 weeks of their research. Moreover, insect food can also be considered a source of protein and a functional component of feed, which can positively affect the histomorphological structure of the gastrointestinal tract of fish and stimulate the expansion of beneficial bacterial populations in the intestine, according to Polish researchers (Józefiak et al., 2019).

Crustaceans

Chicks et al., conducted in 2021 a study of trout fed isoproteins (42%) and isolipids (24%) without fishmeal for 15 weeks, in which 10% of the protein in a mixture of plant ingredients was replaced with cyanobacteria and dried microalgae biomass (*Arthrospira platensis*, *Tisochrysis lutea* and *Tetraselmis suecica*) or Louisiana red swamp crayfish flour (*Procambarus clarkii*). The content of carotenoids and lipids in the fillets was determined. The coloring capacity of the rainbow trout fillet in fish fed with crayfish meal was also compared with that obtained from the

use of commercially available synthetic astaxanthin. For the latter purpose, the test diets were additionally administered for 12 weeks.

Image analysis and colorimetric data indicated that trout fed cyanobacteria and microalgae showed an unwanted yellowish color compared to those fed Louisiana crayfish meal (*P. clarkii*) which showed a desired pink pigmentation.

Another study by Hoffmann et al. (2021) evaluated a combined feeding system, which included the addition of live food (*Artemia salina*) for 0; 3 or 7 days, the addition of insect flour (test diet) and the addition of fishmeal (control diet). Compared to the control diet (based on fishmeal) it was concluded that diets containing 20% larval insect flour can be used as an effective component of feed for juvenile fish.

The application of live feed (brine shrimp) did not significantly affect the growth results, although the type of insect meal had a crucial impact on the growth of the fish.

CONCLUSIONS

The data collected from the studied bibliography show that fishmeal is the main ingredient in trout feed, of paramount importance in its growth and development. However, researchers have tried to replace fishmeal with other ingredients. Some researchers have obtained significant results with vegetable protein diets with enriched mixtures, protein concentrates based on rapeseed or cottonseed meal, with positive results in most cases where fishmeal has been partially and not totally replaced.

The partial replacement of fish oil with vegetable oils does not significantly affect the growth and development of fish, but it does have a negative impact on polyunsaturated fatty acids in salmonid meat. The use of coconut oil did not change significantly the composition of the fish meat, but the flaxseed and sunflower oil adversely influenced the taste of the meat. Spirulina from algae and the protein they contain lowers cholesterol and fat in fish meat but also improves lipid metabolism, while increasing the color of rainbow trout fillets, and can be introduced into fish diets as a natural source of carotenoids to the detriment of synthetic astaxanthin. Insects and insect larvae have given good results in replacing fishmeal with different concentrations in the respective diets. Insect flour can be used in the early stages of growth and development of fish larvae with very good results, having the nutritional qualities as close as possible to fishmeal. Crustaceans (crayfish flour) played an important role in the pink pigmentation of trout, and the Artemia salina crustacean used as a live food in the diets of some trout did not significantly affect the growth results.

Algae, crustaceans, vegetable oils, seeds, various types of flour, insects and larvae are ingredients that could replace fishmeal, thus helping to develop aquaculture, increase sustainability, but also protect the environment.

As a result of the presented aspects that research is necesary in order to replace conventional feed with alternative feed, not only on an experimental scale, but also at high production level, in order to establish all the advantages and disadvantages that would result from this change in the types of raw materials in trout (fish) feed.

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THE EFFECT OF DIETS WITH ADDED GRAPE MARC ON GROWTH PARAMETERS AND MEAT QUALITY OF CARP *(CYPRINUS CARPIO)*

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Abstract

Nutrition is a determining factor in the growth potential of fish. The high costs of fodders have created the need for research of substitutes that will contribute to improving the growth performance and that will achieve high-quality fish products. Many by-products in the food industry are rich in bioactive nutrients, with the potential to serve as functional food ingredients for fish fodder. The aim of this study is to determine how the growth parameters and composition of carp meat (Cyprinus carpio), reared in a recirculating system and fed with diets that have grape marc as a fodder component, are influenced. The inclusion of grape marc in fodders ensures increased growth performance compared to diets without the addition of grape marc, and a feed conversion ratio (FCR) with better values in the experimental lots (1.48 for lot T2, 1.67 for lot T1, 1.62 for lot T3) compared to the control lot C (1.86). Grape marc used as supplement in diets, determined the accumulation of protein and lipids in carp meat, an increased intake of fatty acids, an improvement in the ω3/ω6 ratio, causing an increase in the nutritional value of fish.

Key words*: Cyprinus carpio, fatty acids, grape marc, growth parameters.*

INTRODUCTION

The aquaculture sector is facing diseases that can cause significant losses of biological material. Therefore, strategies focused on disease prevention, rather than treatment of diseased stocks, are recommended. The use of combined immunoprophylaxis may lead to the final protection of the health of aquaculture fish. Many by-products resulting from fruit and vegetable processing are rich in phytonutrients, bioactive nutrients with the potential to be functional food ingredients for fish fodder.

New strategies for rearing technologies are needed for the sustainable development of aquaculture.

The aim of this study is to determine how the growth parameters and composition of carp meat (*Cyprinus carpio*), reared in a recirculating system and fed with diets that have grape marc as a fodder component, are influenced.

By incorporating this by-product, without economic value, the total cost of production in aquaculture can be reduced, knowing that fodder is the largest share in the final cost of fish (Birol & Şennan, 2017).

MATERIALS AND METHODS

The experiment was performed with 1-year-old carp specimens, with an average weight of 22.52±3.92 g/specimen obtained in the Brateș Experimental Laboratory, Galați.

The experiment took place in a pilot system, of recirculating type, which belongs to the Research and Development Institute for Aquatic Ecology, Fisheries and Aquaculture in Galați, for a period of 8 weeks.

We used 200 carp specimens (*Cyprinus carpio*), divided into four fiberglass tanks with a volume of 240 liters of water, with a feed rate of 4-8 L/min/tank.

At the beginning of the experiment, the biological material was sorted to ensure population homogeneity in the four tanks (control tank and three experimental tanks).

The slaughter of fish at the beginning and end of the experiment, for biochemical analysis of carp meat, was done by bathing the fish in a super concentrated solution of anaesthetic (1 mL of clove oil and 10 L of water with a temperature of 23˚C), until the installation of rigor mortis, according to Law no.43/2014, on the animal protection, used for scientific

purposes and Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Fish feeding experiments

For the control lot C, a standard fodder, type ALLER CLASSIC with a grain size of 2 mm, without grape marc, coded FS, was used.

For the experimental lots, the standard fodder was replaced with different percentages of grape marc, as follows:

- 5% of the standard fodder replaced with grape marc (coded with F5% GM), for lot T1;
- 10% of the standard fodder replaced with grape marc (coded with F10% GM), for lot T2;
- 15% of the standard fodder replaced with grape marc (coded with F15% GM), for lot T3.

The amount of fodder administered daily was 2.5% of the weekly measured biomass.

In order to be included in the fodder, fresh grape marc was dried in an oven at a temperature of 600˚C for 18-22 hours, ground and passed through a 1 mm sieve. Fresh grape marc was obtained by processing grapes from the Burgund Mare, Feteasc㠠Neagr㠠and Merlot varieties, after extracting the juice by crushing and pressing, at the Bujoru Research and Development Station for Viticulture and Vinification.

Physical and chemical parameters of the technological water

A portable multiparameter, model HQ40D - Hach, was used to measure pH, temperature, dissolved oxygen and oxygen saturation.

Nitrogen compounds (ammonia, ammonium ions, nitrite ions and nitrate ions) were determined spectrophotometrically according to the Standard Methods for the Examination of Water and Wastewater, 2005, with a Hach Lange DR 1900 spectrophotometer using LANGE kits.

The determination of chemical oxygen consumption (CCO-Mn) was performed using the potassium permanganate method, expressed in mg $KMnO_4$/L, according to the standard SR ISO 6060:1996. A mineralizer, model LT 200 from Hach Lange, was used to determine the organic matter.

Assessment of growth performance and fodder efficiency

Individual Weight Growth (WGi, g) and total Weight Growth (WGt, kg), Food Conversion Ratio (FCR, kg/kg) and Specific growth rate (SGR, %/day), were determined as follows:

WGi = Final weight - Initial weight (g/fish);

WGt = Final lot weight - Initial lot weight (kg/total fish);

FCR = feed fed (kg) / weight gain (kg);

SGR = 100 × [(ln Final fish weight) - (ln Initial fish weight)] / experimental days.

Composition of the fodders and fish meat

The analysis of fodder and samples of fish meat was performed using the procedures indicated by the standard methods of analysis for fodder and fish meat.

The moisture was determined by Standard Official Methods of the AOAC (1990).

The total ash was determined by Furnace Incineration described by AOAC (1990).

The crude proteins content of the samples was determined using the Kjeldah method of AOAC 17th edition, 2000, Official Method 928.08 Nitrogen in Meat (Alternative II), which involved protein digestion and distillation, where F (conversion factor), is equivalent to 6.25.

The total carbohydrate percentage was determined by the difference method.

This method involved adding the total values of crude protein, lipid, moisture, ash and fibre constituents of the sample and subtracting it from 100.

The total fats were determined using the Soxhlet method, equipped with Gerhardt Brand Multistate Controller, with modified ether extraction methods AOAC 960.39.

Increase protein or retained protein (RP) and increase lipid or retained lipid (RL) were calculated using the following formulas:

RP (g protein) = Final specimen mass × [final proteins] - initial specimen mass × [initial proteins]

RL (g lipid) = Final specimen mass × [final lipids] - initial specimen mass × [initial lipids]

Fatty acids profiling

The determination of fatty acids in fish meat and fodder was determined by gas chromatography. To extract lipids, the homogenized samples were dried for 1 h at 105°C. The fatty acid methyl esters were analysed with a Clarus-500 gas chromatograph with a Perkin-Elmer mass spectrometry detector, equipped with a system of injection into the capillary column (ratio of 1:100). The change of the fatty acids from the

sample to the methyl ester was followed by the separation of the components on the capillary column and identification by comparison with a chromatography standard.

Statistical analysis

All determinations were made in triplicate from the prepared average sample of 3 specimens (highest, lowest and average mass). Data are presented as mean±standard deviation (SD). The comparison of several samples was done using the ANOVA test - Single factor followed by T test. The differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSIONS

Chemical analysis of fodder

Grape marc had an acidic pH (3.75±0.13%) which may help prevent the development of pathogenic microorganisms. The major component of grape marc flour is fibre (49.35±0.67 g/100 g). Regarding protein content, the identified value (12.76±2.54 g %) was higher than that reported by Sousa et al. (2014), while for lipid content, the identified value (6.11±0.16 g %) was lower than that reported by Sousa et al. (2014). The composition of grape marc used in the present experiment is similar to that used by Nistor et al. (2014), for the nutrition of sheep and dairy cows. Grape marc is characterized by a high content of PUFA (68.88-75.66%) especially linoleic acid, and poor in SFA (12.30-13.37%) and MUFA (10.09-17.95%) according to Renata et al. (2020).

The standard fodder used, Aller type, contains 30 g% protein and 8 g % lipids. Grape marc flour, which replaced 5%, 10% or 15% of standard fodder, did not significantly alter nutrient composition in experimental diets.

Protein concentration ranged from 29.15± 0.22 g % (F 15% GM) to 29.55±0.12 g% (F 5% GM) and 29.65±0.08 g % (F 10% GM) and fat concentration ranged from 8.15±0.10 g % (F 10% GM) to 8.25±0.12 g % (F 15% GM) and 8.35±0.20 g % (F 5% GM).

The energy value for all four types of fodder was in the range of 356.96 kcal/100 g - 364.53 kcal/100 g. The conversion factors used are 4.1 kcal/g for protein, 9.3 kcal/g for lipids and 4.1 kcal/g for carbohydrates.

Substitution of standard fodder with grape marc flour resulted in a change of the fatty acid profile of the experimental feeding diets (Table 1).

Table 1. Fatty acid profile of fodder in which different percentages of grape marc have been incorporated

Fatty acid %	Standard fodder without grape marc (FS)	Fodder with 5% grape marc (F 5% GM)	Fodder with 10% grape marc (F 10% GM)	Fodder with 15% grape marc (F 15% GM)
Total Saturated Fatty Acids (SFA)	36.64	36.47	36.11	35.88
Total Monounsaturated Fatty Acids (MUFA)	31.37	30.75	30.48	30.04
Polyunsaturated Fatty Acids (PUFA)	30.21	31.53	32.25	32.85
Other Fatty Acids	1.78	1.25	1.16	1.23
Total ω-3 fatty acids	2.95	1.95	1.65	1.95
Total ω-6 fatty acids	27.26	29.58	30.60	30.90
ω6/ω3	9.24	15.17	18.55	15.85
ω3/ω6	0.11	0.07	0.05	0.06

Physico-chemical analysis of water

The physico-chemical parameters of the technological water play a significant role in fish biology and physiology. One of the major advantages of a recirculating system is to ensure the technological water quality conditions necessary for rearing the carp species (*Cyprinus carpio*) involved in the experiment.

Parameters with a significant role in carp biology and physiology were monitored to determine how grape marc diets influence the quality of the aquatic environment (Table 2).

Table 2. Physical and chemical parameters evolution, of the technological water (Average±St. Dev), during the 8 weeks of the experiment

	No. of samples	pH upH	Dissolved oxygen mg/l	Temperature °C	Organic matter mg $KMnO_4$/l	Nitrates, (NO_3^-) mg/l	Nitrites, (NO_2^-) mg/l	Ammonia (NH_3) mg/l	Ammonium (NH_4^+) mg/l
Control tank	56	8.13±0.15	6.65±0.41	25.63±0.73	62.57±12.91	2.85±0.70	0.26±0.17	0.17±0.05	1.73±0.97
Experimental tank 1	56	8.10±0.14	6.38±0.52	25.48±0.60	59.21±13.81	3.74±1.24	0.22±0.09	0.19±0.07	1.95±1.50
Experimental tank 2	56	8.12±0.17	6.52±0.71	25.46±0.56	62.71±12.83	2.94±1.19	0.22±0.07	0.17±0.06	2.11±0.12
Experimental tank 3	56	8.08±0.15	6.42±0.68	25.51±0.91	62.02±16.04	3.86±1.94	0.27±0.12	0.20±0.08	2.18±1.35
CV	56	0.02	0.09	0.03	0.23	0.37	0.45	0.34	0.65

The values of the physical and chemical parameters of water in the four rearing tanks showed that there were no statistically significant differences between the mean values of the parameters measured over the entire experimental period, for any parameter, at the significance threshold of 95% (P- value > 0.05). The values of physico-chemical parameters of water did not register deviations, falling within the optimal interval according to the provisions of Ord. MMGA no. 161/2006 on the classification of surface water quality in order to establish the ecological status of water bodies.

Feeding diets that incorporated different percentages of grape marc, being properly dosed and administered, did not influence the quality of the growing environment, water quality being essential for maintaining the health of fish involved in the experiment.

Analysis of the biologic material involved in the experiment

Growth parameters

At the end of the experiment, the fish in lot T2 fed with 10% added grape marc, had an average mass by 4.58% higher than lot T3 and by 7.63% higher compared to lot T1, results that also influenced the values of specific growth rate (SGR) in the three experimental lots (Table 3).

The amount of fodder consumed during the whole experimental period, to obtain one kg of growth increase (FCR), recorded better values in the experimental groups (1.48 for lot T2, 1.62 for lot T3 and 1.67 for lot T1) compared to control lot C (1.86) (Table 3).

The FCR values for the present experiment are higher than those obtained by Gabor et al. (2012), which studied the effect of phytoadditives on growth parameters and disease resistance in carp fingerlings.

Carp fed with additional grape marc in different percentages, efficiently capitalized on the fodder by reaching a Feed Conversion Rate (FCR) and Specific Growth Rate (SGR) similar to those obtained by Maucieri et al. (2019), in the experimental rearing of carp in conditions of different densities, and by Endut et al. (2016), in the experimental rearing of African catfish in a aquaponic system, but superior to those obtained by Bocioc et al. (2014a), studying the influence of prebiotic pellets on the growth performance of carp fingerlings reared in a recirculating system.

The Feed Conversion Rate (FCR) values obtained in the present feeding experiment of carp with added grape marc for 56 days, are similar to those obtained by Lamichhane et al. (2020), in the 60-day carp breeding experiment, that was fed diets in which *Aloe vera* extract was added, while the values of Specific Growth Rate (SGR) are higher.

The growth performance in the experimental lots showed differences but are not significant (P-value > 0.05), therefore the inclusion of grape marc in fodder ensures a similar or slightly increased growth performance compared to diets without the addition of grape marc.

Mortality was 0% during the experiment, which suggests the possibility of maintaining good health for carp fed with the addition of different percentages of grape marc.

The analysis of technological indicators highlighted the favourable influence of the addition of grape marc on the growth parameters, grape marc being rich in antioxidants, a similar conclusion stated by Dicu (Stroe) et al. (2013), studying the effect of vitamin C.

Table 3. Bioproductive indicators obtained by rearing carp, fed for 56 days on diets with varying concentrations of grape marc

Growth parameters	UM	Lot C Control tank	Lot T1 Experimental tank 1	Lot T2 Experimental tank 2	Lot T3 Experimental tank 3
		SF	(F 5% M)	(F 10% M)	(F 15% M)
		Initial Parameters			
Number of Specimens	-	50	50	50	50
Mean individual weight	*(g/specimen) mass*±SD*	21.86±3.63	22.45±4.45	23.35±3.79	22.40±4.22
Initial Biomass	kg	1.09	1.12	1.17	1.12
Density of the initial population	kg/m^3	4.55	4.68	4.86	4.67
		Final Parameters			
Number of Specimens	-	50	50	50	50
Mean individual weight	*(g/specimen) mass*±SD*	53.82±13.24	56.01±15.73	60.64±16.61	57.86±15.10
Final *Biomass*	kg	2.69	2.80	3.03	2.89
Density of the final population	kg/m^3	11.21	11.67	12.63	12.05
		Growth parameters			
Number of days	days	56	56	56	56
Weight growth individual (WGi)	g	31.96	33.56	37.29	35.46
Weight growth total (WGt)	kg	1.60	1.68	1.86	1.77
Total Shared Food	kg	2.98	2.81	2.76	2.88
Feed Conversion Rate (FCR)	kg/kg	1.86	1.67	1.48	1.62
Daily growth rate (DGR)	g/day	0.57	0.60	0.67	0.63
Specific growth rate (SGR)	%/day	1.61	1.63	1.70	1.69

* *Standard deviation*

The health of the fish was monitored daily, in order to reduce the incidence of disease and minimize the use of chemicals, medicines and antibiotics.

The biochemical composition of the material involved in the experiment

The results of the biochemical analysis of meat, highlight the accumulation of protein and fat throughout the experiment, to the detriment of moisture content for all lots involved in the experiment. At the end of the experiment, there were differences in protein and lipid accumulation between the control lot and the experimental lots fed with the addition of grape marc.

The protein and lipid increase in fish meat, at the end of the experiment, in lot C, has lower values (535.82 g proteins; 103.50 g lipids), compared to lot T1 (611.83 g proteins; 116.13 g lipids), lot T2 (687.60 g proteins; 168.46 g lipids) and lot T3 (652.41 g proteins; 159.64 g lipids) (Table 4).

Data on the biochemical composition of fodder-fed carp meat incorporating different percentages of grape marc from table 4 are similar to data previously published by Georgieva et al., 2019, on the biochemical composition of common carp meat fed with fodder supplemented with phytoadditives.

Table 4. Biochemical composition (Average±St. Dev), of carp meat fed for 56 days on diets with varying concentrations of grape marc

Biochemical parameters	Fish utilized for population of experiment	After 56 days of experiment. Lot C	Lot T1	Lot T2	Lot T3
		SF	(F 5% M)	(F 10% M)	(F 15% M)
Moisture (g %)	79.80±0.20	78.86±0.38	78.15±0.11	77.30±0.08	77.46±0.10
Proteins (g %)	16.00±0.41	16.44±0.53	17.34±0.29	17.50±0.14	17.47±0.18
Fats (g %)	2.68±0.42	3.01±0.31	3.15±0.38	3.81±0.16	3.80±0.41
Ash (g %)	1.32±0.03	1.38±0.07	1.31±0.27	1.33±0.22	1.27±0.13
M/P	4.99	4.80	4.51	4.42	4.43
RP (g proteins)	-	535.82	611.83	687.60	652.41
RL (g lipids)	-	103.50	116.13	168.46	159.64

M/P= Moisture (g %)/ Proteins (g %)

RP=Retained protein; RL=Retained lipid

The concentration of proteins and lipids has the highest value in the experimental lot fed with fodder with the addition of 10% grape marc. It is noted that the accumulation of these components in carp does not vary proportionally with the addition of grape marc in the fodder. Lot T3, which received fodder with 15% grape marc, has approximately equal values for the accumulation of protein and fat in meat with lot T2, which received fodder with 10% grape marc. The composition of carp meat in this experiment shows higher accumulations of

lipids and proteins compared to the composition of muscle tissue in carp juveniles fed the same type of standard feed supplemented with probiotics in the experiment conducted by Bocioc et al. (2014b). These results create the premises for advanced studies on the addition of grape marc in feeding diets and the extension of studies for carp rearing in soil ponds.

The M/P ratio, that characterizes the biological material in terms of nutritional value and maintenance status, decreased in all four lots, evolution determined by the accumulation of protein. The values of this ratio reached 4.80 in lot C, 4.51 in lot T1, 4.42 in lot T2 and 4.43 in lot T3. The proportion of biochemical components in carp meat in this experiment coincides with that identified by Raesen et al. (2017), in the common carp (*Cyprinus carpio*) caught in the wild.

The fatty acid composition of carp meat at the beginning and end of the experiment is shown in Table 5. MUFA monounsaturated fatty acids were predominant in the starter lot and at the end of the experiment in all four lots, the values representing about half of the total fatty acid content (~51.12%).

Table 5. Composition of fatty acids (%) in the meat of carp fed for 56 days with fodder, including varied concentrations of grape marc

Fatty Acids, %	Fish utilized for population of experiment	Lot C Control tank	Lot T1 Experimental tank 1	Lot T2 Experimental tank 2	Lot T3 Experimental tank 3
		SF	(F 5% M)	(F 10% M)	(F 15% M)
Saturated Fatty Acids (%)	28.64	27.97	27.51	26.88	27.02
Monounsaturated Fatty Acids (%)	52.07	51.25	50.58	50.54	51.18
Polyunsaturated Fatty Acids (%)	19.21	20.23	21.65	22.30	21.75
Other Fatty Acids (%)	0.08	0.55	0.26	0.28	0.05
Eicosapentaenoic Acid (C20:5n3) (EPA) (%)	3.18	3.05	4.24	4.42	3.98
Docosahexaenoic Acid C22:6n3 (DHA) (%)	4.38	4.39	5.155	5.82	5.42
Total ω-3 fatty acids (%)	7.55	7.43	9.40	10.25	9.41
Total ω-6 fatty acids (%)	11.66	12.80	12.25	12.05	12.34
ω-3/ω-6	1.54	1.72	1.30	1.18	1.31
ω-6/ω-3	0.65	0.58	0.77	0.85	0.76

The concentration of fatty acids in carp used to start the experiment is similar to that of common carp specimens analysed by Matos et al. (2019), except for the PUFA ω6/ω3 ratio which is 3.5 times lower, which highlights an increased nutritional quality of the specimens with which the experiment was started.

At the end of the experiment, the concentration of polyunsaturated fatty acids showed higher values in the experimental lots (21.65% lot T1; 22.30% lot T2; 21.75% lot T3), compared to the control lot (20.23%) (Table 5).

The PUFA values in the experimental lots are similar, with a slight increase in the lot fed with 10% added grape marc, but the differences are insignificant ($p > 0.05$). The data obtained are similar to those previously presented by Ljubojević et al., 2017, for the wild carp caught in the Danube.

The grape marc used as a supplement in feeding diets comes with an increased intake of fatty acids, leading to an improvement in the ω3/ω6 ratio, an increase in the nutritional value of the fish and a higher resistance of the biological material to environmental conditions and disease.

These results indicate that the incorporation of grape marc into the feeding diets of the carp species (*Cyprinus carpio*) reared in a recirculating system is feasible, with the recommendation to extend the rearing studies in soil basins.

The use of grape marc in aquaculture feeding diets creates an opportunity with great potential, not yet exploited.

CONCLUSIONS

Fish fed with added grape marc has a better fodder conversion ratio and improved weight gain.

This by-product rich in unsaturated fatty acids has the potential to be used as a functional fodder ingredient in carp feeding, as it stimulates the accumulation of polyunsaturated fatty acids in fish meat.

The introduction of grape marc as a source of bioactive compounds in feeding diets is a support for the development of "organic" products, the application of organic technologies, as well as the improvement of the supply with fish of superior quality.

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GROWTH AND SURVIVAL RATE OF STURGEON HYBRID BESTER ♀ × BELUGA ♂ JUVENILES REARED IN A RECIRCULATING AQUACULTURE SYSTEM

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Abstract

Growth performance and survival rates of backcrossed hybrids of bester females × beluga males were assessed after 28 days of rearing. Hybrid larvae were obtained in the conditions of the reproduction station from the Horia branch of Danube Research Consulting SRL. Three experimental groups were created, corresponding to three classes of sizes: V1- the initial weight of 3.2-5.2 g, V2-initial weight of 1.4-2.5 g, and V3- the initial weight of 0.7-1.4 g. To minimize the effects induced by the increase of the degree of heterogeneity among the fish, after 20 days the fish biomass was divided into two classes of sizes: VA: 25.8 - 32.8 g, respectively VB: 7.3 - 16.8 g. The obtained results showed better values of the individual weight gain in V1 compared to V2 and V3, while at the second stage of the experimental period, the values of the individual growth gain for each of the two experimental variants (VA, VB) were almost similar. Due to the high growth performance, as well as the good survival rate obtained in the two stages of the hybrid rearing, the crossing of these two species can be recommended for commercial fish producers for maximum yield and higher profit.

Key words*: aquaculture, growth, hybridization, sturgeons.*

INTRODUCTION

Sturgeons are one of the oldest groups of fish, with a high economic value (Agh et al., 2012; Havelka & Arai 2018; Williot et al., 2018). Unfortunately, due to the destruction of their natural habitat and to intensive international trade of caviar and meat, sturgeon populations around the world are declining (Williot et al., 2002; FAO, 2018) being classified as critically endangered by the IUCN (2013) Red List (International Union for Conservation of Nature). Lately, the sturgeon aquaculture industry is growing worldwide (Chebanov and Billard, 2001; Vasilyeva et al., 2019), being conditioned by the increasing demand of consumers for meat and caviar (Bronzi et al., 2019).

Generally, sturgeon aquaculture is divided into two main directions such as restoking of the wild population and maximizing the efficiency of production, by obtaining valuable products in a short period. To be profitable sturgeon aquaculture strategy is oriented towards obtaining intraspecific hybrids sturgeon lines (Shivaramu et al., 2019), which have higher disease resistance, and increase environmental tolerances and a better food conversion ratio (Williot et al., 2001; Shivaramu, 2019; Nikolova and Bonev, 2020). For example, bester hybrid (a cross between *Huso huso* and *Acipenser ruthenus*) proved to have a faster growth performance than its parental species (Arefjev, 1999; Baradaran, 2009). Also, Dediu et al., 2021, in a study that compares growth performance of bester hybrid and the hybrid obtained by crossing of bester ♀ × beluga ♂ (best beluga), reported a better performance for best beluga.

Over the past years, recirculating aquaculture systems (RAS) become popular for growing fish species with high commercial value, such as catfish, tilapia, rainbow trout, striped bass, sturgeons, etc (Vasilean et al., 2009; Crețu et al., 2019; Amin et al., 2020; Ekawati et al., 2021). RAS systems have the advantage of raising fish in controlled environmental conditions thus obtaining the highest production per unit area (Timmons & Ebeling, 2013). To be profitable the main goal of RAS systems is to obtain

maximum weight gain by all individuals and to increase their survival rate which results in obtaining the maximum biomass. In this context, an important technological aspect is to size-grading fish periodically. Sorting separates small and big fish fingerlings, contributing to the reduction in cannibalism (Ugwem et al., 2016), decrease in size variability among harvested fish and increased growth among small fish (Saoud et al., 2005; Chebanov et al., 2011).

In this context, this study aimed to compare the growth performance of different size classes of the best beluga hybrids, obtained by crossing of Bester ♀ × Beluga ♂, reared in a recirculating aquaculture system.

MATERIALS AND METHODS

Study animals and experimental design. Hybrid sturgeon were obtained after artificial reproduction at the hatchery of a commercial farm belonging to Danube Research Consulting (DRC), Tulcea County, Romania. Healthy Bester females and Beluga males, well developed and without any trauma, were used for artificial reproduction. After 7 days of hatching, about 50 % of the larvae began to swim actively. At this point, the administration of food began. For adaptation to pre-starter feed, with high protein content, a progressive feeding protocol was applied which aimed at the gradual transition from feeding exclusively with zooplankton (in the first 5 days when 40% BW was administered/day to the formulated diet) to mixed feeding of zooplankton and benthic organisms - tubifex (in the next 4 days) and exclusively tubifex up to 12 days. From the 13th day, the feed was gradually introduced, initially in a proportion of 3%, every 3 days doubling the proportion to the detriment of natural food. Thus, after 45 days of hatching, the hybrids were fed exclusively with extruded pellets. In this context, 219 fish with an individual weight between 0.7-5.2 g/fish were randomly distributed in a recirculating aquaculture system to create three experimental variants: V_1 (3.2-5.2 g), V_2 (1.4-2.5 g), and V_3 (0.7-1.4 g), respectively. Each size class was divided into two homogeneous experimental batches ($p>0.05$), thus ensuring an experimental design in duplicate V_1 ($V_{1.1.}$, $V_{1.2}$), V_2 ($V_{2.1}$, $V_{2.2}$), and V_3 ($V_{3.1}$, $V_{3.2}$).

To minimize the effects induced by the increase of the degree of heterogeneity among the sturgeon hybrids, after 20 days from the beginning of the experiment, the fish biomass was regrouped in two classes of sizes. In this context, 183 best beluga specimens are redistributed in two experimental variants, in triplicate VA (VA_1, VA_2, VA_3): 25.8-32.8 g, respectively VB (VB_1, VB_2, VB_3): 7.3-16.8 g.

During the 28 days of the experimental period, the fish were fed *ad libitum*, with a feed of 0.5 mm granulation, 56% crude protein, 15% lipids (first 20 days), respectively 1 mm, 54% crude protein, 20% lipids (last 8 days). Water quality was monitored daily for temperature (20.46 ±1.06°C), pH (7.36±0.16), dissolved oxygen (7.51±0.23 mg L^{-1}), weekly for nitrogen compounds (total ammoniacal nitrogen 0.20±0.09 mg L^{-1}, un-ionized ammonia was 0.012±0.006 mg L^{-1}) and was kept in the optimal range for specie and stage of development.

Calculations. At the end of each trial growth performance and feed utilization parameters were calculated according to the following equations:

- Survival rate (SR, %) = $(N_t/N_0)\times100$, where N_t represents the fish number at the end of the experiment and N_0 -number of fish at the initialization of the experiment;
- Weight gain (WG, %) = [(BWf−BWi)/BWi] ×100, where BWi and BWf are the initial and final average body weight (g) of fish sampled from each tank, and t is the experimental period in each trial (days);
- Relative growth rate (RGR, g/g/day) = (BWf-BWi)/ t /BW) (g/ g/day);
- Specific growth rate (SGR, %/day) = [(lnBWf−lnBWi)/t] ×100;
- Feed conversion ratio (FCR) = FI (g)/BG (g), where FI stands for food consumption (food provided – uneaten food) and BG is biomass gain per tank;
- Protein efficiency ratio (PER) = BG/protein consumed;
- The coefficient of variability (CV) = CvBW (%) = 100 (SD/mean BW), and was calculated for the body weight on the initial (CvBWi) and final (CvBWf) days of the experiment.

Statistical analysis. All data were analyzed using SPSS for Windows, Version 21.0 (SPSS Inc., Chicago, United States). Growth parameters were presented as means ± SD of the replicates. Before statistical analyses, both normality and homogeneity of variance were confirmed by Levene's tests. In the first trial if significant effects (ANOVA, $p<0.05$) were detected, treatment means were compared by the Duncan test ($p<0.05$). In the second trial, significant effects between treatments mean were tested using T-test

RESULTS AND DISCUSSIONS

The growth performance of the hybrids during the first 20 days is briefly presented through the technological indicators summarized in Table 1. At the beginning of the experiment and after redistribution of the biological material there were no significant differences ($p>0.05$) between the duplicates/triplicates of each experimental variant (V1, V2, V3) / (VA, VB).

Table 1. Growth performance of fish at the end of the first experimental stage and second experimental stage

Technological indicators	Experimental variants				
	Stage I			Stage II	
	V1	V2	V3	V1	V2
Experimental period (days)	20	20	20	8	8
Crude protein from feed (%)	56	56	56	54	54
Survival (%)	100	98.34±2.35	98.36	100	90
Initial average weight (g/ex)	3.93±0.19	1.88±0.12	1.09±0.02	30.51±2.56	10.90±0.15
Initial stocking density (g/m^2)	205.50±2.01	160.45±10.02	188.49±6.01	868.06±648.83	617.91±8.51
Individual weight gain (g/ex)	21.84±0.73	9.67±0.26	5.36±0.11	10.43±0.41	10.73±0.47
DGR - (g/day/fish)	1.09±0.04	0.48±0.01	0.27±0.01	1.30±0.05	1.34±0.06
RGR - (g/g/day)	0.28±0.004	0.25±0.02	0.25±0.01	0.04±0	0.12±0.01
CV initial - weight (%)	14.63±1.54	18.435±1.22	19.22±0.01	5.51±0.45	23.94±1.03
CV final - weight (%)	7.42±0.37	11.55±0.31	14.94±0.30	17.96±2.76	26.38±1.51
SGR (% BW/day)	9.42±0.06	9.075±0.37	8.89±0.22	3.79±0.18	8.57±0.29
FCR	0.60±0.02	0.48±0.03	0.25±0.03	0.72±0.05	0.58±0.4
PER (g/g)	3.01±0.13	3.69±0.29	7.17±0.81	2.57±0.17	3.29±0.22

Note: The values are presented as mean±standard deviation of the duplicates (Stage I), triplicates (Stage II), respectively.

In terms of body mass, the homogeneity of the lots was statistically verified with the Levene test ($p>0.05$). Also, the checking of the representativeness of the average was performed using the coefficient of variation (CV). The coefficient of variation of body mass (CV-weight) has a significant degree of significance in the analysis of the degree of homogeneity/heterogeneity for the fish population. Thus, taking into account the first stage of the experimental period, a downward evolution of CVM was observed in each of the three variants (V1, V2, V3). This aspect highlights an increase in the homogeneity of the experimental groups, as a result of the high nutritional recovery capacity of individuals with body biomass below the group average.

Also, at the end of the first 20 days of the experimental period, there is an inverse relationship between the average individual biomass of the biological material within the tested variants and the heterogeneity of the group of hybrids. As a result, as hybrids grow in biomass, there is an increase in competition for food consumption between the individuals from the same experimental variant. This aspect is also confirmed in the second stage of the experiment (last 8 days), where a significant increase of CV-weight values was observed in the case of the variant with the highest average of the individual biomass (VA).

From Table 1 it can be observed that the best individual weight gain was registered in the variant V1 compared to V2 and V3. Given the fact that differences between the values of the relative growth rate (RGR) associated with each of the three experimental variants are very close, the higher values of the individual weight gain from V1 can be justified by the higher individual biomass of the fish from this variant at the initial moment. At the second stage of the experimental period, the values of the individual weight gain

recorded for each of the two experimental variants (VA and VB, respectively) were close, but the higher RGR values for VB show a higher growth performance for specimens from this variant, justified by their higher metabolic activity, induced by the need for nutritional recovery.

The feed efficiency, expressed as the feed conversion factor (FCR), is better in the experimental variant V3, followed by V2 and V1. The same higher trend of feed utilization is observed, with lower individual biomass variants, and in the second stage of the experimental period, where higher FCR values are recorded for VA, compared to VB.

To evaluate the fish condition, we calculate the allometric condition factor F ($F=W/L^b$), where "b" is an allometric exponent experimentally determined. From Figure 1 and Figure 2 it can be observed that the allometric exponent "b" recorded values lower than 3, which indicate a negative allometric increase, higher in length compared to body weight.

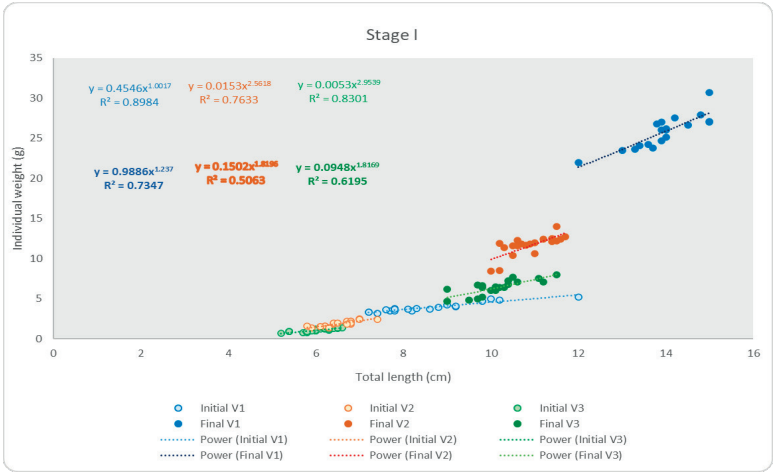

Figure 1. Length-weight regressions for different size classes at the first experimental stage (initial and final moment)

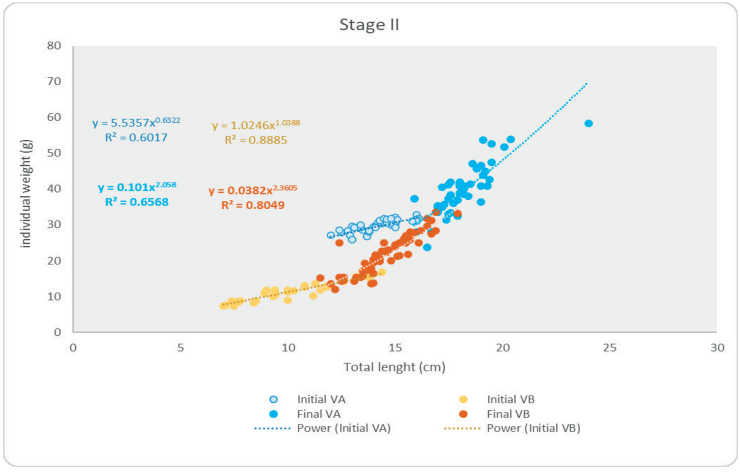

Figure 2. Length-weight regressions for different size classes at the second experimental stage (initial and final moment)

Following the comparative analysis of the experimental variants, it can be observed a higher value of the allometric condition factor, higher in VB compared to VA, suggesting an

isometric increase in VB and a negative allometric increase in VA. The better initial condition recorded in the VB variant, in conjunction with the smaller initial size of fish can be an argument for the better results obtained for all the technological indicators (Table 1). Therefore, appreciable robustness can be reported in the case of VB variants, compared to VA, which confirms the positive influence of sorting and redistribution of biological material, respectively, the ability to recover the biomass deficit. At the same time, it was observed that, at larger sizes, food competition is more pronounced, an aspect that induces a more pronounced intra-group heterogeneity.

CONCLUSIONS

The results from this study indicated that the periodically sorting of fish and dividing in less competitive subclasses can be a condition of recovering as many individuals as possible. That`s why periodically sorting of sturgeon larvae by size classes is extremely important because it increases the efficiency of the subsequent processing stages and thus the ultimate yield. This, therefore, helps most fish farmers, especially the fish hatchery operators, to achieve a high survival rate and a better efficiency of food, at early fingerlings production.

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EFFECT OF SOME WATERBORNE PHARMACEUTICALS ON FISH HEALTH

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Abstract

Pharmaceutically active compounds (PhACs) occurred in aquatic environments and have the potential to adversely affect the homeostasis of the endocrine axis leading to immunological, developmental, neurological and reproductive disarray at the organism level. These compounds are frequently discharged or run-off into the water stream and consequently is an increasing concern in research for the threat posed by PhACs on aquatic fauna. Fishes are vulnerable to different compounds, but the effects depend on specie, toxic concentration and exposure time. The concentrations of PhACs detected in the aquatic environment are relatively low but depending on the compound, they can reach up to a few hundreds of ng/g. The detection of pharmaceuticals in the aquatic environment has predominantly been reported in the developed world (USA, EU, Japan, and Australia) which are dominant countries in global pharmaceutical sales, but their presence tends to become ubiquitous. The aim of this paper is to highlight the main adverse effects of most common PhACs found in Danube river on fish health in order to assess the PhAC with the higher risk for fishes.

Key words*: Endocrine disruptors, fish health, pharmaceutically active compounds.*

INTRODUCTION

In the aquatic environment, pharmaceutically active compounds (PhACs) are increasingly present and are expected to rise over time. PhACs include human pharmaceuticals, veterinary medicines and aquaculture drugs. There are worldwide studies in the aquatic environment to determine the concentrations for some pharmaceuticals, but not all of them are considered a real risk for the environment (Corcoran et al., 2010). Depending on the predominant human and animal diseases there are categories of pharmaceuticals more often used and more common in the aquatic environment. The most used classes of pharmaceuticals are antibiotics, nonsteroidal anti-inflammatory drugs, beta-blockers, antiepileptics, antihypertensives, antidepressant (Corcoran et al., 2010; Yang et al., 2014; Ebele et al., 2017). Pharmaceuticals are ingested directly by fish through specific treatments, applied in aquaculture industry to control diseases, or indirectly due to the discharge of insufficiently treated wastewater to eliminate medicines for human and animal use (Kibenge et al., 2020). The main condition for restoring the affected aquatic environment or protecting it is to observe the traceability and monitoring these medicines, to assess the risks and identify the sources of pollution (Bobrowska-Korczak et al., 2021). Most of these pharmaceuticals reaching the aquatic environment act as endocrine disruptors (ED) on fish populations (Biswas et al., 2021). Pharmaceuticals are generally nonpolar and are able to pass through biological membranes by diffusion, to targets within specific cells and tissues (Khetan & Collins, 2007).

The most spread pharmaceuticals in aquatic ecosystem are anti-inflammatories (paracetamol, diclofenac, ibuprofen), antibiotics (trimethoprim, sulfamethoxazole, erythromycin, clarithromycin), anticonvulsants (carbamazepine), beta-blockers (metoprolol) (Kibenge et al., 2020). In the last two decades, a lot of experiments and researchers have been carried out to find which are the common PhACs in the aquatic environment and to evaluate their effects on fish. The concentrations of the most PhACs detected in the aquatic environment are relatively low, in the range ng/L to µg/L (Corcoran et al., 2010).

The legal status of PhACs vary at global level as there are countries with well-developed legislation where PhACs in the aquatic environment are observed for their biological effects (Europe, Japan, North America) (EMEA, 2006), and countries where the legislation is more permissive or poorly developed (India, China) (Corcoran et al., 2010).

In the last 20 years, the occurrence of some pharmaceuticals has been periodically reported also in the Lower Danube basin, in special in the last years, due to the increased sensitivity of the used analytical methods (Patel et al., 2019). Therefore, the most frequently founded pharmaceuticals were carbamazepine (3.94–945 ng/L), diclofenac (0.8–255 ng/L), sulfamethoxazole (30-204 ng/L), trimethoprim (0.8-223 ng/L) and ibuprofen (3.32-346 ng/L) (Chitescu et al., 2021).

Diclofenac (DCF) is an important nonsteroidal anti-inflammatory drug (NSAID) of high consumption suspected of causing damage in nontarget organisms (Parolini, 2020). It has a well-known safety and efficacy profile. In aquatic environment are found high concentrations of diclofenac that indicate an unacceptable risk in terms of environmental risk assessment (Schwaiger et al., 2004).

Ibuprofen (IBU) is the third most popular drug in the world with a relatively high therapeutic dose of 600 to 1200 mg/day (USEPA 2009). Paracetamol (PRM) is one of the most commonly used analgesic and antipyretic drug around the world available alone and also in combination with other medicines (Nunes et al., 2015). Toxic effects induced by IBU and PRM has been emphasized for different aquatic organisms such as invertebrates (Parolini, 2020; Muniz-Gonzales, 2020) and fish (Stancova et al., 2017).

Carbamazepine (CBZ) is frequently measured in river and seawater, even though it is not a pharmaceutical of high consumption. Due to low elimination rate of 4-8% (Clara et al., 2004) the most prevalent drugs are antiepileptics and antidepressants like CBZ and venlafaxine (Huerta et al., 2018). In aquatic animals CBZ affected homeostasis and physiological systems in various ways modifying reproduction, feeding behaviour and even growth and survival (Baali & Cosio, 2022).

Sulfamethoxazole (SMX) is a bacteriostatic sulphonamide antibiotic that is commonly used in human and veterinary medicine used across the globe, first produced in 1962. Sulphonamides competitively inhibit bacterial enzyme dihydropteroate synthase (DHPS) (Corcoran et al., 2010).

Although the occurrence of various antibiotics in the aquatic environment, are frequently reported, toxic effects of these drugs in fish are poorly studied.

Due to its wide use and low biodegradability, sulfamethoxazole (SMX), has been detected in aquatic environment and the toxic effect has been reported for different aquatic organisms (Zhang et al., 2021; Yang et al., 2014).

The monitoring of antibiotic residues including sulfamethoxazole in catfish Pangasius imported in Thailand (Jansomboon et al., 2017) evidenced sulfamethoxazole maximum concentrations of 245.91 µg/kg fish.

Metoprolol (MTP) is prescribed for hypertension, relieve angina and prevent heart attacks (Martinez-Rodriguez et al., 2018). Metoprolol is one of the most useful beta-blockers (Triebskorn et al., 2007 being frequently detected in aquatic systems, surface water and municipal water (Kümmerer, 2010).

MATERIALS AND METHODS

The present study is based on a literature survey on publications acquired for the information related to the target pharmaceuticals and their impact on fish physiology. The platformed used included mainly Google Scholar and Web of Science and the most used key terms were “fish health”, “fish toxicity”, “toxicologic impact” “pharmaceutically active compounds”, “paracetamol”, “carbamazepine”, “diclofenac”, “metoprolol”, “ibuprofen”. It has been selected and reviewed a number of 54 published articles focusing on targeted pharmaceuticals from the perspective of their impact on freshwater and marine fish species.

RESULTS AND DISCUSSIONS

Impacts of DCF on fish health

Diclofenac is NSAID and is one of the most detected compounds in aquatic environment. 28 days experiments on 2 years old rainbow trout

exposed to diclofenac in concentrations between 1 µg/L and 500 µg/L highlighted histopathological changes in the kidney and the gills (Schwaiger et al., 2004; Triebskorn et al., 2004). In the kidney, it was observed a distinct proliferation of the renal interstitial tissue, a severe hyaline droplet degeneration, accumulation of proteinaceous material and necrosis of tubular epithelial cells. In the gills, changes were represented by a degenerative and necrotic of the pillar cells, a dilation of the capillary walls. The researchers concluded that the damage increased significantly after exposure at 5 µg/L. As a threshold for no observed effect concentration (NOEC) could be established to be 1 µg/L. The general health of the fish can be affected at a concentration of 20 µg/L because it has been observed changes in respiratory epithelial cells, which could interfere with normal respiratory function, plus renal changes. (Schwaiger et al., 2004).

The histological research was followed by the cytological research of rainbow trout exposed to different concentrations of diclofenac (Triebskorn et al., 2004). The conclusions were that ultrastructural studies provide more detailed information. For example, diclofenac induced cellular reactions indicating activated hepatic metabolism had already become visible in the trout liver after exposure to 1 µg/L (Triebskorn et al., 2004). To these are added 50% effect concentration (EC_{50}). EC_{50} (*Daphnia magna*, motility) for diclofenac was shown to be 22.43 mg/L (Triebskorn et al., 2007).

Of interest to some researchers were the feeding performance and swimming speed, which were studied on 3-month-old medaka fish under the action of 1 mg/L diclofenac for 9 days (Nassef et al., 2010). As a result, feeding behaviour was altered by exposure to DCF.

In contrast to other experiments performed on specimens of about 3-month-old or 2 years, it was performed on zebrafish embryos exposed for 24, 48 and 72 h (van den Brandhof & Montforts, 2010). The aim of this experiment was to highlight the effects on embryonic mortality, yolk deformity, heartbeat, head malformation, otoliths and heart, tail movement and detachment, pigmentation, scoliosis, and successful hatching. After 72 h, NOEC was 1.5 mg/L. Above this concentration appeared specific effects on hatching, yolk sac and tail deformation. The EC_{50} for 72 h was 5.3 mg/L.

There were also experiments performed over a longer period of time. For example, to quantify the chronic toxicity and bio-concentration of DCF there were realised early life stages (ELS) test for 95 days exposure rainbow trout and 35 days for zebrafish (Memmert et al., 2013).

Following these experiments, it is concluded that concentrations of DCF above 320 µg/L endanger the two species of fish.

Given the experiments with contradictory conclusions regarding the effects of diclofenac on fish at different stages of life, there have been experiments to clarify this (Schwarz et al., 2017). Thus, fertilized brown trout eggs and 6-month-old brown trout were subjected to different concentrations of diclofenac at temperatures of 7°C. It was concluded that the early life stages of brown trout were not affected while the brown trout brood was affected by concentrations of up to 10 µg/L diclofenac (Schwarz et al., 2017).

These experiments showed that exposure time is a very important parameter. Thus, for a relatively short exposure time, a higher concentration of diclofenac is required to see harmful effects, while for a long period exposure time, the effects occur at lower concentrations.

Impacts of IBU on fish health

Studies on fish have shown that ibuprofen can induce changes in various tissues and systems at both sublethal and aquatic environment concentrations. Thus, studies performed on model fish *Danio rerio* exposed to concentrations up to 25 mg/L for 28 days showed the installation of oxidative stress (Bartoskova et al., 2013), while in embryos neurotoxicity and locomotor changes were observed in the context of 6 to 96 hours post-fertilization exposure to concentrations up to 500 µg/L (Xia et al., 2017). In fact, similar studies on zebrafish embryos have shown that ibuprofen is metabolized and excreted in a similar way to mammals (Jones et al., 2012) and that it affects organogenesis, larval growth and survival (David & Pancharatna, 2009). Oxidative stress in liver, blood, and gill was also observed in other fish species such as common carp, when exposed to ibuprofen between 12 and 96 hours at 17.6 mg/L (Islas-Flores et al., 2013).

Moreover, histopathological deformities in the gills, liver, and kidney were described for african catfish exposed at 3.78 mg/L for 30 days (Ogunwole et al., 2021).
The toxic effect of ibuprofen was also evaluated for much lower concentrations found in aquatic ecosystems. Thus, the exposure of freshwater species *Rhamdia quelen* for 14 days to ibuprofen concentrations up to 10 µg/L induced immuno suppressive effect and nephrotoxicity (Mathias et al., 2018) while for zebrafish impaired the cardiovascular development at concentrations ranging between 0.91 and 21.9 µg/L (Zhang et al., 2020).

Impacts of CBZ on fish health

Due to its resistance to degradation, even in the most modern wastewater treatment plants, CBZ is considered a persistent contaminant in the environment (Saggioro et al., 2018). In short-term studies (96 h) the exposure of zebrafish (*D. rerio*) at low concentrations of 1 µg/L carbamazepine resulted in significant morphological effects: accelerated yolk sac absorption, greater body length, increased swim bladder appearance (Qiang et al., 2016). On the other hand, chronic exposure of zebrafish to CBZ (10 µg/L for 63 days) caused alterations in the gonad's follicular stages, increasing enzymatic activity in some organs (acetylcholinesterase (AChE) activity in the head and muscle, glutathione S-transferase (GST) activity in gills and lactate dehydrogenase (LDH) activity in the liver) or inhibiting enzymatic activity in other structures (GST activity in the intestine) (Silva Santos et al., 2018). Similar studies revealed that exposure of adult zebrafish to 10 µg/L CBZ for 6 weeks decreases not only reproductive output of zebrafish but also have transgenerational effects impacting as well unexposed progeny up to the F4 generations (Fraz et al., 2019).
In other species, such as common carp, sublethal concentration of 5.97 mg/L (1/10th of LC_{50} value) induced alterations in the activities of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and LDH in various organs (Malarvizhi et al., 2010). Exposure of rainbow trout at sublethal concentrations of CBZ (up to 2.0 mg/L) for 42 days induced antioxidant responses (thiobarbituric acid reactive substances (TBARS), carbonyl proteins (CP), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx)) and inhibition of energy metabolic parameters ($RNA - DNA$ ratio, adenosine triphosphatases ($Na^+ - K^+ - ATPase$)) in muscle (Li et al., 2010).
Besides enzymatic and metabolic effects, CBZ acts also as endocrine disruptor inducing significant decrease of vitellogenin (VTG) concentration in striped catfish females exposed at concentrations of 25 µg/L and 50 µg/L for 4 months (Ortiz et al., 2021). Recent study emphasized that CBZ, even in low environmentally relevant concentrations, induces DNA damage and apoptosis in Chinese rare minnow (Yan et al., 2021).
However, even though short-term exposure at CBZ low environmental concentrations induced reversible oxidative damage in the liver of *Carassius carassius* unrecoverable neurotoxicity potential was also observed (Nkoom et al., 2020) underlining the risk associated with this residue in the environment.
Regarding the metabolization of CBZ by fish, a study measuring the accumulation and biotransformation of CBZ in *Jenynsia multidentate* (Valdes et al., 2016) showed that the brain and liver accumulated the highest quantity while gills, intestine and muscle the lowest. The identified metabolites were 2-hydroxycarbamazepine (2-OH-CBZ) and carbamazepine-10,11-epoxide (CBZ-EP).

Impacts of PRM on fish health

The paracetamol effects on a neotropical fish species (guaru), after 96 h of exposure to concentrations ranging from 0.008, to 80 mg/L, were observed only at the highest concentration and just at behavioural level (Matus et al., 2018). This observation is partially in line with the results reported by other researchers (Nunes et al., 2014), after exposing eel, *Anguilla Anguilla,* 96 hours to paracetamol concentrations ranging from 5 to 3125 µg/L. This experiment indicated that exposure to paracetamol was not capable of inducing a response in liver and gills and did not cause oxidative stress. However, at higher concentrations signs of neurotoxicity were observed.
On the other hand exposure of embryos and larvae of *Danio rerio* to paracetamol at

concentrations of 0.005-3.125 mg/L affected CAT, GST, cholinesterase, GPOx, and LPO activities (Nogueira et al., 2019).
Testing much lower environmental concentrations of paracetamol (0, 0.25, 2.5 µg/L) on male fish of *Rhamdia quelen* held in a semi-static bioassay for 21 days other authors (Guiloski et al., 2017) emphasized haematological, enzymatic (hepatotoxicity) and hormonal alterations (disrupted the hypothalamic-pituitary-gonadal) in special at the highest tested concentration.
This set of results shows that the paracetamol toxicity is multilevel exerted on fish impairing several key physiological functions and relationships among them.

Impacts of SMX on fish health

Most of the studies conducted to evaluate the impact of sulfamethoxazole on fish were emphasized, using zebrafish, alteration of growth, development and reproduction function. Thus, in a chronic exposure of 150 days using SMX concentrations of 2, 20, and 200 µg/L an increase in ethoxyresorufin-O-deethylase (EROD) activity was observed (Yan et al., in 2016). In terms of reproduction, egg production has declined, and the hatching success rates of embryos were reduced. Other signs of abnormal development like weak pigmentation, pericardial edema, yolk sac edema, blood clotting, lordosis was observed. Environmentally relevant levels of SMX (5.6 µg/L) tested on male zebra fish for 21 days revealed thyroid dysfunctions, an increase in thyroxin (T4) hormone concentration, an increase in expression of thyrotropin-releasing hormone receptor (trhr1) and thyroid-stimulating hormone (Kwon et al., 2016). Other acute trail testing a larger range of SMX levels (0.1, 1, 10, 100 µg/L for 120 h) showed that the zebrafish larvae's antioxidant system was affected through up-regulating the activities of antioxidant enzymes and down-regulating the reactive oxygen species (ROS) and that the ability of zebrafish to resist pathogen was suppressed (Liu et al., 2019). It is noteworthy to mention that, in some fish species like catfish Pangasius, imported in Thailand, the concentrations of sulfamethoxazole reached the maximum level of 245.91 µg/kg fish (Jansomboon et al., 2017).
In much higher doses, SMX delivered by diet (20 up to 1000 mg/kg) to Nile tilapia negatively affected growth performance, reduced intestinal biological diversity and changed the structure of the intestinal flora (Fang et al., 2021).

Impacts of MTP on fish health

Experiments realised on Nile tilapia fish from fertilized egg until 80 days post-hatch to 0.12 µg/L, 1.20 µg/L, 11.61 µg/L and 116.86 µg/L of MTP (Groner et al., 2017) highlighted changes consisted in alteration in gene expression patterns of pituitary gonadotropins luteinizing hormone (lh), follicle stimulating hormone (fsh) and increase in expression of VTG. It was observed an increased occurrence of hypertrophy, infiltration by leucocytes (at highest metoprolol concentration), proliferation of mucous and chloride cells, epithelial lifting, hyperplasia and hypertrophy of mucus and chloride cells.
Experiments performed on zebrafish in order to evaluate the effects of MTP on embryo mortality, tail movement, malformation of head, pigmentation, heartbeat, deformity of yolk, scoliosis, and hatching success at 24, 48 and 72h evidenced scoliosis at 25.3 mg/L MTP (van den Brandhof et al., 2010). At higher concentrations the effects were represented also by growth retardation and heart abnormalities.
In other experiment performed on freshwater teleost *Common carp* to highlight the toxicity induced by MTP in concentrations of 10 ng/L, 10 µg/L and 10 mg/L for the following exposure times 12, 24, 48, 72 and 96 h (Martinez-Rodriguez, 2018) SOD and CAT activity increased significantly in all organs. The most affected organs were gills and liver, while the least susceptible to these compounds was the brain (Table1).

Table 1. The effects of some PhACs on fish health

PhACs	Autors and year	Fish species	Experimental conditions	Effects on fish health
Diclofenac (DCF)	Schwaiger et al., 2004	rainbow trout	concentrations ranging from 1 µg/L to 500 µg/L	✓ kidney: a distinct proliferation of the renal interstitial tissue, a severe hyaline droplet degeneration, accumulation of proteinaceous material and necrosis of tubular epithelial cells ✓ gills: a degenerative and necrotic of the pillar cells, a dilation of the capillary walls.
	Hoeger et al., 2005	brown trout	concentrations of 0.5-50 µg/L	✓ head kidney: hinders the stimulation of prostaglandin synthesis
	Triebskorn et al., 2004;	rainbow trout	concentrations of 1-5 µg/L	✓ kidney: induction of glomeruloneophritis, necrosis of endothelial cells, and hyaline droplet degeneration ✓ gills: pillar cell necrosis, epithelial lifting, hyperplasia, and hypertrophy of epithelial chloride cells
	Memmert et al., 2013	rainbow trout	concentrations of 3.2, 10, 32, 100, 320, and 1000 mg/L for 95 days	✓ kidney: hyaline droplet degeneration of the tubular epithelial cells and the occurrence of an interstitial nephritis
		zebrafish	concentrations of 10, 32, 100, 320, 1000, and 3200 mg/L for 34 days	✓ gills: a necrosis of pillar cells leading to damage of the capillary wall within the secondary lamellae
	Nassef et al., 2010	medaka	concentrations of 1 mg/L for 9 days	✓ effects on feeding behavior and swimming speed
	Brandhof et al., 2010	zebrafish	2.9 - 5.8 - 11.6 -23.3 - 46.5 mg/L for 72h	✓ 2.9 mg/L and upwards hatching and growth retardation was recorded together with a more profound pigmentation of the yolk sac
	Guiloski et al., 2017	silver catfish	0.2-20 µg/L for 21 days	✓ 5.8 mg/L effects were recorded for heartbeat
	Triebskorn et al., 2007	rainbow trout common carp	1, 5, 20, 100, or 500 µg/L diclofenac, for 28 days under flow-through conditions (water flow rate 9 L/h)	✓ oxidative stress ✓ testosterone levels in male fish ✓ liver: collapse of cellular compartmentation, glycogen reduction, membrane material, dilation and vesiculation of ER, increased amount of macrophages ✓ kidney: glomerulonephritis with thickened basal lamina, shortening of pedicels and retraction from basal lamina, necrosis of endothelial cells, hyaline droplet degeneration ✓ gills: epithelial lifting, pillar cell necrosis, hyperplasia and hypertrophy of chloride cells

PhACs	Autors and year	Fish species	Experimental conditions	Effects on fish health
Paracetamol (PRM)	Nunes et al., 2014	Anguilla anguilla	5, 25, 125, 625 and 3125 µg/L for 96 hours	✓ the paracetamol did not cause oxidative stress
	Guiloski et al., 2017	catfish	0.25, and 2.5µg/L	✓ anti-androgenic effect, a decrease in testosterone levels of male fish and an increase in the levels of estradiol on silver catfish
	Matus et al., 2018	guaru	0.008, 0.08, 0.8, 8.0 and 80 mg/L	✓ the paracetamol effects observed were not in terms of swimming patterns of fish
Carbamazepine (CMZ)	Nassef et al., 2010	medaka	6.15 mg/L for 9 days	✓ effects on feeding behavior
	Malarvizhi et al., 2011	common carp	0.2, 2, and 20 mg/L concentrations for 2 h	✓ oxidative stress in spermatozoa and decreases sperm motility and velocity
	Hampel et al., 2014	atlantic salmon	7.85 µg/L for 5 days	✓ upregulation of mRNA expression level of pituitary hormones like somatolactin, prolactin, and growth hormone somatotropin
	Triebskorn et al., 2007	rainbow trout, common carp	1, 5, 20, 50, or 100 µg/L carbamazepine for 28 days under flow-through conditions (water flow rate 9 L/h)	✓ liver: increased amount of macrophages, membrane material ✓ kidney: vesiculation and dilation of endoplasmic reticulum (ER) in posterior portions (PII) and distal tubules (DI), enlarged mitochondria in DI, increased amount of macrophages in PII and DI, increased amount of cellular debris in intercellular spaces and secondary lysosomes in basal portions of cells ✓ gills: epithelial lifting, hyperplasia and hypertrophy of mucus cells
	Qiang et al., 2016	zebrafish larvae and embryos	1, 2, 5 µ/L of carbamazepine for 72 h and 96 h	✓ accelerated the development of embryos zebrafish and perturbed their behaviours in larvae and embryos zebrafish
Metoprolol (MET)	Triebskorn et al., 2007	rainbow trout, common carp	1, 5, 20, 50, or 100 µg/L for 28 days under flow-through conditions (water flow rate 9 L/h)	✓ liver: at 1 µg/L collapse of cellular compartmentation, glycogen reduction, membrane material, dilation and vesiculation of ER, increased amount of macrophages, cellular disintegration at the spaces of Disse ✓ trunk kidney: slight thickening of the basal membrane in the RC, slightly elongated and more branched endocytotic channels in the PI, and an increased amount of macrophages in all investigated kidney portions ✓ gills: epithelial lifting, hyperplasia and hypertrophy of mucus and chloride cells, dilation of ER in chloride cells with electron dense-lumen
	Brandhof et al., 2010	zebrafish	6.25, 12.5, 25, 50, 100 mg/L for 72 h	✓ 25.3 mg/L with scoliosis as the sensitive effect, and growth retardation and heart abnormalities at higher concentrations

PhACs	Autors and year	Fish species	Experimental conditions	Effects on fish health
Metoprolol (MET)	Groner et al., 2017	nile tilapia	0.12-116.86 µg/L 80 days post-hatch	✓ alteration in gene expression patterns of pituitary gonadotropins (lh, fsh) and increase in expression of VTG ✓ increase of hypertrophy, proliferation of mucous and chloride cells, as well as infiltration by leucocytes (at highest metoprolol concentration) ✓ epithelial lifting, hyperplasia and hypertrophy of mucus and chloride cells
	Martinez-Rodriguez et al., 2018	common carp	10 ng/L, 10 µg/L and 10 mg/L for 12, 24, 48, 72 and 96 h	✓ SOD and CAT activity increased in all organs. The most affected organs were gill and liver, while the least susceptible to these compounds was brain.
Sulfamethoxazole (SMX)	Kwon et al., 2016	male zebra fish	5.6 µg/L for 21 days	✓ thyroid dysfunctions, an increase in thyroxin (T4) hormone concentrationan ✓ increase of thyrotropin-releasing hormone rece ptor (trhr1) and thyroid-stimulating hormone, beta subunit (tshb) mRNA in the zebra fish brain
	Yan et al., 2016	zebra fish	2, 20, and 200 µg/L for 150 days	✓ increase in EROD activity, depressed the egg production of the parental fish by 13.2 to 26.4 % by the highest concentration ✓ the hatching success rates of embryos were reduced by 15.7 to 26.7% ✓ lower survival rates of the larvae were observed after the parents exposed to 200 µg/L SMX ✓ induced a higher development abnormality rate: pericardial edema, weak pigmentation, blood clotting, yolk sac edema, underdeveloped eye and ear, and lordosis
	Liu et al., 2019	zebrafish larvae	0.1, 1, 10, 100 µg/L for 120 h	✓ affect the young fish's antioxidant system through up-regulating the activities of antioxidant enzymes and down-regulating the ROS ✓ suppresses the ability of zebrafish to resist pathogen
	Fang et al., 2021	nile tilapia	LS - 20 mg/kg day; MS - 200 mg/kg day; HS - 1000 mg/kg day) for 4 weeks and then fed with normal feed for 4 weeks	✓ reduced the biological diversity, changed the structure of the intestinal flora, affected the number and proportion of dominant bacteria, without changing the main dominant bacteria at the phylum leve ✓ changed the growth performance of nile tilapia MS>LS>NS, while HS antibiotics significantly retarded growth the structure of intestines flora

CONCLUSIONS

The problem concerning the presence of PhACs in the aquatic environment is significant. The experiments performed in the last decades showed that the PhACs may alter aquatic ecosystem equilibria by the negative effects on fish populations. It should be noted though those experimental trials analysed here were performed during short periods of time from a few hours, days to weeks. However, the effect of analysed products was significant even at low environmental concentrations estimating deeper effects in fish species with longer life cycle. This illustrates the need for more long-term chronic studies to evaluate the effect of different PhACs in fish, either individually or in combinations found in the aquatic environment. These studies are relevant not only for environmental risk assessment but also from the perspective of aquaculture industry, in special for open production systems.

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OBSERVATION ON SOCIAL BEHAVIOUR OF OSTRICH (*STRUTHIO CAMELUS*) IN CAPTIVITY

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Abstract

The ostrich, the flightless Ratite, is the world's largest bird. Ostrich has been reared in Romania for many years, but we have limited information on this species. The aim of this paper is to provide information on the social behavior of the ostrich (Struthio camelus) in captivity. Ostrich products, meat skin, and eggs are considered luxury products not only in Romania but also abroad. Observations on the social behavior of captive ostrich were made on two extensive farms in Romania in Arad County. The most common behavior patterns are walking, sitting, standing, foraging, pecking, dancing, coprophagia and aggression. The captive ostriches spend most of their time standing, resting or bathing, and nest-making. In the summer period, ostriches show the greatest difference in their behavior, they are more inactive and sit more in rainy weather than in dry periods. In the breeding season, they become more aggressive. They are violent toward humans, juveniles, even each other and other species. Some individuals may be showing abnormal behavior such as feather pecking, anorexia, coprophagy, and dietary indiscretion. Understanding the feelings in animals through behavioral observations is a vital step in improving their welfare.

Key words*: captivity, social behaviour ostrich, Struthio camelus.*

INTRODUCTION

The growth of ostriches in Romanian farms is in continuous development, as a new branch in agriculture. The ostrich feather trade, however, is several centuries old, dating back to primitive civilizations. From ancient times, ostrich feathers have been used for decorative purposes. In ancient Egypt, they were a symbol of justice. The main targets for ostrich growth are 60% for skin, 15% for eggs, 14% for meat, and 11% for feathers (Dragan et al., 2019; Fericean et al., 2013; Fericean, 2017).

The ostrich (*Struthio camelus*) is the largest, tallest, and heaviest flightless bird which has 2.75m in height and weight from 70 to 150 kilograms, and its feathers are fluffy and symmetrical (Mushi, 2008; Kreibich, 1995; Csermely, 2007; Newberry, 2007).

The ostrich is an herbivorous bird, a sole member of its order, is endemic to Africa and can be found in a range of open habitats (Hallam, 1992; Kreibich, 1995; Davies, 2003; Alden et al., 1996, Schaller et al., 2011; Birau, 2013; Croney, 2016). They have long necks and legs and are the fastest running bird achieving a speed up to about 60-70 km/h. At a month-old ostrich chicks can run at speeds approaching 50 kilometers per hour (Davies, 2003). They can lay the largest eggs that can weigh up to 1.4 kilograms. They can live until 50 years (Birau et al, 2013).

MATERIALS AND METHODS

Observations on the social behavior of captive ostrich were made for three consecutive days, three times a day: morning, lunch, and afternoon (period 1 - from 6:00 a.m. to 09:30 a.m.; period 2 - from 10:00 a.m. to 1:30 p.m. and period 3 - from 2:00 p.m. to 5:30 p.m.) in April 2021, in a two farm from Arad County. Observations were made on ostriches raised in the trio breeding system (Figure 1) and colony group (Figure 2). No rainfall was recorded during the observation days.

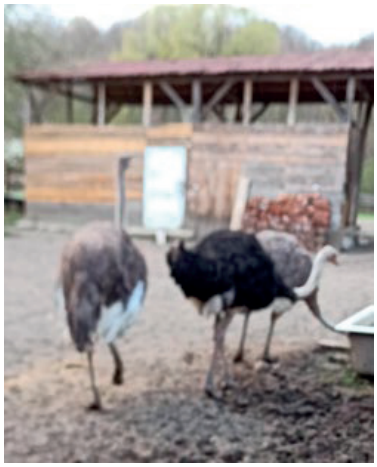

Figure 1 The trio families - one male and two females

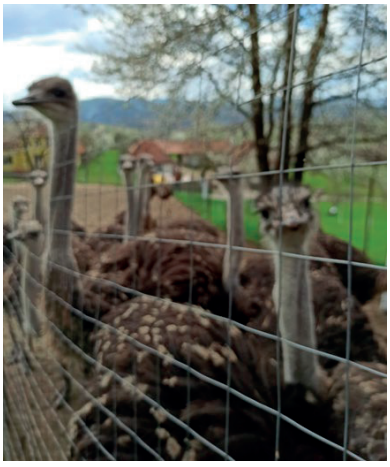

Figure 2. The colony groups

To produce the ethogram, a few behaviors were observed as feeding, courtship, dust-bathing, resting standing, sitting, walking, and aggression.

RESULTS AND DISCUSSIONS

The social behavior of ostriches is as complex as that of some social mammals with dominance among individuals (Schaller et al., 2011).
The ostrich (*Struthio camelus*) is a diurnal species, very active during the day. In the wild, a male ostrich has a territorial range and a small harem, and also, they can be violently protective of their territory.
In captivity, they are separated into categories: ostrich chicks of different ages, young ostrich, productive and reproductive families a group that included a male and more ostrich females. For safety reasons, it should you never have more than one male in a paddock.
They stand during the day unless they rest, bathe in the dust, mate, or nest. They remain inactive during the night if they are not disturbed (Degen & Rosenstrauch 1989). The resting behavior of ostriches is present both during the day and at night when they stop doing any activity.
This behavior includes sitting, standing, and sleeping. Sitting was made with the foot bent under the body, and the neck straight up in some cases (Figure 3), the head and neck tilted to the ground.

Figure 3. The sitting behaviour

Observations during the day show that the most commonly observed resting behavior was standing (Figure 4), followed by sitting. The sleeping behavior was observed only at night at trio families and at colony group was observed in the afternoon and at night.

Figure 4. The standing behaviour

Locomotion behavior is one of the most common behaviors (20%) in trio families and at colony group, it is much higher reaching 30%

and was observed in the morning, afternoon and evening and includes walking was with their necks straight up, was the most common locomotion observed behavior (93%) followed by running (7%).
The ingestive behavior of ostrich was divided into four categories: feeding on green fodder, feeding with concentrated feed (Figure 5), drinking (Figure 6), and pecking (Figure 7).

Figure 5. The feeding with concentrate

In the wild, the diet of ostrich consists of plants, insect eggs, fruits, small mammals, and sand. The ostrich spent a lot of daylight hours feeding with continuous ingestion.
Feeding with green fodder (35%) was the most frequent ingestive behavior observed, followed by feeding with concentrated feed (32%) followed by pecking (27%), and drinking (6%). Clean and fresh water must be available for the ostrich free choice.

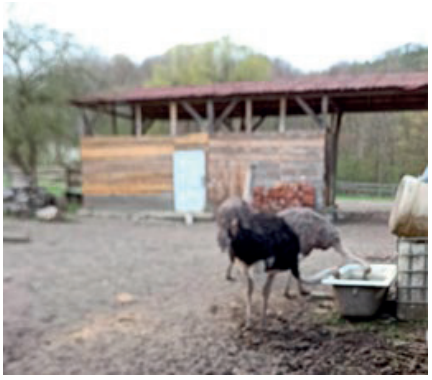

Figure 6. The drinking behaviour

Pecking has a lower percentage in breading families and higher in the colony group. Ostriches frequently arrange their feathers with their beaks and do so while walking, sitting, and standing.

Figure 7. The pecking behaviour

In natural habitats, the ostriches live in groups especially for water and food, with a dominant male defending and establishing territory, and a few other females of which a dominant female is called the "main hen".
The courtship has a high percentage of 30% and was observed only in breeding families of ostrich.
The hen ostrich is sexually mature at 4 years and the male matures at 5 years (Schaller, 2011). The male ostriches have a black-and-white plumage, while females and immature birds females have grayish-brown feathers.
Madekuroza et al. (2006) studied the sexual behavior of adult ostriches and mentioned that females showed violent behavior against other females and to impress their potential mates made pre-nuptial courtship by posturing (Figure 8 a, b). Young ostriches had a submissive behavior by lowering head and neck in S-shape. Males in mix-groups showed a dominant and aggressive behavior by posturing usually with tails held erect.
In reproductive families during the breeding season, males made ‘the breeding dance’ to attract the female for breeding. The ritual is very complex, he sinks slowly to the ground, by swelling their feathers, crouching, and using his wings to swing.

a.

b.

Figure 8. The femele ostriches mating dance a, b

The male scraped in the ground a nest who contains one or more eggs. The main hen mates with the male and puts her eggs in the center of the nest where have the best chance of hatching. The other female may mate with the male and then lay their eggs in the same nest made by the male. Both the main hens and the males incubate the eggs and care for the chicks, the female sits on the eggs at night and the male during the day. An ostrich egg is very shiny and has the color of bone-white (Figure 9).

Figure 9. The ostrich egg

In the early stages of raising the chicks, the male is very protective and aggressive. High aggression towards the caregiver or other males was observed during the breeding period.

In Romania, the breeding season depends on climatic conditions, the quality of food, and state birds and commences in early spring.

In reproductive families, aggression had a high share of 7% (Figure 10) and was observed especially in males compared to the caregiver or other strangers both during egg collection and during the administration of feed. The males kept in the neighboring shelters showed aggression.

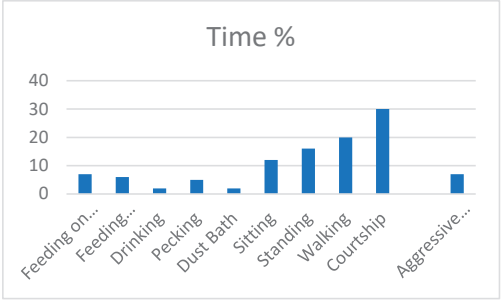

Figure 10. The behaviour of trio families

At the productive ostrich, the aggression had a very low weight of 1% (Figure 11) and was observed only during the administration of concentrated feed.

Comfort behaviors were low during the research period, especially in breeding families, and include

the care of feathers and other bodies, bathing in the sand when the ostrich would lie down in dry sand. The aggressive behavior was lower in the afternoon and evening compared to the morning.

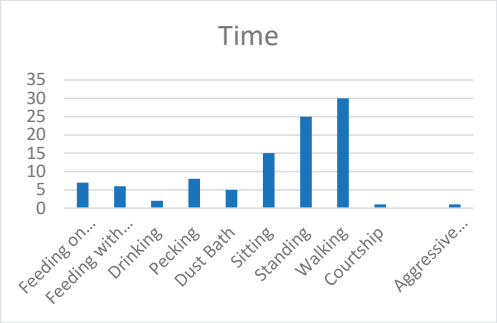

Figure 11. The behaviour of colony group

Bolwig (1973) and Kock (1996) investigated interspecific behaviors of the ostrich with mammals and birds and observed that the ostrich tolerates other species.

Amado et al. (2011) mentioned that the behavior of ostriches diverges according to the day period and age. In addition to the behaviors mentioned, a variety of maintenance activities were observed, including stretching and yawning.

Magige (2008) and Vyver (1992) observed that frequency of behavior changes in the winter and summers seasons.

Due to inadequate growth techniques, ostrich age groups can engage in abnormal behaviors such as stinging, feather picking, dietary indiscretion, behavioral stargazing, anorexia, and aggression.

CONCLUSIONS

During captivity, the ostrich can also experience stress so it is necessary for the conditions in the shelters to be as close as possible to the natural environment. In captivity, the ostrich improves the feed concentrates very well.

In the breeding season, the aggressivity is higher, the males are violent toward each other, humans, or caregiver.

Some individuals may show abnormal behavior such as feather pecking, anorexia, coprophagy, and dietary indiscretion. Understanding the feelings in animals through behavioral observations is a vital step in improving their welfare.

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THE IMPACT OF THE COVID 19 PANDEMIC ON THE PRODUCTION PRICE OF CARP RAISED ON FLOATING CAGES

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Abstract

Fish is an important food in human nutrition, with high biological value, which does not produce adverse side effects, being easily digestible. In this study, it is examined how the Covid-19 Pandemic and the measures taken to limit the spread of the virus affect and transform the production costs of the carp raised on floating cages, having a direct effect on the sales price. The sector is expected to resume expansion known in 2019 over the next four years, although rising costs are a challenge to be overcome. As a result of comparison, we find a considerable increase in the production price, which is largely given by the increase in the feed price.

Key words*: aquaculture, economy, fish, management, production costs.*

INTRODUCTION

The main aim of fish culture is to produce and control production of fish and other aquatic organisms for human consumption (Diaconescu, 2003). Fish is an important part of human nutrition, healthy food, with high biological value (Anin et al., 2021).

The importance of fish farming in the global economy is related to the great potential of inland waters in the production of aquatic organisms for human and industrial consumption (FAO, 2022).

Floating cages can be placed in natural and artificial pools, being suitable for intensive fish breeding (Pricope et al., 2013).

At the beginning of 2020, the Corona-virus pandemic was declared. After the beginning of the pandemic, measures were implemented to limit the spread of the virus, which led to limiting the movement of people, goods, and increasing prices. As a result, the aquaculture sector was also directly affected (Alam et al., 2022).

With the increase in the cost of production, the profit rate decreased, because it was desired that the sale price to be directly proportional to the purchasing power. The purpose of this paper is to highlight the evolution of economic indicators during the pandemic, compared to the pre-pandemic period.

MATERIALS AND METHODS

In the present study, a comparative analysis of the economic indicators for a production of 100000 kg carp in floating cages was made. The analysed period is the period of the pandemic, respectively the year 2021, compared to the pre-pandemic period, respectively the year 2019.

In order to carry out this analysis were used indicators of effect, effort and economic efficiency, respectively production expenses, production cost, profit, unit profit and the rate of capital consumed or the rate of profit.

The following calculation formulas have been used (Niculaie & Costaiche, 2014):

- for *Unit cost (Cu)*:

$$Cu = Capm / Q \quad (1)$$

where:
Capm = production costs;
Q = production quantity.

- for *Profit (P)*:

$$P = V - Capm \quad (2)$$

where:

V = sales income;
Capm = production costs.

- for *Unit profit (Bu)*:

$$Bu = Pvu - Cu \quad (3)$$

where:
Pvu = sale price/kg;
Cu = unit cost.

- for *Profit rate (Rr)*:

$$Rr = (B/Capm) \times 100 \quad (4)$$

where:
B = benefit/profit;
Capm = production costs.

For the calculations, the euro was used at the rate of 4.9 lei (average exchange rate euro / leu in 2021).

RESULTS AND DISCUSSIONS

In order to make the comparison, the production cost was analyzed, including the following: fodder, medicines, electricity, salaries, cages maintenance, service contracts, car and boat maintenance, telephone and building maintenance contracts. In the pre-pandemic year (2019) the total expenditure was 1430051 lei (291847 euro) (Table 1), and in the pandemic year 2021 the expenses amounted to 1787947 lei (364887 euro) (Table 2).

Table 1. Expenditures in 2019 for the production of 100,000 kg of carp

Name of expenses	Calculation	Expenses value during production period	Expenses value expressed as a percentage
Fodder	190000 kg×3.6 lei (0.73 euro)	684000 lei (13959 euro)	47.83%
Veterinary drugs		7185 lei (1466 euro)	0.50%
Electricity		16800 lei (3429 euro)	1.17%
Salaries	8 fishermen×3000 lei (612 euro)/month 1 engineer×4000 lei (816 euro)/month 1 manager×4000 lei (816 euro)/month 3 guards×3000 lei (612 euro)/month 1administrator×3500 lei (714 euro)/month	462000 lei (94285 euro) (net salary) Gross salary= net salary x 40.93% 651096 lei (132877 euro) (gross salary)	45.53%
Cages maintenance		10000 lei (2041euro)	0.70%
Service contracts		9800 lei (2000 euro)	0.68%
Maintenance cars/boats	4 cars 6 boats	21000 lei (4285 euro)	1.50%
Communication		20000 lei (4082 euro)	1.40%
Maintenance of buildings and annexes		10170 lei (2075 euro)	0.70%
Total		1430051 lei (291847 euro)	

Following the total production expenses in 2021, we can see an increase of 20 percent compared to 2019, the fodder having the largest share in 2019 followed by salaries. In 2021, the highest percentage of expenses was recorded by salaries (Figure 1).
The percentage analysis indicates a 16% increase in fodder expenses in 2021, while the salary expenses register an increase of 25% in the pandemic year (Figure 2).
Keeping the upward trend, the other categories of expenses register increases of maximum 1% in 2021 compared to 2019, which have a small influence on the selling price of about 6% of the total expenses (Figure 3).

Table 2. Expenditures in 2021 for the production of 100,000 kg of carp

Name of expenses	Calculation	Expenses value during production period	Expenses value expressed as a percentage
Fodder	190000 kg×4.3 lei (0.87 euro)	817000 lei (16673 euro)	45.7%
Veterinary drugs		8000 lei (1633 euro)	0.44%
Electricity		18000 lei (3673 euro)	1.00%
Salaries	8 fishermen×3500 lei (714 euro)/month 1 engineer×4500lei (918 euro)/month 1 manager×4500lei (918 euro)/month 3 guards×3500lei (714 euro)/month 1administrator×4000lei (816 euro)/month	618000 lei (12612 euro) (net salary) Gross salary= net salary x 40.93% 870947 lei (177744 euro) (gross salary)	48.71%
Cages maintenance		11000 lei (2245 euro)	0.61%
Service contracts		10000 lei (2041euro)	0.57%
Maintenance cars/boats	4 cars 6 boats	21000 lei (4285 euro)	1.18%
Communication		20000 lei (4082 euro)	1.12%
Maintenance of buildings and annexes		12000 lei (2449 euro)	0.67%
Total		1787947 lei (364887 euro)	

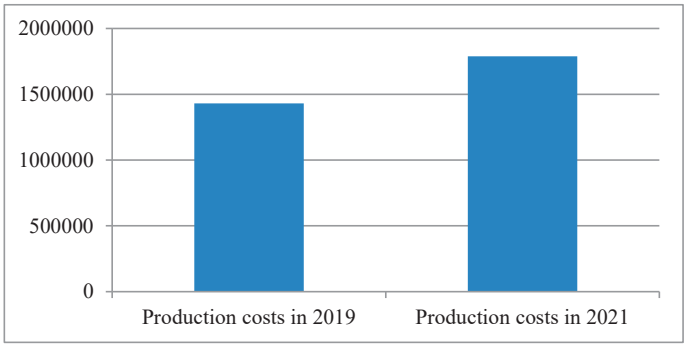

Figure 1. Analysis of production costs in 2019 and 2021

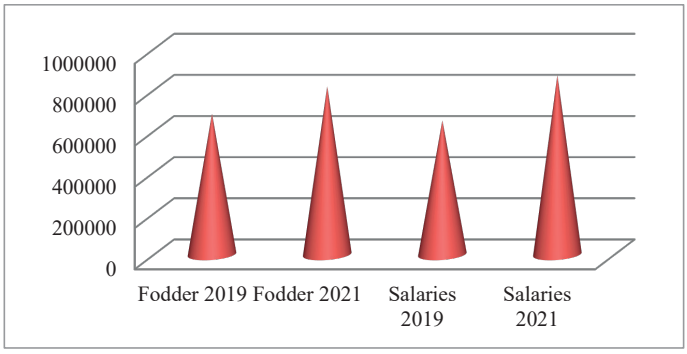

Figure 2. Analysis of costs for feed and salary in 2019 and 2021

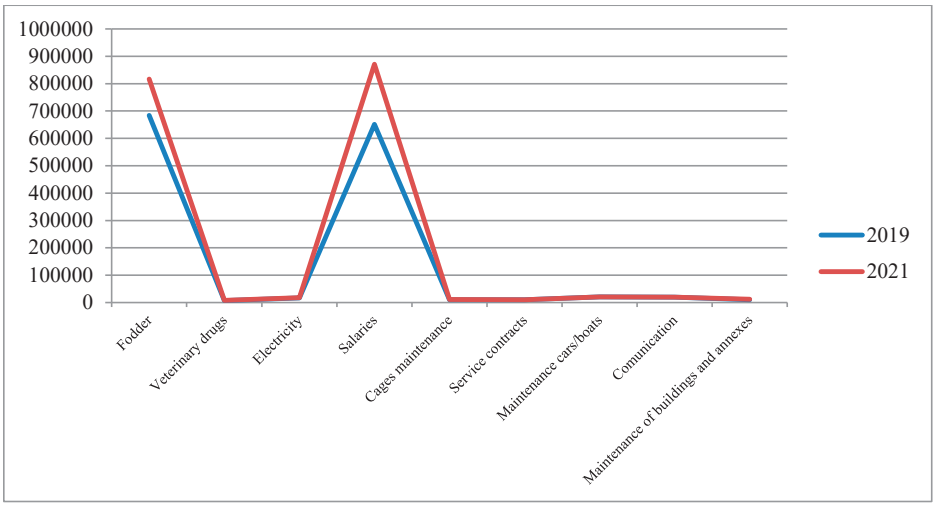

Figure 3. Other cost categories in 2019 and 2021

The income is an amount of money that a company receives from its normal business activities, usually from the sale of goods and services. The income obtained from the capitalization of the 100000 kg of carp grown in floating cages in 2019 is 1700000 lei (346939 euro), the sale price being 17 lei (3.45 euro)/fish kg, while in 2021 the sale price was 19 lei (3.88 euro)/fish kg registering an income of 1900000 lei (387755 euro) (Figure 4).

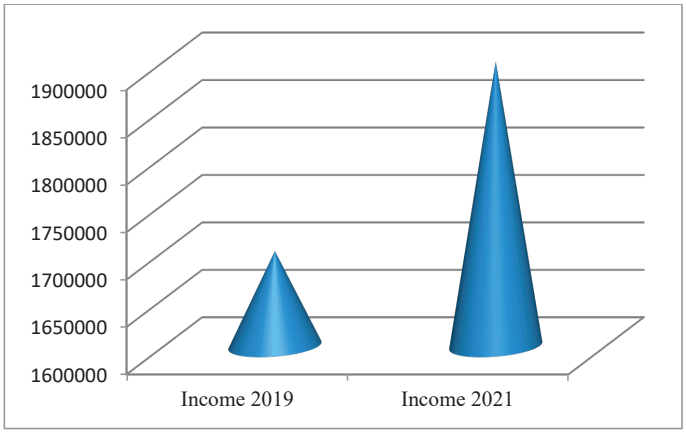

Figure 4. Income analysis in 2019 and 2021

The profit is the amount of money obtained by making the difference between the value of production of goods at sale price, and the value of production of goods at the cost of production. The profit obtained from the sale in 2019 was 269949 lei (55092 euro), representing 15.9% of the total revenues, while in 2021 there is a profit of 112053 lei (22868 euro), representing 5.9% of the total revenues (Figure 5).

By the end of 2021, there is a profit of 63% lower than the pre-pandemic year, 2019.

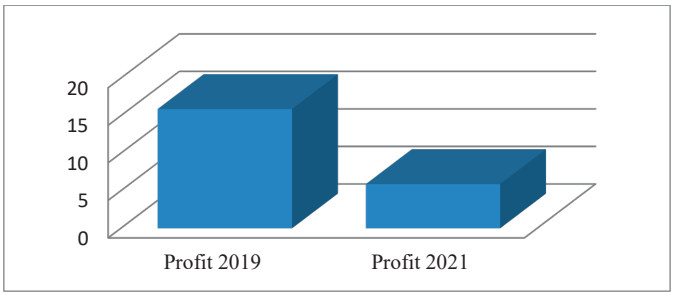

Figure 5. Profit analysis in 2019 and 2021

Economic efficiency is a notion with a complex content, which expresses the way of transformation in the production process of the economic effort performed (production costs and expenses generated by the consumption of economic resources), the resulting economic effect (quantity, quality and value of the realized production) (Table 3).

Table 3. Economic efficiency

Specification		2019 year	2021 year
Sale price/kg	lei	17	19
	euro	3.47	3.88
Price of feed/kg	lei	3.6	4.30
	euro	0.73	0.87
Unit cost/kg	lei	14.30	17.87
	euro	2.92	3.65
Profit	lei	269949	112053
	euro	55092	22868
Sales revenue	lei	1700000	1900000
	euro	346939	387755
Unit profit	lei	2.7	1.13
	euro	0.55	0.23
Profit rate %		18.9	6.26

The economic efficiency was increased in 2019 with a rate of profitability of 18.9% and a unit benefit of 2.7 lei, while in 2021 we recorded a rate of profitability of only 6.26% and a unit benefit of 1.13 lei (Figure 6).

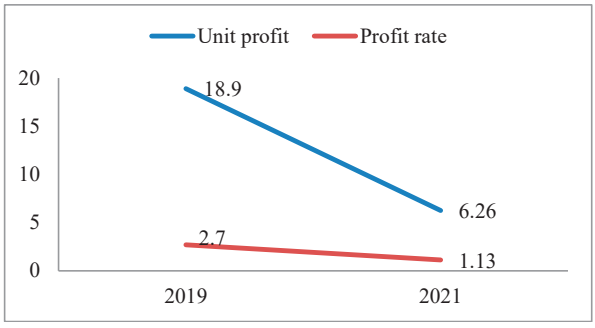

Figure 6. Economic efficiency analysis in 2019 versus 2021

CONCLUSIONS

Analysing the economic indicators of effect, effort and economic efficiency for a production of 100000 kg carp in floating cages, during the pandemic year, respectively 2021, compared to the pre-pandemic period, respectively 2019, the following conclusions can be drawn:

- The indicators of effect, respectively the production expenses in 2021 register an increase of 20% compared to 2019, an increase that is due to the increase in fodder and salary expenses.
- The effort indicators, respectively, the revenues registered an increase of 11.7% in 2021 compared to 2019, due to the increase of the selling price.
- Efficiency indicators, respectively:
  - the unit cost registered an increase of 24.9% in 2021 compared to 2019, which represents a negative aspect from an economic point of view;
  - the profit recorded a decrease of 58.5% in 2021 with 2019 a profit rate decreased by 66.9% in 2021 compared to 2019 considering the increase in expenses and the decrease in revenues;
  - the profit rate registered a decrease of 66.9% in 2021 compared to 2019 considering the increase of expenses and the decrease of revenues.

ACKNOWLEDGEMENTS

This research work was a part from the PhD thesis of Anin Ionut Alexandru - "The influence of thinning and subdivision on carp for consumption increased in floating ponds", and was carried out with the support of Faculty of Animal Productions Engineering and Management, University of Agronomic Sciences and Veterinary Medicine of Bucharest, Pelicanul Distribution carp farm (Giurgiu County) and Romanian Fish Board.

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STUDY REGARDING THE EVOLUTION OF SOME SEDENTARY GAME POPULATIONS IN GIURGIU COUNTY

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Abstract

The purpose of this paper is to analyse the situation of some native game species in Giurgiu County, based on official assessments, using data from the Ministry of Environment. The analysis of the official data from the evaluations of sedentary game will give the possibility to assess if the data presented at the evaluations are correct and if the hunters' fears are justified. The analyses also took into account the creditworthiness keys of the hunting areas in order to perform a comparative analysis between the real and the optimal number. Following the analyses performed, and the resulting conclusions, some recommendations were formulated, among which we mention: the active involvement of the administrator of the national hunting fund by participating and supervising the evaluation actions of the sedentary game herds, or the obligation of the managers of the hunting areas to maintain an ascending trend until the optimum number is reached.

Key words*: hunting, pheasant, sedentary game, wildlife.*

INTRODUCTION

After hatching, the young bee feeds on honey in the last decade hunters from all over the country have drawn attention to declining populations of game, especially for hare and pheasant populations (Hill & Robertson, 1988).

For a period of more than half a century, the hare was the main game in Romania, due to the important share it had in the total hunting harvest, due to both large numbers and the spread of the species throughout the country (Comșia, 1961; Cotta et al., 2008).

In the last decades, the number of hares has rapidly decreased, the situation being as obvious as possible, especially for those who enjoy the practice of this noble sport, respectively hunting (Weterings, 2018).

The situation is much more obvious in the plain areas of southern Romania, areas where intensive agriculture is practiced, as is the case of Giurgiu County. The causes that led to this are many and various. It is mentioned here the intensive agriculture (large share of monoculture on huge areas, the use of machines with impressive working widths, excessive herbicide etc.), climate change (late frosts, cold rains followed by frost even during birth etc.), and increasing of raptors populations (Babutia, 1964; Cukor et al., 2018). It seems that, at present, in Romania, the pheasant took the place of the rabbit, as the main game, but we must point out that in the case of the pheasant we are dealing with the same problems.

All over the world the hunter's associations and other organizations (scientific or not), collaborate in the direction of environmental protection implicitly for conservation of biodiversity and protection of wildlife. Of course, this conservation of the environment and biodiversity cannot be carried out without maintaining a prey-predator balance, a balance whose maintenance is hampered by the protection of predator birds (which have multiplied significantly). More than that, a new predator appeared and developed rapidly - the golden jackal, which also contributes significantly to the decline of prey game populations. However, the official data that formed the basis of the study shows a very good situation, which makes us ask some questions about the veracity of the information.

MATERIALS AND METHODS

The analysed material it is represented by two prey species - pheasant and hare - and two predators species - fox and the golden jackal. It was analysed the official data from Ministry of Environment, Hunting Department, more exactly the evaluation of sedentary game in Giurgiu County area, between 2016 and 2021 years.

In order to have a better view of situation it was calculated some statistics, like average population, standard deviation, error of average, and variability coefficient.

It was taken in to account the optimal population, the real population, the hunting harvest and the number of animals approved for hunting (quota). It must specify that the hunting areas in Giurgiu County total more over 300000 ha. All this hunting area is divided in 42 hunting funds (districts at over 5000 ha each), which are managed by National Forest Authority, hunter's associations and associations for conservation of biodiversity (as in the case of the Neajlov Delta reservation).

RESULTS AND DISCUSSIONS

The situation of hare population, in Giurgiu County, between 2016-2021, is presented in Table 1.

Table 1. Dynamics of hare population in Giurgiu County, in 2016-2021 period

Year	Evaluated population	Hunted	Approved for hunting (quota)	Optimal effective
2016	31003	2384	3240	28090
2017	29831	1705	3115	28090
2018	28707	1671	2640	28090
2019	28204	1432	1785	28090
2020	27332	1252	1775	28090
2021	25934	1514	1710	28090
X	*28501.83*	*1659.67*	*2377.5*	*28090*
St. dev.	*1795.3047*	*391.4295*	*709.4143*	*0*
Sx	*802.8847*	*175.0526*	*317.2593*	*0*
CV%	*6.3*	*23.58*	*29.84*	*0*

It is obvious a descendant tendency of population as it is showed by the variability coefficient. The biggest ambiguity is given by the relatively small differences between the optimal and real numbers as presented by the official data, and which do not correspond to the reality on the ground (Figure 1). The hunted and approved number of individuals for hunt creates same ambiguity also.

In 2016, the approved number of hares for hunt (3240 individuals) represent 10.45% from evaluated population, or the real number as it calls. From this number of 3240 individuals approved to be hunted, only 2384 was hunted (73.58%), which means a share of 7.69% from the evaluated effective.

In 2017 the evaluated population has decreased with 3.78%. The number of approved individuals for hunting decreased with 3.86% which highlights the interest of the national authorities to maintain a well balanced management plan. From 3115 approved individuals to be hunted, it was harvested only 1705 which means only 54.74%. Transpose to the real (evaluated) population we talk about a share of just 5.72% in comparison with 7.69% in 2016 year.

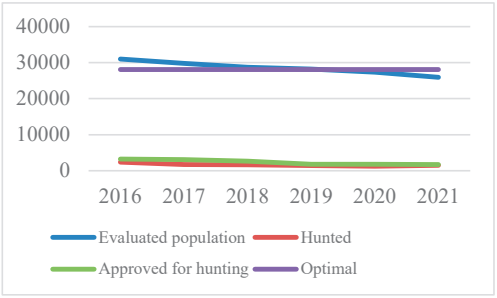

Figure 1. Hare population situation, in Giurgiu County, in 2016-2021 period

In 2018 year, the number of hunted hares decrease again with 2%, and the evaluated population with 3.77%. The share of hunted hares represents 63.30% from approved quota and 5.82% from evaluated population. At the level of 2019 year, hunted individuals represents a share of 80.22% from quota and only 5.08%. The population records a decreasing at only 1.75%.

The descending trend continue in 2020, when the population became smaller with 3.09%. The number of hunted hares is the smallest number for entire analysed period, 1252, and represent 70.54% from quota and 4.58% from real population. In 2021 it registered the smallest effective - 25934 hares, the lowest quota but not the lowest number of hunted hares - 1514 (88.54% from quota and 5.84% from evaluated

effective). This entire situation presented above, regarding hunted individuals in relation with quota and evaluated effective is much more visible in Figure 2.

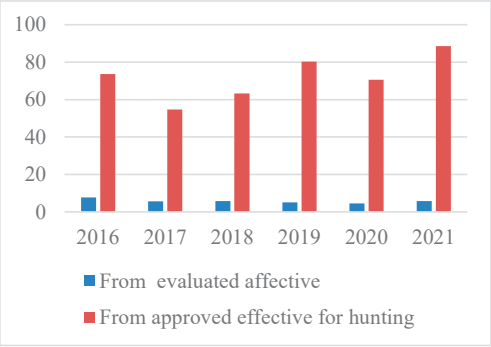

Figure 2. Situation of hunted hares as a share from quota and from evaluated effective

The pheasant dynamic situation during 2016-2021 period is presented in Table 2, and the graphic representation is presented in Figure 3.

Table 2. Dynamics of pheasant population, in Giurgiu County, in 2016-2021 period

Year	Evaluated population	Hunted	Approved for hunting (quota)	Optimal effective
2016	12843	2661	3765	3570
2017	12678	1946	3758	3570
2018	12553	2335	3630	3570
2019	12399	2709	3731	3570
2020	12243	2431	3513	3570
2021	11135	3355	3756	3570
X	*12308.50*	*2639.67*	*3625.33*	*3570*
St. dev.	*611.8143*	*611.3917*	*163.7030*	*0*
Sx	*273.6117*	*273.4227*	*73.2102*	*0*
CV%	*4.97*	*23.16*	*4.52*	*0*

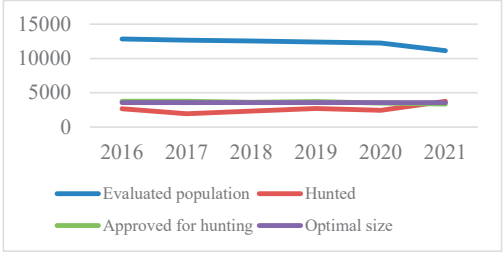

Figure 3. Pheasant population situation in Giurgiu County, in 2016-2021 period

The strangest thing comes from the enormous differences between the evaluated effective and the optimal number of pheasant. It looks like the real effective it is 3.5 times bigger than the optimal effective. In the same time, if it considers 3570 pheasant in 300000 ha it means that it is possible to see a pheasant in 100 ha (1 square kilometre) which is hilarious if it compared with what happens in other countries (Castiov, 2010; Gheta et al., 2020). It is obvious that the ranking keys for establishing the quality of hunting territories must be change at least for some species (Maftei et al., 2017).

In 2016 year, it is registered, the largest evaluated effective from entire analysed period. The hunting harvest (2661 individuals) representing 70.68% from quota and 20.72% from evaluated population. In 2017 year, the evaluated population decrease with only 1.3%. The number of hunted individuals represent 51.78% from quota and 15.35% from entire evaluated population. Year 2018 year continue in the same style: the real effective decrease insignificant with only 0.99%, the hunting harvest represents 64.33% from quota and 18.6% from evaluated population. Same indicator, hunting harvest, in the real effective (evaluated) decree se in 2019 year with 1.23% and the hunting harvest represent 72.61% from quota and 21/85% from entire evaluated population.

In 2020 and 2021 years, the population has reduced with 1.26% respectively 9.05% (biggest values registered in decreasing of population from one year to another). The hunting harvest represent 69.2% from quota and 19.86% from evaluated population in 2020 year. In 2021 year, the same indicator had a share of 89.32% from quota and 30.13% from evaluated population. This entire dynamic it is represented in Figure 4.

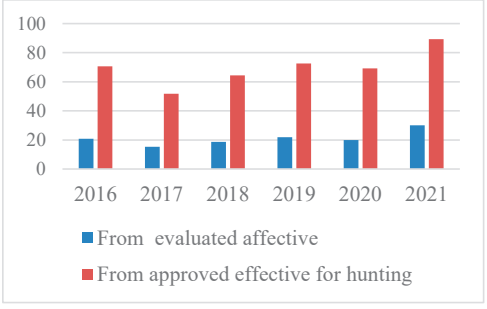

Figure 4. Situation of hunted pheasants as a share from quota and from evaluated effective

The situation, presented so far, would be just a simple presentation if we did not analyse the

evolution of most important mammal predators: gold jackals and foxes.

The evolution of fox population in Giurgiu County is presented in Table 3 and in Figure 5. From 2016 year, until 2021 year the fox population increased with 141.97%.

Table 3. Dynamics of fox population, in Giurgiu County, in 2016-2021 period

Year	Evaluated population	Hunted	Approved for hunting (quota)
2016	641	321	589
2017	899	422	775
2018	831	494	736
2019	905	583	771
2020	877	455	870
2021	910	569	877
X	*843.83*	*474*	*769.67*
St. dev.	*103.5073*	*97.7548*	*105.2533*
Sx	*46.2899*	*43.7173*	*47.0707*
CV%	*12.27*	*20.62*	*13.68*

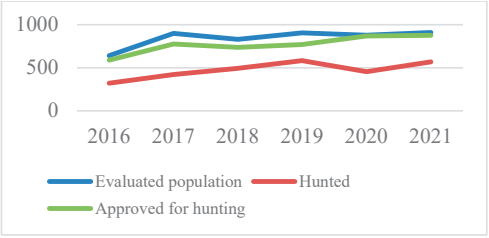

Figure 5. Fox's evolution in Giurgiu County, in 2016-2021 period

The hunting harvest represents, as an average, only 61.64% from admitted quota and just 56.17% from evaluated population, in the conditions when the hunting harvest must represent 91.2% from real (evaluated) effective.

The situation becomes more complex when it considers the number of golden jackals (Table 4, Figure 6).

The hunting harvest is negligible compared with quota. Analysing just the average value it founds a hunting harvest, which represents only 50.91% from quota and just 49.25% from entire evaluated effective, instead of 91.68%.

The situation of analysed predators and prey populations is presented, separately, in Figures 7 and 8.

Table 4. Dynamics of golden jackal population, in Giurgiu County, in 2016-2021 period

Year	Evaluated population	Hunted	Approved for hunting (quota)
2016	261	111	223
2017	300	145	261
2018	294	195	290
2019	377	193	318
2020	388	102	372
2021	400	197	388
X	*336.67*	*157.17*	*308.67*
St. dev.	*58.589*	*43.8744*	*63.7986*
Sx	*26.2018*	*19.6212*	*28.5316*
CV%	*17.4*	*27.92*	*20.67*

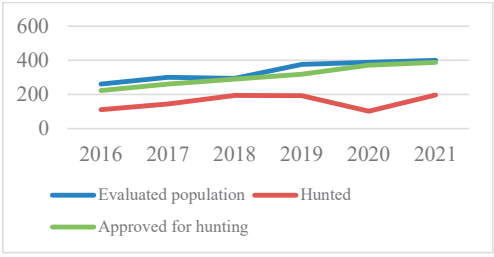

Figure 6. Golden jackal evolution in Giurgiu County, in 2016-2021 period

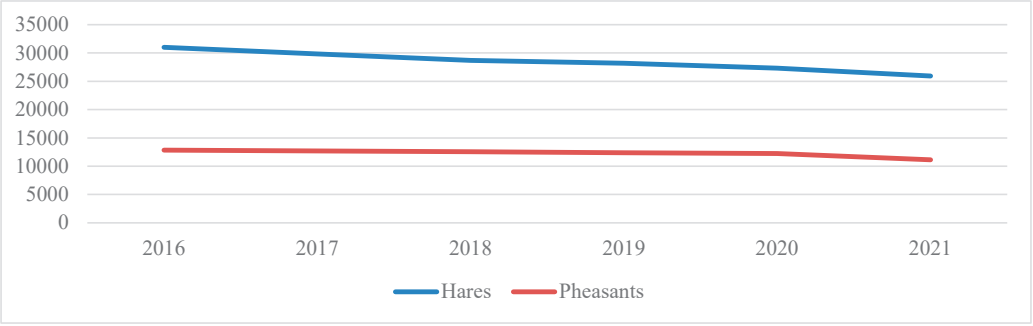

Figure 7. Evolution of analysed non-predator populations in Giugiu County, in 2016-2021 period

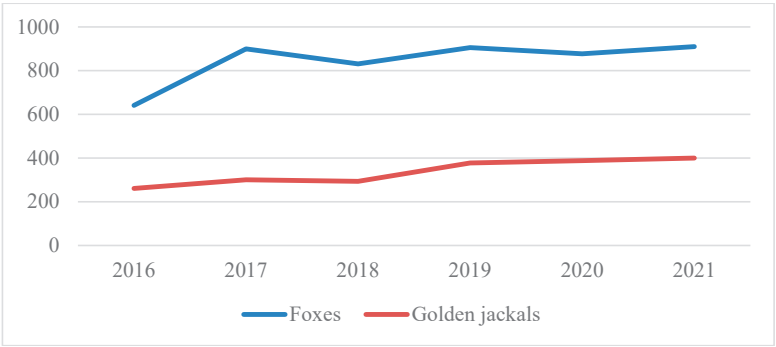

Figure 8. Evolution of analysed predator populations in Giurgiu County, in 2016-2021 period

It is obvious that the decreasing of hare and pheasant population is influenced directly by the increasing of predators populations, in this case fox and golden jackal populations. Achieving hunting quotas for these predatory species, in a share of only 50% of the quota, can be a good explanation for the decrease in hares and pheasant populations, especially if it analyses the percentage of hunting extraction compared to the quota granted to prey populations analysed in this study (72.81% for pheasant, and 69.81% for hare). That this percent of hunting harvest from quota, for analysed pray populations, it is only at this level because the situation on the ground is different from the one reflected in the evaluation works, and presented as official data by the relevant ministry. Due not forget something very important about the golden jackal: it is a fox competitor and, due to superior physiological and morphological characteristics (Micu, 2004). It became the predominant predator, from plain and hill area, where the wolf is missing, for small game but also for the roe deer and even red deer, preferring the youth, but not getting back in front of the mature specimens, especially in the case of roe deer (Maftei et al., 2017).

CONCLUSIONS

Unfortunately, the official data from evaluation of wild animal populations is unclear. The hare is almost absent in free hunting area or, in a better case, is very low represented.

According to official data, the evaluated hare population, at the level of 2021 year, for Giurgiu County, represents 92.32% of the optimal herd. Moreover, if analyse the average values calculated in the present study, we can conclude that the real average population, calculated for the analysed period, is even 1.46% higher than the optimal size of population calculated for the same period. All this situation is inappropriate in the conditions in which we observe a decrease of the hunting harvest related to the granted quota. And no, the preferences of hunters have not changed, hare being the most wanted game for over 80% from hunters, but the real population only that the actual numbers are much smaller than those on the paper.

Regarding the pheasant population it is very clear that an optimal population at 3570 individuals, for all Giurgiu County (over 300000 ha) is very small even if we subtract from the total of this surface the unproductive areas from the hunting point of view. The pheasant is a species that adapts to intensive agriculture, and this fact is presented in numerous specialized scientific papers, especially in the USA but also in Europe.

The predator species have an ascendant trend, populations growing rapidly due to the lack of interest in achieving the quotas. The situation reveals two aspects: on the one hand, the lack of interest of hunters for harvesting these species, and/or the disinterest of the administrators of the hunting funds to keep these predators under control, implicitly to ensure a balance of prey-predator.

Regarding the analysed situation, the following are recommended:

- Reanalysing the criteria for ranking of hunting funds;
- Elaboration of a hunting management plan at national level;
- Active involvement of the administrator of the national hunting fund in the evaluation of the populations of hunting interest;

- The entrustment of the hunting area after the elaboration of a management plan, the sustainability of which will be verified by the administrator of the national hunting area, and which must be in accordance with the national hunting management plan;
- Active involvement and reward of hunters participating in the hunt for foxes and golden jackals (and not only);
- Introducing the obligation, for managers, to maintain populations at the optimal level;
- Making fence areas intended exclusively for the population from free hunting area;
- Modification of specific legislation in the direction of easier accessibility to European funds.

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THE INFLUENCE OF THE STOCKING DENSITY ON THE SURVIVAL RATE OF THE PIKE-PERCH (*SANDER LUCIOPERCA* LINNAEUS, 1758) DURING THE COLD SEASON IN INDUSTRIAL AQUACULTURE SYSTEMS

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Abstract

The survival rate of one summer old pike-perch's (Sander lucioperca L., 1758) during the cold season is low (50-75%) due to lower temperatures which lead to a decrease in metabolism. The feed sources are becoming scarce and, as a result, the fish are consuming their adipose tissue accumulated during the warm season, becoming prone to disease due to weight loss. The research was carried out during 2018, 2019 and 2020 years at the Fish Culture Research and Development Station, Nucet. The fish wintering was done in ponds, in two experimental versions: Variant 1 - no feed was administered and Variant 2 - live food was administered (fish species without economic importance). The experiments for each variant were performed in three densities, as follows: 200 kg/basin (2000 kg/ha), 500 kg/basin (5000 kg/ha) and 700 kg/basin (7000 kg/ha). The best results for survival rate were obtained in variant 2 in 2020 at 200 kg/basin density, with a 98.8% survival rate. The weight gain rate in variant 2 for 2018 at 500 kg/basin density, obtained a growth rate of 137 kg/wintering season.

Key words*: density, pike-perch, survival, wintering.*

INTRODUCTION

The pike-perch (*Sander lucioperca* L., 1758) is both a commercial fishing valuable species and for recreational sport fishing (Rundberg, 1977; FAO, 1984; Lehtonen, 1985; Ibanescu, 2020; Dobrotă, 2021). This species is used as a biomanipulation tool in order to reduce the number of undesirable fish, usually cyprinids without economic value (Van Densen & Grimm, 1988; Benndorf, 1990; Dörner et al., 1999; Mehner et al., 2001; Wysujack, 2003).

The pike-perch habitat preferences differ throughout the year and vary depending on age and environmental conditions (Lappalainen et al., 1995, FAO, 2022).

Water temperature influences many variables, such as: metabolism, oxygen content in water, the amount of oxygen that organisms need, including food availability (Hokanson, 1977), so growth is related to temperature which is correlated with latitude (Ferguson, 1958; Hokanson, 1977).

The weight and numerical losses reduction during the cold season in the one summer old pike-perch, is an important factor for obtaining a larger population and an additional income for aquaculture farmers.

MATERIALS AND METHODS

The researches were carried out in 2018-2020 period at the Fish Culture Research and Development Station Nucet, Dâmbovița County. The experimental basins are located in the major riverbed of the Ilfov brook downstream of the Ilfoveni accumulation dam. For the wintering of the pike-perch, the material base consisted of wintering ponds with an area of 1000 m². The depth of the wintering ponds is between 1.5-2.4 m.

The harvest fishing was carried out between 10 and 25 November each year. The one summer old pike-perch, with an average weight between 142-362 g/ex, had been stocked for wintering in 1000 m² earth ponds, in monoculture. During the

warm season, the wintering ponds were left to dry and were disinfected with lime chloride, more intensely in wet areas.

The water supply was made from a common channel, through a concrete monk outlet structure, where metal grids were installed with a 4 mm screen was mounted, in order to prevent the penetration of other fish species. Because the supply channel was common, it can say that in all experimental basins the physio-chemical characteristics of the water were identical.

The stocked fish for wintering were made in two variants, in each variant three densities were experienced, as follows:

- Variant 1 (where no fish feed was administered), with three fish densities: 200 kg/basin (2000 kg/ha), 500 kg/basin (5000 kg/ha) and 700 kg/basin (7000 kg/ha);
- Variant 2 (where live food composed of fish with no economic importance was administered), with three fish densities: 200 kg/basin (2000 kg/ha), 500 kg/basin (5000 kg/ha) and 700 kg/basin (7000 kg/ha).

The harvest fishing of pike-perch at the end of the cold season, in the three experimental years, was carried out between March 22 and April 11, after approximately 135 wintering days.

The methods for determining the chemical parameters of water were:

- pH, dissolved oxygen and chlorides were determined using the portable multiparameter HQ 40d;
- Dissolved organic matter (CCO-Mn), hardness, calcium ion concentration, magnesium ion concentration and alkalinity were determined in the laboratory by volumetric methods;
- The concentration of nitrogen anion, nitrite anion, phosphate anion and ammoniacal nitrogen were determined in the laboratory by the spectrophotometric method (Griess-Ilosvay method).

The growth rate was calculated by subtracting the quantity of stocked fish from the harvested quantity.

The results of stocking density on the survival rate of the pike-perch experimental groups, during the cold season, were statistically analyzed using descriptive statistics and Microsoft Excell (Office 2010) for Windows.

RESULTS AND DISCUSSIONS

During the experiments, the water physico-chemical parameters were monitored. The obtained results interpretation was performed in accordance with the provisions of the "Norm regarding the classification of surface water quality", correlated with the data from the specialized literature in waters for fish use (OMMGA no. 161/2006) (Table 1).

In those three years of study (2018-2019; 2019-2020 and 2020-2021) the cold season was similar, with high temperatures compared to the normal period. The exception was the year 2020-2021, which recorded positive air temperatures in January and February with low amounts of rainfall, the month of March was cold, with negative temperatures both day and night with snowfall episodes. In April 2021 it was recorded lower temperature for this period than normal.

During the experiments, the water temperature recorded average values in the range of 2.2-8.9°C, and during the day positive average air temperatures of up to 11°C were recorded (Table 2). The nights were cold with average temperatures of -15.0°C. During certain periods of the cold season, an ice bridge with transparent ice was formed, for 10-15 days, without loss of fish.

In Variant 2 (with feeding), in all the three experimental seasons, live food was composed of fish species without economic value and small dimensions (5-20 g/ex), like Prussian carp (*Carrassius gibelio*); rudd (*Schardinius erytrophthalmus*); European bitterling (*Rodeus amarus*) and stone maroko (*Pseudorasbora parva*). The live food administration was performed monthly, in an amount of 10% of the total stocked quantity. Live food was administered on December 2018 and in the first decade of January, February, March of 2019. The total food amount administered in the 2018-2019 season was as follows: at 200 kg/basin density - 80 kg; at 500 kg/basin density - 200 kg; and at 700 kg/basin density - 280 kg. The same amount of live food was administered in the 2019-2020 and 2020-2021 winter periods of study.

In the 2018-2019 winter season, the stocking actions were made between 12-20 November 2018, with pike-perch (*Sander lucioperca*) with

an average live weight, as follows: in Variant 1 at 200 kg/basin - 142 g/ex, at 500 kg/basin density - 318 g/ex and at 700 kg/bazin density - 154 g/ex. In Variant 2, the average live weight was: at 200 kg/basin density - 234 g/ex, at 500 kg/basin density - 151 g/ex and at 700 kg/basin density - 343 g/ex. The specimens number was different depending on the average weight of the fish and the stocked quantity, which was between 855-4545 ex/pond, in both variants.

Table 1. Average values of the physico-chemical parameters of the water in 2018-2020 period

No.	Chemical parameters		U.M.	Parameter values		
				Source	Experimental ponds	Optimally according to quality standards
				Optimally according to quality standards		
1	pH		unit pH	7	7.6	7-7.8
2	Alkalinity		mg/l	140	166	200-400
3	Calcium (Ca^{2+})		mg/l	44.2	43.8	90-120
4	Magnesium (Mg^{2+})		mg/l	20.7	19.8	10-40
5	Ca^{2+}/ Mg^{2+}		mg/l	2.1	2.2	5
6	Organic matter		mg $KMnO_4$/l	14	28.5	20-60
7	Oxygen		mg/l	14.6	9.7	5-10
8	Ammonia (NH^{+}_3)		mg/l	missing	missing	missing
9	Nitrates (NO^{-}_3)		mg/l	missing	0.21	2.5-4
10	Nitrogen (NO^{-}_2)		mg/l	0.001	0.002	0.03
11	Phosphates (PO^{3-}_4)		mg/l	missing	0.04	0.05-1.5
12	Chlorides	Cl^{-}	mg/l	8.13	8.23	30
		Na Cl	mg/l	14.21	14.04	20
13	Ammonia (NH^{+}_4)		mg/l	missing	0.017	0.5-1
14	Total hardness		(^{0}D)	13.6	15.5	12

Table 2. Water and air temperature during the study

Month and year	Water temperature			Air average temperature
	T min. (°C)	T max. (°C)	T med. (°C)	T air (°C)
Season 2018-2019				
December 2018	1.5	4	2.2	-2.2
January 2019	1.5	3	2	-0.4
February 2019	2.5	5	3.6	4.3
March 2019	3.5	13	8.9	11
Season 2019-2020				
December 2019	2	5	3.8	5
January 2020	1.5	2.5	2.3	0.9
February 2020	2.5	6.5	4.1	5.9
March 2020	5	12.5	9.6	10.5
Season 2020-2021				
December 2020	3.5	5	4.2	2.3
January 2021	2	5	3.5	3.2
February2021	3	7.5	4.7	4.3
March 2021	5	10.5	7.7	9.9

The spring fishing was carried out between April 4 and 11, 2019, with the following results (Table 3):

- The average weights were: in Variant 1 at 200 kg/basin density - 131 g/ex, at 500 kg/basin density - 287 g/ex and at 700 kg/basin density - 137 g/ex, and in Variant 2 at 200 kg/basin density - 276 g/ex, at 500 kg/basin density - 196 g/ex and at 700 kg/basin density - 377 g/ex.;
- The quantities harvested were as follows: in Variant 1 at 200 kg/basin density - 128 kg, at 500 kg/basin density - 325 kg and at 700 kg/basin

density - 422 kg, and in Variant 2 at 200 kg/basin density - 225 kg, at 500 kg/basin density - 637 kg and at 700 kg/basin density - 743 kg;
- The survival rate had lower values in Variant 1 (at 200 kg/basin density - 69.4%; at 500 kg/basin density - 72.0%; and at 700 kg/basin density - 67.7%), with an average of 69.7%, compared to Variant 2 (at 200 kg/basin density - 95.5%; at 500 kg/basin density - 98.2%; and at 700 kg/basin density - 96.6%), with an average of 96.8%.

In the winter season 2019-2020, the wintering stockings were made between November 19 and 25, 2019, with pike-perch (*Sander lucioperca*) that had an average weight, as follows: in Variant 1 at 200 kg/basin density–241 g/ex, at 500 kg/basin density - 321 g/ex and at 700 kg/basin density - 167 g/ex, and in Variant 2 at 200 kg/basin density - 222 g/ex, at 500 kg/basin density - 354 g/ex and at 700 kg/basin density–151 g/ex.

The number of specimens was different, depending on the average weight of the fish and the stocked quantity, between 830-4636 ex./pond, in both versions.

The spring fishing was carried out between March 26 and April 3, 2020, with the following results (Table 4):
- The average weights were: in Variant 1 at 200 kg/basin density -212 g/ex, at 500 kg/basin density - 287 g/ex and at 700 kg/basin density - 137 g/ex, and in Variant 2 at 200 kg/basin density - 267 g/ex, at 500 kg/basin density - 372 g/ex and at 700 kg/basin density - 186 g/ex.;
- The harvested quantities were as follows: in Variant 1 at 200 kg/basin density - 146 kg, at 500 kg/basin density - 283 kg and at 700 kg/basin density - 393 kg, and in Variant 2 at 200 kg/basin density - 231 kg, at 500 kg/basin density - 483 kg and at the 700 kg/basin density - 842 kg;
- The survival rate had lower values in Variant 1 (at 200 kg/basin density - 82.8%; at 500 kg/basin density - 63.4%, at 700 kg/basin density - 68.5%) compared to Variant 2 (at 200 kg/basin density - 95.9%; at 500 kg/basin density - 91.9%; and at 700 kg/basin density -97.6%).

In the winter season 2020-2021, the wintering stocking were made between November 10 and 17, 2020, with pike-perch (*Sander lucioperca*) (Figure 1), which had the average weight, as follows: in Variant 1 at 200 kg/basin density - 348 g/ex, at 500 kg/basin density - 168 g / ex and at 700 kg/basin density - 233 g/ex, and in Variant 2 at 200 kg/basin density - 360 g/ex, at 500 kg/basin density - 146 g/ex and at 700 kg/basin density - 231 g/ex. The number of specimens was different depending on the average weight of the fish and the stocked quantity, which was between 556-3425 ex/pond, in both variants.

The spring fishing was carried out between March 22 and 30, 2021, with the following results (Table 5 and Figure 2):
- The average weights were: in Variant 1 at 200 kg/basin density - 312 g/ex., at 500 kg/basin density - 141 g/ex. and at 700 kg/basin density - 212 g/ex., and in Variant 2 at 200 kg/basin density - 401 g/ex., at 500 kg/basin density -168 g/ex. and at 700 kg/basin density - 267 g/ex.;
- The quantities harvested were: in Variant 1 at 200 kg/basin density - 119 kg, at 500 kg/basin density - 319 kg and at 700 kg/basin density - 391 kg, and in Variant 2 at 200 kg/basin density - 220 kg, at 500 kg/basin density - 559 kg and at 700 kg/basin density - 798 kg;
- The survival rate had lower values in Variant 1 (at 200 kg/basin density - 66.5%; at 500 kg/basin density - 76.0%, and at 700 kg/basin density - 61.4 %) compared to Variant 2 (at 200 kg/basin density - 98.8%; at 500 kg/basin density - 97.1%, and at 700 kg/basin density -98.6%).

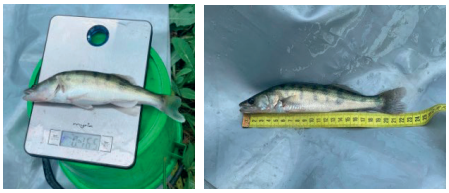

Figure 1. Pike-perch in autumn, 2020

Figure 2. Pike-perch fishing in spring, 2021

Weight loss and growth increase (g) per year and experimental variants

In the 2018-2019 season, in Variant 1 (without feeding) in all three densities was a decrease in individual weight, the most pronounced being recorded in the 500 kg/basin density of 31 g/ex.,

the lowest being recorded at 200 kg/basin density of 11 g/ex., and at the 700 kg/basin density a loss of 17 g/ex. was registered. In Variant 2 (with feeding) it was an increase in individual weight, the highest being recorded at 500 kg/basin density of 45 g/ex., the lowest being recorded at 700 kg/basin density of 34 g/ex., and at 200 kg/basin density was recorded an increase of 42 g/ex. (Table 3).

Table 3. The results obtained on experimental versions in 2018-2019 winter season

Registered parameters	Variant 1 (without feeding)			Variant 2 (with feeding)		
Density (kg/basin)	200	500	700	200	500	700
Number of specimens	1408	1572	4545	855	3311	2041
Ex./ha	14085	15723	45455	8547	33113	20408
kg/ha	2000	5000	7000	2000	5000	7000
W med. autumn (kg)	0.142	0.318	0.154	0.234	0.151	0.343
Nr. specimens collected	978	1132	3078	816	3251	1972
W med. spring (kg)	0.131	0.287	0.137	0.276	0.196	0.377
Survival (%)	69.4	72	67.7	95.5	98.2	96.6
Fish quantity harvested (kg)	128	325	422	225	637	743

Table 4. The results obtained on experimental versions in 2019-2020 winter season

Registered parameters	Variant 1 (without feeding			Variant 2 (with feeding)		
Density (kg/basin)	200	500	700	200	500	700
Number of specimens	830	1558	4192	901	1412	4636
Ex./ha	8299	15576	41916	9009	14124	46358
kg/ha	2000	5000	7000	2000	5000	7000
W med. autumn (kg)	0.241	0.321	0.167	0.222	0.354	0.151
Nr. specimens collected	687	987	2871	864	1298	4526
W med. spring (kg)	0.212	0.287	0.137	0.267	0.372	0.186
Survival (%)	82.8	63.4	68.5	95.9	91.9	97.6
Fish quantity harvested (kg)	146	283	393	231	483	842

Table 5. The results obtained on experimental versions in 2020-2021 winter season

Registered parameters	Variant 1 (without feeding			Variant 2 (with feeding)		
Density (kg/basin)	200	500	700	200	500	700
Number of specimens	575	2976	3004	556	3425	3030
Ex./ha	5747	29762	30043	5556	34247	30303
kg/ha	2000	5000	7000	2000	5000	7000
W med. autumn (kg)	0.348	0.168	0.233	0.36	0.146	0.231
Nr. specimens collected	382	2261	1845	549	3326	2989
W med. spring (kg)	0.312	0.141	0.212	0.401	0.168	0.267
Survival (%)	66.5	76	61.4	98.8	97.1	98.6
Fish quantity harvested (kg)	119	319	391	220	559	798

In the 2019-2020 season in Variant 1 (without feeding) in all three densities was a decrease in individual weight, the most pronounced being recorded in 500 kg/basin density of 38 g/ex., the

lowest being recorded at 200 kg/basin density of 29 g/ex. and at the 700 kg/basin density a loss of 30 g/ex. was registered. In Variant 2 (with feeding) was an increase in individual weight, the highest being recorded at 200 kg/basin density of 45 g/ex., the lowest being recorded at 500 kg/basin density of 18 g/ex. and at 700 kg/basin density was recorded an increase of 35 g/ex.

In the 2020-2021 season in Variant 1 (without feeding) in all three densities there was a decrease in individual weight, the most pronounced being recorded in 200 kg/basin density of 36 g/ex., the lowest being recorded at 700 kg/basin density of 21 g/ex. and at the 500 kg/basin density there was a loss of 27 g/ex. In Variant 2 (with feeding) there was an increase in individual weight, the highest being recorded at 200 kg/basin density of 41 g/ex., the lowest being recorded at 500 kg/basin density of 22 g/ex. and at 700 kg/basin density was recorded an increase of 36 g/ex.

Weight loss and growth increase (g) per year and experimental variants are presented in Figure 3.

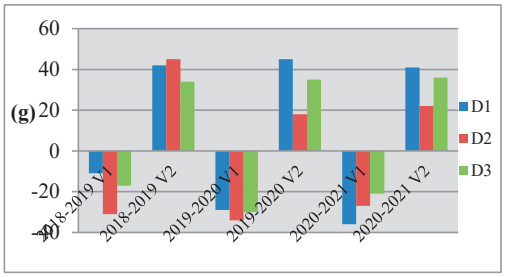

Figure 3. Weight loss and growth increase (g) by year and experimental variants

The numerical losses during on three years of study on experimental variants

In the 2018-2019 season in Variant 1 (without feeding) the survival rate registered values between 67.7-72.0%, the lowest being registered in the700 kg/basin density where the survival was of 67.7%, the highest being registered at the 500 kg/basin density where the survival was 72.0%, and at the 200 kg/basin density a survival of 69.4% was registered. In Variant 2 (with feeding), the highest survival was recorded at 500 kg/basin density of 98.2%, the lowest being recorded at 200 kg/basin density of 95.5%, and at 700 kg/basin density there was a survival of 96.6 %.

In the 2019-2020 season in the Variant 1 (without feeding) the survival registered values between 63.4-82.8%, the lowest being registered in the 500 kg/basin density where the survival was 63.4%, the highest being recorded at 200 kg/basin density where the survival was 82.8%, and at 700 kg/basin density a survival of 68.5% was recorded. In Variant 2 (with feeding), the highest survival was recorded at 700 kg/basin density of 97.6%, the lowest being recorded at 500 kg/basin density of 91.9 %, and at 200 kg/basin density there was a survival of 95.9 %.

In the 2020-2021 season in Variant 1 (without feeding) the survival registered values between 61.4-76.0%, the lowest being registered in the 700 kg/basin density where the survival was 61.4%, the highest being registered at the 500 kg/basin density where the survival was of 76.0%, and at the 200 kg/basin density a survival of 66.5% was registered. In Variant 2 (with feeding), the highest survival was recorded at 200 kg/basin density of 98.8%, the lowest being recorded at 500 kg/basin density of 97.1%, and at 700 kg/basin density there was a survival of 98.6%.

The survival rate (%) during on three years of study on experimental variants are presented in Figure 4.

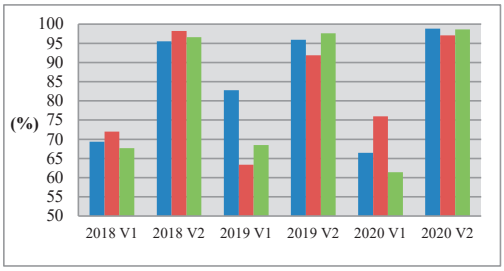

Figure 4. Survival rate variation (%) on experimental variants in 2018-2020 period

Quantitative losses by years and experimental variants

In the 2018-2019 season in Variant 1 (without feeding) it was found that the most accentuated decrease of fish material was registered in the 700 kg/basin density of 40%, the smallest being registered at the 500 kg/basin density of 35 %, and at the 200 kg/basin density recorded a loss of 36%. In Variant 2 (with feeding) the largest decrease in the amount of fish material was recorded at 500 kg/basin density of 27%, the smallest being recorded at 700 kg/basin density

of 6%, and at 200 kg/basin density there was a decrease of 13%.
In Variant 1 of the 2019-2020 season (without feeding) it was found that the most accentuated fish material decrease was registered in the 700 kg/basin density of 44%, the smallest being registered at the 200 kg/basin density of 27%, and at the 500 kg/basin density it was recorded a loss of 43 %. In Variant 2 (with feeding), the largest amount decrease in fish material was recorded at 700 kg/basin density of 20 %, the smallest being recorded at 500 kg/basin density of 3%, and at 200 kg/basin density there was a decrease of 15%.
In the 2020-2021 season, in Variant 1 (without feeding) it was found that the most accentuated fish material decrease was registered in the 700 kg/basin density of 44%, the smallest being registered at the 500 kg/basin density of 36%, and at the 200 kg/basin density recorded a loss of 40%. In Variant 2 (with feeding), the largest amount decrease in fish material was recorded at 700 kg/basin density of 14%, the smallest being recorded at 200 kg/basin density of 10%, and at 500 kg/basin density there was a decrease of 12%.
The pike-perch survival rate in Variant 2 was clearly superior to Variant 1, in all densities and in all three years of study (Figure 5).

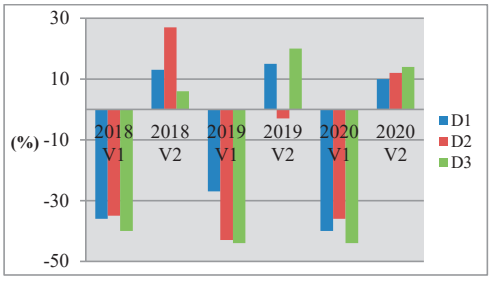

Figure 5. The fish material lost amount (%) and growth increase by years and experimental variants

In Variant 1 it was between 61.4-82.8%, compared to Variant 2 where was 91.9-98.8%, showing that when the pike-perch is fed even if the temperature is low, the losses in the cold season are lower.

CONCLUSIONS

From the weight losses point of view, in Variant 1 it was found that the highest losses were in the 2020-2021 season at 200 kg/basin density with the value of 36 g/ex., and the lowest losses were in the 2018-2019 season at 700 kg/basin density with the value of 17 g/ex. In the case of Variant 2, there is an increase in average weight in all study years and at all densities, with values between 18-45 g/ex.
The fish material total amount (weight and numerical losses) in Variant 1 decreased, the percentage of losses was not influenced by the average weight. In Variant 2, the fish material amount registered increases in all years of study and in all densities, except for the year 2019 (500 kg/basin density), where, in the spring the pike-perch had a higher average weight comparing to the stocking moment (autumn), it recorded a survival rate of 91.9%, which led to a loss of 3% of the total population.
In conclusion, feeding the pike-perch during the cold season with live fish with no economic value, leads to a high survival rate and an increase in individual weight, so that, in the spring, resulted a vigorous stocking biological material with very few numerical losses and from a quantitative point of view there have been increases.

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