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GENETICS AND BREEDING

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THE COMBINATIVE CAPACITY OF HYBRIDS FROM CROSSBREEDING LINES AT THE SILKWORMS BREED BANEASA 75 (*Bombyx mori* L.)

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Abstract

The results of former research concerning the development of inbred lines in native silkworms breeds, established the technologic formation procedures and pointed out reduced values of inbreeding depression in the productive characteristics. The object of the present research aims are the identification and selection of the most productive inbred lines based on crossing with tester constant lines, namely the research of general combining capacity; establishing specific combining capacity by direct and reciprocal crossing and estimation of heterosis effect. The results of research emphasize that from those 14 inbreeding lines tested for combining capacity, 7 and 10 maternal lines and 3 paternal lines obtain heterosis results that demonstrate superior possibilities of turning into account the crossbreeding system in silkworms raising. The heterosis effect is obvious important foe shell weight.

Key words*: inbreeding; heterosis; tester constant lines*

INTRODUCTION

The formation and highlighting of inbreeding effects on morphofunctional traits in silkworms have constituted the first works for using artificial populations in capitalization of hybridization effects. The objectives of this research have the purposes: identifying and selection of the most productive inbreeding lines from the 14 lines formed in the frame of Băneasa 75 race on the basis of crossings with tester races (lines), respectively, the research of general combining capacity; finding specific combining capacity by direct and reciprocical crossings and the evaluation of the heterosis effect.

On the basis of the data from world specialty literature, the data of Romanian researches and experiments and our own researches, resulted that the quantitative and qualitative performances in silkworm change in positive sense from generation to generation and the substantial contribution of this progress is obtained by the maximum capitalization of genetic improvement theory and practice.

From this point of view, the hybridization of artificial populations obtained by management and supervision of formation and using inbred lines constitutes a foundation of currently scientific and practical concerns.

Without constituting a practice in silkworm breeding, in our country, the creation of inbred lines and their selection on the basis of general and specific combining capacity has been reported in some speciality papers: Craiciu and Otărăşanu,1969; Craiciu et al.,1970; Craiciu et al., 1971; Craiciu and Tiţescu, 1971a; Craiciu and Tiţescu, 1971b; Craiciu et al., 1974a; Craiciu et al., 1974b.

The selection of the most productive hybrid combinations on the basis of dialele crossings between pure races, also made the object of some researches in our country: Braslă et al., 1992; Matei et al., 2001; Matei et al., 2002a; Matei et al., 2002b.

Worldwide, the researches in which the heterosis is obtained by decomposition of populations into inbred lines, the selection between lines on the basis of general combining capacity and the obtaining of hybrids by crossing the lines with high specific combining capacity, are mentioned in the papers elaborated by: Kremky, 1983; Pershad

et al., 1986; Datta and Pershad, 1988: Jeong et al., 1990; Nagaraja and Govindan, 1994; Ho Zoo Lea, 1998; Kalpana and Sreerama Reddy, 1998; Singh et al., 2001, Mirhosieni et al., 2004; Rahman et al., 2015.

Instead, the selection between the existent races, non-inbred, on the basis of their specific combining capacity tested in crossings with other populations, represents a current technique in sericiculture: Petkov N. şi col. (1984); Pershad G. D. (1986); Datta R. K. şi col. (1988); Lin Jianrong (1989); Xue-Weihua (1989); Jeong W. B. (1990); Jung D. S. (1990).

The final purpose of the silkworm improvement program is the increasing of silk production. The hybridization represents one of the ways to achieve this objective. However, choosing the lines used in hybridization is difficult, due to their different behaviours. Because of this, the actual achievement of the crossings is preceded by the selection between lines, related to the additive and non-additive The selection of additive variations relates to the appreciation of reproductives improvement value by their general combining capacity in different crossing couples.

The selection of non-additive variations refers to the specific combining capacity manifested in crossings between lines.

In this context, the experiences purpose in this stage consists in:

- identifying the most productive inbred lines in crossings with tester races (finding the general combining capacity of inbred lines in I_4);
- finding specific combining capacity by direct and reciprocical crossings between selected lines on the basis of general combining capacity and the evaluation of heterosis effect.

MATERIALS AND METHODS

In the present synthesis there are shown the results regarding two of the most important productivity traits in silkworm: the shell cocoon weight and the silk content; we mention that the traits studied in the whole paper have been 9, as an expression of the most works in this field.

The biological material has been constituted by 14 hybrid combinations between the inbred lines I_4 and the race considered content tester J90.

Samples size on which the determinations were made, was in the eggs stage of 500 eggs/experimental variant (hybrid) and in the larval stage for each hybrid were made 3 lots x 200 larvae/lot at the beginning of the third larval age.

In the stage of raw cocoon, the determinations were made on 100 cocoons, representing both sexes, and in the stage of dried cocoon and filament, on 50 cocoons/hybrid.

The work methods were those used in experimental sericicultural technique and have been grouped into three categories: those used in obtaining experimental data in egg, larva, cocoon stage; those used in larvae incubation and rearing; those used in finding general and specific combining capacity.

The inbred lines with high general and specific combining capacity have been selected and used in the obtaining of commercial hybrids.

RESULTS AND DISCUSSIONS

The performances of inbred lines in I_4.

In the assortment of analyzed lines have been included lines that differ by traits direct related to the cocoon production, as: raw cocoon weight, shell weight, silk cover.A classification of the inbred lines on the basis of average values of their traits in Băneasa 75 race is presented in Table 1.

From their analysis results that in the group of inbred lines from Băneasa 75 race, a number of 9 lines presents for shell weight values above lines average, highlighting 4 of them.

From the same table it is noted that the lines average for the silk percentage was 18.54%, a number of 11 lines presenting values above average.

Between the inbred lines that came from the founding race Băneasa 75 the following lines stood out: B 75 -12 line; B 75 -10 line; B 75 -14 line; B 75 -7 line.

The performances of combinations resulted from crossing the inbred lines with the tester race.

The combining capacity of inbred lines is one of the important operations of organization and realization of an interlinear hybridization program. Making a whole series of possible crossings between a high number of inbred

lines, as the subsequent experimentation of the obtained hybrids assume great efforts and costs. From this reason, there have been elaborated procedures that allow a prior sorting of the lines on the basis of their value in crossings with an analyzer race (tester), following that the selection on the simple hybrids level to be made only between the combinations of the selected lines, much less in number.

The experimental material is represented bythe hybrid group with 14 direct simple combinations realized between 14 inbred lines in I_4extracted from Băneasa 75 race, which played the role of maternal genitor in the hybridization formula and the tester race J 90.

The general combining capacity has been directly appreciated by determination of the character value at the descendants resulted from hybrids creation.

The analysis of general combining capacity of the set of hybrids resulted from crossings between inbred lines and tester race gave the possibility of identifying a number of hybrid combinations with high values of parameters which were on the basis of their evaluation. The obtained results are shown in Table 2.

The shell cocoon weight varied between 0.304 – 0.221g, with a group average of 0.273 g.

From the group of hybrids Băneasa 75 x Tester in which the silk content varied between 22.01 – 17.75% we noticed the hybrid combinations between Băneasa 75 L10 x J90, Băneasa 75 L12 x J90, Băneasa 75 L2 x J90.

The performances of hybrids resulted from direct and reciprocal crossing (specific combining capacity) between the lines selected on the basis of general combining capacity

The results of the researches concerning hybridization between inbred lines selected on the basis of general combining capacity are presented in Table 3.

A first conclusion resulting from the data's analysis is that the shell cocoon weight is medium to strong influenced by the heterosis effect, in positive sense, while the silk cover is negatively influenced by the heterosis effect.

Regarding the results of hybrid combinations in Băneasa 75 race, the shell weight presents positive effects in the case of all combinations of selected lines. The greatest heterosis effects for this character are recorded for the combinations in which the lines 10 and 7 are maternal forms and the other selected lines are paternal forms.

Table 1.Classification's order of inbreeding lines selected for crossbreeding with tester breed

Line	Shell weight (g)	Difference ± out of average	Line	Silk cover (%)	Difference ± out of average
I 12	0.304 ± 0.020	+ 0.050	L 10	22.01 ± 2.47	+ 3.47
I 10	0.303 ± 0.040	+ 0.049	L 12	21.34 ± 1.28	+ 2.80
I 14	0.294 ± 0.034	+ 0.040	L 2	20.88 ± 2.08	+ 2.34
I 7	0.287 ± 0.032	+ 0.033	L 14	20.82 ± 2.03	+ 2.28
I 2	0.286 ± 0.030	+ 0.032	L 7	20.60 ± 1.41	+ 2.06
I 13	0.284 ± 0.037	+ 0.030	L 5	20.45 ± 1.95	+ 1.91
I 5	0.283 ± 0.030	+ 0.024	L 4	20.01 ± 1.51	+ 1.47
I 2	0.282 ± 0.026	+ 0.028	L 13	19.90 ± 1.98	+ 1.36
I 6	0.279 ± 0.035	+ 0.025	L 6	19.67 ± 1.64	+ 1.13
I 8	0.250 ± 0.030	- 0.004	L 8	19.16 ± 1.39	+ 0.62
I 9	0.244 ± 0.026	- 0.010	L 11	18.68 ± 1.65	+ 0.14
I 11	0.241 ± 0.030	- 0.013	L 9	18.32 ± 1.50	- 0.22
I 15	0.221 ± 0.030	- 0.033	L 15	17.75 ± 1.98	- 0.79
X	0.254 ± 0.020		X	18.54 ± 1.87	

Table 2.Performances of combinations at the inbreeding lines (Băneasa 75) and tester breed

Inbreeding line x Tester	Shell weight (g)	Silk cover (%)
	$X \pm s_x$	$X \pm s_x$
I 1 x J 90	-	-
I 2 x J 90	0.286 ± 0.030	20.88 ± 2.08
I 4 x J 90	0.282 ± 0.026	20.01 ± 1.51
I 5 x J 90	0.283 ± 0.034	20.45 ± 1.95
I 8 x J 90	0.250 ± 0.030	19.16 ± 1.39
I 6 x J 90	0.279 ± 0.035	19.67 ± 1.64
I 7 x J 90	0.287 ± 0.032	20.60 ± 1.41
I 9 x J 90	0.244 ± 0.026	18.32 ± 1.50
I 10 x J 90	0.303 ± 0.040	22.01 ± 2.47
I 11 x J 90	0.241 ± 0.030	18.68 ± 1.65
I 12 x J 90	0.304 ± 0.020	21.34 ± 1.28
I 13 x J 90	0.284 ± 0.037	19.90 ± 1.98
I 14 x J 90	0.294 ± 0.034	20.82 ± 2.03
I 15 x J 90	0.221 ± 0.030	17.75 ± 1.98

Table 3. Performances of hybrids from direct and reciprocal crossing (specific combinative ability) between the selected lines on their general combinative ability in Băneasa 75 breed

Parents		Shell weight			Silk cover		
P_1	P_2	MP	F_1	H (%)	MP	F_1	H (%)
7 x	7	0.287	-	-	20.60	-	-
7 x	10	0.295	0.302	2.37	21.30	22.03	3.42
7 x	12	0.295	0.323	9.49	20.97	21.89	4.38
7 x	14	0.290	0.362	24.82	20.71	21.36	3.13
10 x	10	0.303	-	-	22.01	-	-
10 x	7	0.295	0.323	9.49	21.30	22.29	4.64
10 x	12	0.303	0.342	12.87	21.67	19.77	- 8.76
10 x	4	0.298	0.344	15.43	21.41	21.24	- 0.79
12 x	12	0.304	-	-	21.34	-	-
12 x	7	0.295	0.308	4.40	20.97	19.79	- 5.62
12 x	10	0.303	0.335	10.56	21.67	20.60	- 4.93
12 x	14	0.299	0.304	1.67	21.08	19.70	- 6.54
14 x	14	0.294	-	-	20.82	-	-
14 x	7	0.290	0.310	6.89	20.71	19.40	- 6.32
14 x	10	0.298	0.319	7.04	21.41	20.44	- 4.53
14 x	16	0.299	0.308	3.01	21.08	20.36	- 3.41

Regarding the silk content, it is noted that the heterosis effect is generally low in value, in some cases insignificant, therefore absent, with negative values in most combinations and with positive, but small values, in the combinations of line 7 as maternal form and lines 10, 12, 14 as paternal forms.

Taking into consideration the two characters, results that the shell weight is a trait mainly controlled by genes with non-additive action, and therefore suitable for obtaining the heterosis effect, while the silk cover is generally controlled by additive genes.

CONCLUSIONS

The study of inbreeding and hybridization effects in silkwormwas materialized in completion of experiences on inbred lines behaviour under the report of general combining capacity and specific combining capacity, respectively of estimate the crossing and heterosis effects.

The selection of inbred lines on the basis of generalcombining capacity was done using the ranking on the basis of the results of their

crossing with an analyzer race (constant tester), respectively J90 of Japonese provenance.

From the 14 hybrid combinations realized between the inbred lines and the tester race, were selected 4 inbred lines, taking as selection criterion the shell weight and the silk cover.

It results that about 30% of the formed inbred lines constitutes higher biological material in the obtaining of commercial hybrids.

The shell weight is medium to strong influenced by the heterosis effect, in positive sense, while the silk cover is less influenced by the hybrid vigour effect and in most cases in negative sense.

Taking into consideration the two characters based on which the evaluations were made, it results that the shell weight is a trait controlled in substantial proportion by non-additive genes with major action and so suitable for obtaining the heterosis effect, while the silk content is in generally controlled by additive genes.

The obtained results and the differences evaluated between various combinations highlight the possibility of parental from selection in order to obtain commercial hybrids.

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ASSESSMENT OF THE EXTERIOR OF FIRST-CALF HEIFERS OF HOLSTEIN BREED

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Abstract

These are the results of studies on the assessment of the exterior of first-calf heifers of Holstein breed under the conditions of the Republic of Moldova. The aim of the presented scientific paper is the study of exterior features and morphological and functional indicators of the udder of Holstein breed in the herd of Holstein cattle of the breed of LLC "DokSanCom", v. Tomay, ATU Gagauzia. Exterior features were studied at 91 of first-calf heifers. As a result of the research, it was established that the first-calf heifers of Holstein breed were well-defined type of dairy cattle, which is confirmed by their proportional body shape, the development of the middle part of the trunk, and strong bone structure. The first-calf heifers in the herd of SLL "DokSanCom" were quite tall – 139.1 cm height at the withers and 146.6 cm – in the sacrum. The slanting length of the trunk is 198 cm on average. The prolixity index was 117.0%, which is by 2.5% less than compared to the standard for breeds of dairy direction of productivity. A compact physique with a consistency index of 122.1% characterizes the estimated the first-calf heifers. Measurements of the udder along the girth, length and width were on the average 137.1; 45.9 and 32.6 cm respectively.

Key words*: Holstein breed, the first-calf heifers, exterior, body indexes, udder.*

INTRODUCTION

Exterior assessment of animals, in spite of its subjectivity and conventionality, occupies an important place in dairy cattle breeding (Servakh et al., 2008; Basonov et al., 2010). Kudryashov (1950) notes that the evaluation of the exterior is necessary for judging the strength of the animal's constitution and the conformity of this body to the conditions in which the animal exists and the productivity for which it is bred. He points out that such an assessment is necessary for proper selection and selection of animals in order to prevent a gap between their health and productivity.

Currently, the best specialized dairy breed in the world is Holstein, which, together with high milk productivity, is characterized by excellent qualities of the exterior. Holsteins have the highest genetic potential for milk productivity, a strong constitution and an excellent form of the udder (Prokhorenko, 2013). Since the exterior is closely related to dairy productivity, when selecting animals by the exterior, take place an indirect selection of them and by productivity (De Jong Gerben, 2014; Abylkasimov et al., 2011; Prakhov et al., 2010; Lyashenko, 2003; Novikov et al., 2011).

The shape and morphological properties of the udder are one of the important exteriors of high milk yield and cows' technological ability, (Kotenji et al., 1996; VonKeyserlingk and Weary, 2012).

The purpose of the work- to study the exterior features, morphological and functional parameters of the udder of the first-calf heifers of the Holstein breed in the herd LLC "DokSanCom".

MATERIALS AND METHODS

The material for research were the first-calf heifers of Holstein breed of Dutch breeding (n=91) in the herd Limited Liability Company (LLC) "DokSanCom", v. Tomay, Administrative and Territorial Unit (ATU)

Gagauzia. For evaluating the exterior indicators, the sampling technique was used, since it is the most objective. The exterior of the researched animals was studied by the development of the main physique state: height at withers, height at the sacrum, chest depth, chest width behind the shoulder blades, width of the croup, width of the hip joints, slanting body length, girth of the chest behind the shoulder blades, girth of the pastern.

Measurements were taken by measuring instruments for 2–3 months after calving (Basovskii, 1983; Belozertzova, 2011). On the base of measurements, the body build indices were calculated: long-legged, prolixity, pelvic thoracic, thoracic, consistency, outgrown, osseous according to the standard method (Kostomakhin et al., 2007). Important indicators that characterize the quality of the udder are its shape and size. They are determined by its girth and the relationship of length, width and depth. Morphological assessment of the udder was carried out for 2-3 months of lactation for 0.5 – 1.0 hours before milking by the method (Garkavoy, 1974).

Udder capacity is a very important breeding attribute, especially in transfer of cows to double milking. The difference in milk yield with three and double milking depends on the capacity of the udder. At cows with a well-developed udder, the difference is only 2-3% and has no practical value. At cows with a small udder capacity, transfer of cows to double milking reduces the yield by 8-10%.

Important indicators that characterize the quality of the udder are its shape and size. They are determined by its girth and ratios of length, width and depth. On the basis of the taken measurements, an assessment of udder was performed in accordance with the requirements (Table 1).

Table 1. Requirements for assessment of udder of heifers

Measurements, cm	Points			
	5	4	3	2
Udder width	29 and >	25-28	21-24	16-20
Udder Length	32 and >	29-32	25-28	21-24
Udder Girth	110 and >	95-109	80-94	65-79
Front Quarter Depth	27 and >	23-26	19-22	16-18
The length of the front nipples	6-8	6-8	4-5	4 and < 5 and >
The diameter of the front nipples	2.2-2.6	2.7-3.0	3.1-3.5	3.6-4.0 1.7-2.1

The obtained results of scientific research were processed using variation statistics methods (Merkurev and Shangin-Berezovsky, 1983; Plohinsky, 1969) using the Excel program, the reliability of the indicators was estimated by Student.

RESULTS AND DISCUSSIONS

The analysis of the physique measurements of the first-calf heifers of Holstein breed in the herd of LLC "DokSanCom" showed that they have a well-defined type of dairy cattle, which is confirmed by their proportional body shape, mid-body development, strong bone (Table 2).

Table 2. Measurements of the body of the first-calf heifers

Measurements	M± m, cm	Cv, %
Height at withers	139.1±0.25	1.72
Height at the croup	146.6±0.25	2.78
The depth of the thorax	68.9±0.43	4.8
The width of the thorax	42.2±0.16	3.65
The width of the croup	51.5±0.1	1.94
The width of the croup at Ischia	32.6±0.17	5.01
Length of the body	162.8±0.32	1.88
The thorax perimeter	198.8±0.85	4.08
The whistle perimeter	20.4±0.09	4.22

Thus, the first-calf heifers in the herd of LLC "DokSanCom" were tall enough – 139.1 cm in height at the withers and 146.6 cm in the sacrum. They have a well-developed depth of the thorax - 68.9 cm, width – 42.2 cm, girth – 198.8 cm, and the width of the croup – 51.5 cm, respectively. The thorax perimeter of the first-calf heifers of the herd is 198.8 cm.

It should be noted that the use of physique indexes allows us to obtain the relative numerical values that characterize the exterior type of dairy cattle in the relative harmony of all physique articles.

The indexes of the constitution of the first-calf heifers of Holstein breed of the animals under study are presented in Table 3.

The received high index of long-legged at animals of Holstein breed of the herd LLC "DokSanCom" averaged 50.5%, which characterizes the good development of the organism in the postnatal ontogenesis of animals.

Table 3. Indexes of the constitution of the first-calf heifers (%)

Index	Values	The breed standard of various productivity directions		
		dairy	meat	dairy - meat
Long-legged	50.5	45.2	42.2	48.2
Prolixity	117.0	120	122	118.4
Pelvic-thoracic	81.9	80.2	83.5	85.5
Thoracic	61.2	61.8	79.6	68.8
Consistency	122.1	118	132.5	121.3
Overgrowth	105.4	100.9	103.2	102.5
Osseous	14.7	14.6	13.9	15.4

The lower level of the prolixity index or format is inherent to dairy cattle with the best quality characteristic of the exterior type. As evidenced by the values of the indicators of our research, at the first-calf heifers of the Holstein breed of the herd of LLC "DokSanCom" the index of lengthiness is correspondingly 117.0%, which is by 8.6% less in comparison with the standard for dairy breeds, which characterizes them by their good quality of the exterior.

The chest index averaged 61.2%, which is by 0.9% lower than the standard for dairy breeds, and speaks of the "narrow-chest" of the estimated heifers. On the overall development of the body and body weight it can be judged by the index of consistency or compactness. It should be noted that the estimated heifers of Holstein breed characterizes a compact physique with an index of consistency of 122.1%, which is characteristic to them in the studied period of development.

The ratio of height in the sacrum to the height at the withers is characterized by an index of overgrowth, which is a good indicator of growth and development of the organism in the postembryonic period. The average index of our studies on this index was 105.4%, which indicates a good development of the physique of the animals of the analyzed herd (Kibkalo et al., 2015).

The index of osseous was at the level of the standard for breeds of dairy direction of productivity (14.7%), while the proportions of the physique of the animals of the analyzed breed are preserved.

Thus, the results of visual and index assessment showed that the heifers of Holstein breed in the herd of LLC "DokSanCom" had a pronounced milky body type (Adushinov et al., 2011; Shatalov et al., 2013). They are characterized by a good form of build and strong constitution, on which the level of milk productivity, health status and duration of productive operation largely depends.

The shape and morphological properties of the udder is one of the important exterior features, since the positive relationship between milk production and the size of the udder allows for effective selection aimed at increasing milk production.

The parameters of the measurements, which are shown in Figures 1-3, characterize the development of the morphological features of the udder at the assessed the first-calf heifers.

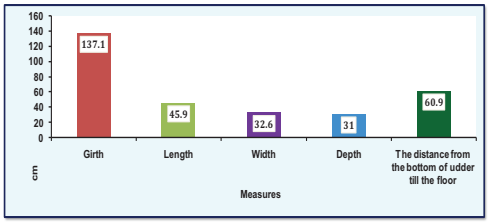

Figure 1. The size of the udder of the first-calf heifers

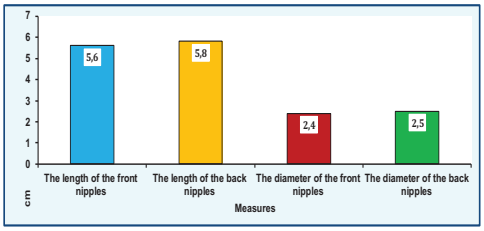

Figure 2. The size of nipples

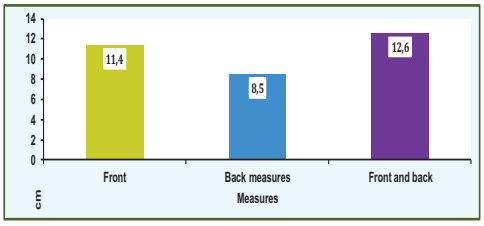

Figure 3. Distance between nipples

It follows from the figures that the measurements of the girth, length and width of the udder at the first-calf heifers averaged 137.1 ± 0.61 cm, 45.9 ± 0.33 cm and 32.6 ± 0.22 cm, respectively which is characteristic to animals of Holstein breed.

Evaluation of the morphological properties of the udder of the estimated the first-calf heifers are given in Table 4.

From the materials of Table 4 it follows that the estimated animals had the desired shape of the udder - bath-shaped. Evaluation by udder measurements - girth, length, width and depth had five points - the maximum score. The size of the nipples - the length and diameter of the front nipples, their assessment had 4 and 5 points respectively.

Table 4. The expressiveness of morphological signs of udder of the first-calf heifers

Signs	Indicators		Points
	M±m (cm)	Cv	
Udder size, measurement:			
Girth	137.14±0.61	4.24	5
Length	45.9±0.33	6.92	5
Width	32.6±0.22	6.42	5
Depth	31.0±0.18	5.5	5
Distance from the bottom of the udder to the ground	60.9±0.21	3.23	-
The size of the nipples:			
The length of the front nipples	5.6±0.06	10.25	4
The length of back nipples	5.8±0.05	9.36	5
Diameter of the front nipples	2.42±0.01	4.13	5
Diameter of back nipples	2.5±0.01	4.07	5
Distance between nipples:			
Front	11.4±0.18	15.3	-
Back	8.5±0.15	17.47	-
Front and back	12.6±0.19	14.29	-
Udder Form:			
Bath-shaped - 100%			

The location, length and diameter of the nipples of the heifers corresponded to the standard arrangement, convenient for milking at high-performance milking installations.

The distance from the bottom of the udder to the floor averaged 60.9 ± 0.21 cm, which indicates a large depth of the udder at these animals. It is considered that the distance from the bottom of the udder to the floor should be 45-50 cm, since a too hanging udder interferes with the free movement of the cow.

All the estimated the first-calf heifers of the Holstein breed had the desired bath-like shape of the udder, the development of the udder quarters is symmetrical, uniform, the attachment to the trunk is dense, the udder's bottom is horizontal, and the shape of the nipples is cylindrical (Lyashenko, 2013.)

Thus, the udder of the Holsteins breeds is bulky, with developed lobes, mostly densely attached. Organoleptic, the outer structure of the udder of these animals differs by more extensive length of the belly and with a sufficient depth.

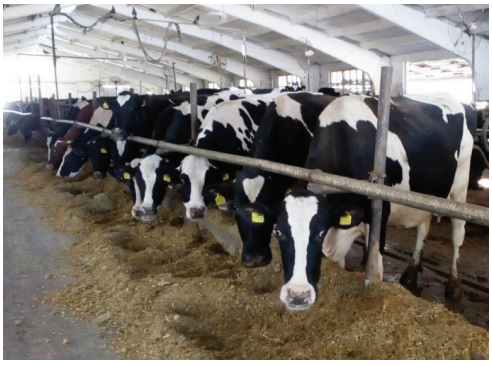

Figure 4. Herd of cattle of a Dairy Farm LLC „DokSanCom”

For further breeding in the breeding kernel of the herd of LLC "DokSanCom" 79 of the evaluated the first-calf heifers were selected.

CONCLUSIONS

At the estimated Holstein breed heifers, the chest depth is well developed - 68.9 cm, width - 42.2 cm, girth - 198.8 cm, and the width of the croup - 51.5 cm, respectively.

At the first-calf heifers of Holstein breed, the stretch index is respectively 117.0%, which is by 8.6% less compared with the standard for dairy breeds productivity.

The measurements of the girth, length and width of the udder averaged 137.1 ± 0.61, 45.9 ± 0.33 and 32.6 ± 0.22 cm, respectively, which is characteristic to Holstein animals.

The location, length and diameter of the nipples at the first-calf heifers corresponded to a standard arrangement convenient for milking on high-performed milking machines.

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STUDY ON INDICES OF REPRODUCTION AT POPULATION OF THE HOLSTEIN COWS OF DIFFERENT ORIGIN IN THE SOUTH AREA OF REPUBLIC OF MOLDOVA

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Abstract

Breeding indices and their interrelations were studied with the Dutch and German Holstein cow populations, exploited in the southern part of the Republic of Moldova. It was established that regardless of origins of cow population both duration of service-period as well as during of calving interval significantly prevail admitted optimal value. The best results regarding the time of service -period and interval between calving is registered at the population of Holstein cows of Dutch origin. The calving interval, in the average on population of cows of Dutch origin exceeds the optimal admissible value by 16.93%. Under similar conditions, maintenance and operating on cows of the German origins the calving interval duration exceeds the target value by 21.2%, being higher comparing with the results established at cows of Dutch origins by 3.5%. The coefficient of reproductive capacity utilization on average at cows in cows of Dutch origin is 0.86 and that of German origin is 0.83. In the investigated populations, were established links under medium, weak and very weak, both positive and negative, between breeding indices and the age of cows.

Key words*: service period, calving interval, dry period, reproduction rate, correlation.*

INTRODUCTION

Reproduction influences the rate of breeding of cattle, the level of economic output, the genetic structure of the populations, the state of animal health and the economic efficiency of cattle breeding. Breeding parameters (fecundity, birth, the calving-interval, etc.) directly affect the rate of breeding of cattle.

The maximum use of cow breeding capacity is among the most important conditions that determine the large efficiency of cattle specialized for milk production. The genetic potential of productivity can be achieved only under the conditions of a high level of the functionality of the reproductive apparatus (Leading, 2003; Lavelin, 2009; Frolova, 2014).

As with other animal species and at cattle, the function of the reproductive apparatus being very complex is subjected to the influence of a multitude of factors (Mishchenko et al., 2005; Frolova, 2014).

The most important points that can influence the brain activity in the process of releasing hormones are large milk production, fodder mistakes, the form of the stable, but also early sowing, seasonal influences, etc. (Azarov et al., 2009; Firsova, 2012).

The role of endocrine status on the cow reproductive function has been exposed in numerous researches (Nezhdanov and Safonov, 2008).

Starting of the many factors that influence the deploy of the reproduction function and its results, in the following we have proposed presentation and analysis of breeding index dynamics on successive lactations and interdependence with the age of Holstein cows of different origin exploited in the southern area of the Republic of Moldova.

MATERIALS AND METHODS

It was experimenting with cow populations of Dutch Hostein race exploited in the production activity course of LLC "DokSanCom", district Ceadir-Lunga and two populations of Dutch and German Hostein cow exploited on the course production activity of JSC "AYDYN", Komrat.

The study was targeted the assessment of the duration of the dry period (RM), service-period (SP), calving interval (CI), reproductive capacity coefficient (CRC) on successive lactations and interrelationships of reproduction indices depending of populations age to such animals.

Statistical processing of the experimental results was performed by computer, through mathematical analysis of biological phenomena.

RESULTS AND DISCUSSIONS

Of the four categories of qualities to which it refers, mainly, the improvement of dairy cows, along with production, body conformation, conformation and functionality of the mammary gland, a special place occupies the breeding indexes, because mammalian females at the end of the reproduction cyclograma ensure the outgrowth of the biological potential of the sire males fertility through a higher natality and prolificity with the highest values (Bogdan et al., 1984). In accordance with the proposed purpose, we evaluated the duration of dry period at cows population of Holstein race (Figure 1).

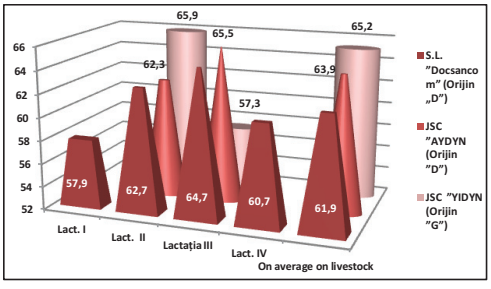

Figure 1. Breast restenosis duration at Holstein cows of different origin, days

The analysis of the presented data reveals that regardless of the origin of the animals, the number of lactation, as well as the conditions of exploitation and maintenance, the duration of the breast restenosis, in the average per capita, is smaller in the population exploited within the LLC "DokSanCom", the maximum being at cows of German origin exploited in JSC "AYDYN" (the optimal duration being considered 60 days), the differences are statistically commensurable. On the basis of these data, we can conclude that in the investigated zootechnical units, the preparation of the females for a new breeding cycle, as well as a high productivity in the later lactation, begins in the period of breast restenosis.

Duration of the service-period represents practical interest, because it directly influences the duration of the calving-interval, the number of obtained calves per 100 cows over a year, and the milk productivity. The results of the service-period study (Figure 2) are presented below.

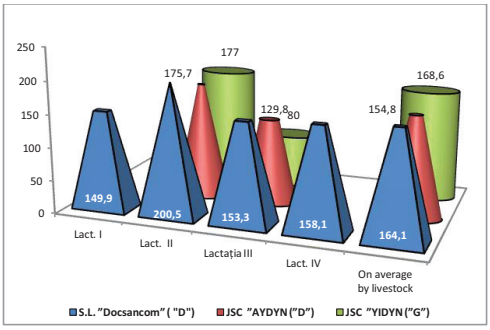

Figure 2. Service-period duration at Holstein cows of different origin (days)

The duration of the service-period in both the livestock and the successive lactations is well above the optimal admissible value. On average, the highest value is found at cows of German origin. In the similar conditions of exploitation and maintenance condition at Dutch origin cows the service-period is higher by 8.19%. Dutch origin cows exploited in different livestock units are showing a difference of - 6.01% in favor of the contingent of JSC "AYDYN". The longest duration is at the exploited population in LLC "DokSanCom", prevailing the indices of the population of the same origin in JSC "AYDYN" by 12.37% and that of the German origin by 11.72%. As a result of the service-period examination, at the investigated cows' populations, being in the III lactation, a decrease in both the average herd scores and with the cows being in the II lactation. At cows of Dutch Holstein in LLC "DokSanCom" the result is lower by 23.54% compared to those of II lactation. At the Holstein Dutch population of JSC "AYDYN" the decrease in comparison to the animals in the IInd lactation is 27.07%. At

Holstein of German origin in the III[rd] lactation, the duration of service-periods is lower than in II[nd] lactation by 47.81% or about twice lower. At these, being the best results, the achieved performance is practically approaching the admissible limits, taking into account the average level of milk productivity per unit. According to the maintenance and exploitation standards at Dutch Holstein cows, the results are better for the population of JSC "AYDYN". Here the duration of the service-period is by 15.33% lower compared to the population of LLC "DokSanCom".

The length ofservice-period of the cow is influenced by genetic factors (breed, individual, etc.) (Leading, 2003; Artyukh et al., 2004.). Limits admitted for service-period, depending firstly on the level of milk production at cows is considered to be: 45 days for those with low milk production; 60 days, for those with average milk production; 70 days for those with high production. Even so, in our case, the length of the service-period is over, at least, 2 times the maximum admissible value for cows with high milk yields.

In continuation, we present the results regarding the duration of the calving interval (Figure 3).

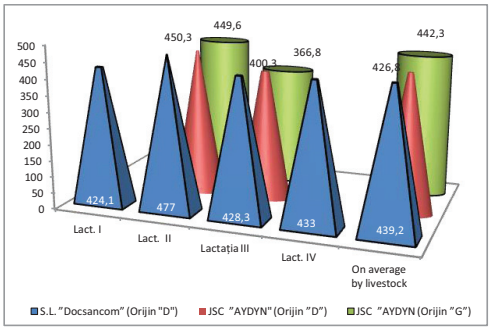

Figure 3. The duration of the calving interval at Holstein cows of different origin (days)

According to the length of the service-period, the interval between calves exceeds significantly the optimal value of 365 days. On average, on the analyzed populations, the duration of the calving interval of the Holstein German population exceed by 21.18% the Holstein Dutch of the LLC "DokSanCom", by 20.33% that of the JSC "AYDYN" and by 16.93% the optimal value of 365 days. The highest value is found at cows of II lactation. The Dutch Holstein population of the LLC "DokSanCom" surpassed by 30.7%, that of the JSC "AYDYN" by 23.37% and Holstein German by 23.18% optimum for 365 days.

At the cattle breed getting each year, from each cow of a healthy calf, represents at the same time the only chance of re-dairying, with all its consequences. But for this, an important link is represented by the normal organization and development of the breeding activity. In the following figure we present the coefficient of reproductive capacity at cows (Figure 4)

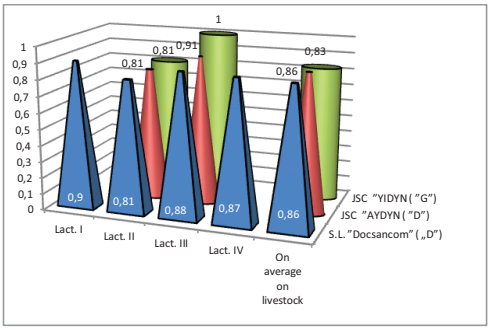

Figure 4. The value of Reproductive Capacity Coefficient at Holstein breed cows of different origin

Reproductive capacity at cows is normally 1.0. Analyzing the data presented in Figure 4, we conclude that in the average per capita, the best results are attested at the Holstein Dutch populations, regardless of the exploitation and maintenance conditions. In correlation with the lactation order number at all populations, the results are similar and the lowest at cows of the II lactation. At cows of the III lactation the best results are at German type of JSC "AYDYN". The minimum value is recorded at cows exploited in LLC "DokSanCom". Although the preparation per unit of the uterus for the next breeding cycle begins during breast restenosis, there are also still unused reserves of cow's reproductive capacity.

In the organization of breeding and improvement it is important to know the interrelationships between reproduction indices and milk yields at cows, since dairy secretion is a consequence of the reproduction function (continuity of the breeding) (Kononov, 2013; Firsova et al., 2012). In continuation we present some results on this subject (Figure 5).

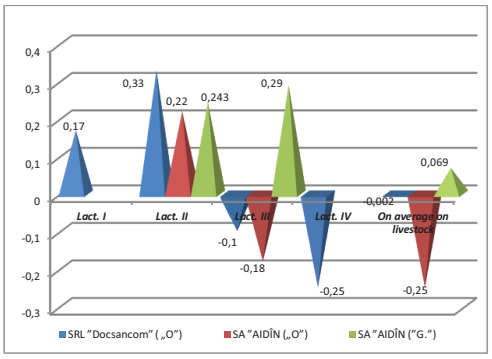

Figure 5. Interrelations service-period duration –the milk productivity on successive lactations at Holstein cows

In the average, the cow population of Dutch origin between the duration of the service period and the quantum of milk on the normal lactation, there are established negative correlative relationships, sub-media and weak, both negative and positive. Depending on the lactation order number, the same trend is observed.

In the following we present the interrelations between the duration of the interval between the calves and the milk yield according to the number of lactating order (Figure 6).

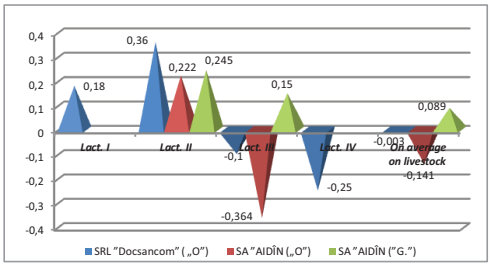

Figure 6. Interrelations the length of calving interval – the milk productivity on successive lactations at Holstein cows

Regarding the interrelations between the calving interval and milk productivity at Holstein cows, regardless of their origin, as well as the conditions of exploitation and maintenance, is found the presence of the positive sub-media correlation at animals of the II lactation. At cows of the III lactation, we observe the presence of the negative correlative bond sub-mediated at the Dutch Holstein population exploited in LLC "DokSanCom" and IVth at those exploited in JSC "AYDYN".

Next we present the interrelations character of the reproductive capacity utilization coefficient at the investigated cows population and milk production according to the lactation order number (Figure 7).

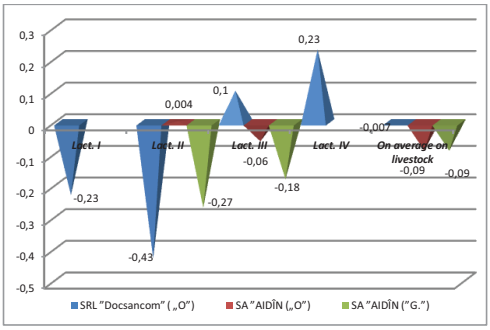

Figure 7. Interrelations the duration of the use of the coefficient of capacity of reproduction – the milk productivity at Holstein breed cows

Between the coefficient of the use of reproductive capacity at the cow populations of Holstein breed and the milk yield on successive lactations predominate negative correlative sub-media relationships and very weak, with the exception of cows of the IIIrd and IVth lactation cows exploited within the LLC "DokSanCom" at which there is a positive sub-media correlation.

CONCLUSIONS

At Holstein breed population of indifferent origin, as well as the conditions of maintenance and exploitation of the main breeding indices (the duration of the service and the interval between the calves) prevail significantly over the optimally admitted values, with the exception of the duration of the dry-period, being within optimal limits.

On average, per capita the best results of the use of reproductive capacity at cows is attested at cows of Holstein Dutch population (0.86), regardless to the conditions of exploitation and maintenance. In relation to the lactation order number, at all populations the results are similar and the lowest at cows of the II lactation (0.81); at cows of the III lactation the best results are at Holstein of German origin.

In the investigated populations, were established average correlative links, weak and

very weak both positive and negative between breeding indexes and milk productivity.

Between the coefficient of reproductive capacity utilization at cows of the Holstein breed population and milk productivity on successive lactations predominates negative correlative relationships sub-media and very weak.

The results obtained with regard to breeding indexes and their interrelationships with the milk productivity indices in the studied populations argue the necessity of organizing selection and organizational works in order to improve them.

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THE RELATIONSHIP BETWEEN HOLSTEIN COWS EXTERIOR AND DAIRY PRODUCTIVITY BY VARIOUS BREEDING

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Abstract

The article presents the results of studies of the relationship between measurements and milk production at Holstein cows of German and Dutch breeding in a herd of Joint-Stock Company „Aydyn", Comrat, Administrative and Territorial Unit Gagauzia, Republic of Moldova. By the level of milk productivity, the cows of German breeding were significantly superior to cows of Dutch breeding by 507 kg of milk (II lactation, P<0.05) and 1529 kg of milk (III lactation, P<0.001), respectively. At cows of the Dutch breeding by the size of the milk yield and the connection with the measurements, dependence was found from the slightly negative −0.008 (metacarpus girth) to a high positive +0.665 (width in jobbers). The average milk yield relationship was established at cows of German breeding with a chest girth behind the shoulder blades (+0.364) and a width of a croup (+0.336), which indicates a positive dependence of milk production for carrying out breeding according to the size of exterior region.

Key words*: Holstein breed, German selection, Dutch selection, lactation, correlation.*

INTRODUCTION

The exterior and body physique of animals plays an important role in milk production, as it reflects the intensity and direction of metabolism, the duration of use of cows. The constitution of animals makes it possible to have an idea about the expressiveness of breed signs, the direction of productivity and health (Gridin, 2015; Lyubimov, 2002; Kogut, 2016). Each breed is characterized by specific exterior features, which are created as a result of appropriate selection and sorting of animals on exterior indicators, taking into account specialization, as well as under the influence of certain environmental conditions (Efimova, 2018; Martynova et al., 2004; 2009).

In many countries with developed dairy cattle breeding (the USA, Canada, European countries), body physique along with indicators of milk productivity is the main breeding attribute when improving dairy breeds (Kondratieva, 2002; Harder, 1989; Corea-Guillen, 2008). Of great importance in the selection work with dairy cattle is the correlation between economic and useful features. When selecting Holstein cattle, it was found that in the selection process, the variability of one of the phenotypic parameters depends on the variability of other economically useful signs. Interest in the improvement of external forms is due to the existence of correlation variability in the development of individual traits and body proportions with the main selectable signs of milk production of cows, the duration of their lifelong use, reproductive qualities and health (Baranov et al., 2008). About the presence of positive reliable relationship between measurements and milk production of cows has been reported by many scientific studies.

The aim of the research was to study the relationship between measurements and milk production in Holstein cows of German and Dutch breeding.

MATERIALS AND METHODS

Studies on the correlation between milk production and exterior characteristics of Holstein cows of various breeds were carried out in the (J.S.C) Joint-Stock Company herd „Aydyn", Comrat, Administrative and Territorial Unit Gagauzia, Republic of Moldova. Were used the materials of the obtained results of studies of the exterior of the main measurements of cows after the third calving (Foksha et al., 2018). Between all indicators of the assessment of the exterior and milk production of animals, the relationship was determined by calculating the correlation of the coefficient (r).

Basic data on the milk production of animals were taken from the forms of zootechnical and pedigree accounting. For the assessment, data on the milk productivity of cows with at least three completed lactations was taken: German breeding - the first and second lactation (n=22), the third lactation (n=20); Dutch selection - the first and second lactation (n=42), the third lactation (n=39).

Static processing of research materials was carried out according to the methods of Plohinsky (1978), Merkuryev and Shangin-Berezovsky (1983).

The data obtained in the course of the research were processed biometrically on a personal computer using Microsoft Excel programs; the accuracy of the indicators was determined by Student test.

RESULTS AND DISCUSSIONS

According to the results of the assessment of the indexes of the physique of cows of the German and Dutch breeding after the third calving, it was found that the cows of the German selection exceeded their peers on the index of high-legged by 0.2%; pelvic – by 1.6%, thoracic - by 2.1%, consistency by - 1.8% and overgrowth by 0.5%, (Foksha et al., 2018).

Therefore, the cows of German selection have relatively better development of the breast in depth, respectively, of the chest organs or, they have more developed chest organs, which provide a higher metabolism and what causes higher milk productivity. This is confirmed by the analysis of the level of milk production of animals in the dynamics of the three lactations, (Table 1).

Table 1. Productive qualities of cows of various breeding of Holstein breed for 305 days of lactation, J.S.C. "Aydyn"

Indicators		Breeding	
		Dutch	German
I lactation		n=22	n=42
Yield of milk, kg	M±m_x	8058±176.4***	7207±147.0
Fat, %		3.71±0.05	3.73±0.04
II lactation		n=22	n=42
Yield of milk, kg	M±m_x	8192±157.9	8699±191.5*
Fat, %		3.79±0.03	3.78±0.03
*** III lactation**		**n=20**	**n=39**
Yield of milk, kg	M±m_x	9636±307.8	11165±204.4***
Fat, %		3.84±0.04	3.91±4.5

Note: *** III lactation** - assessed by measurements; * - P<0.05; *** - P<0.001

Analysis of indicators table 1 indicates that the milk production on the first lactation at cows of German breeding is lower than at their peers from Holland. Thus, for 305 days of the first lactation from German heifers, was received an average of 7,207.6 kg of milk, while from the Dutch - 8058.3 kg, which is by 851 kg less, the difference is significant at P<0.001. From the second lactation at cows of German breeding, a significant increase of milk productivity has been observed, therefore for the next 2 lactations they exceeded their peers in milk yield by 507 kg of milk (II lactation, P<0.05) and 1529 kg of milk (III lactation, P<0.001) accordingly (Figure 1).

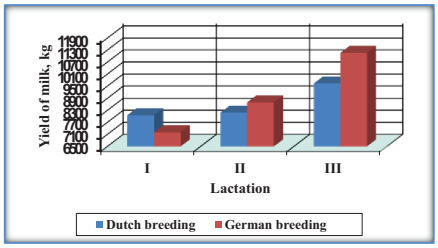

Figure 1. Dynamics of milk productivity of cows of various selections of the herd of J.S.C. „Aydyn"

It should be noted that the change of the conditions of keeping and feeding did not lead to a decrease of the productive abilities of animals of the Dutch and German breeding. Under the new conditions of keeping a cow of Holstein and German breed, realize their genetic potential at a high level.

A comparative analysis showed that at the tested heifers' population, the realization of the genetic potential differs insignificantly in comparison with the average indicators for the herd (Foksha et al., 2018) and constitutes 80.2 and 69.2% Dutch and German selection, respectively (Figure 2).

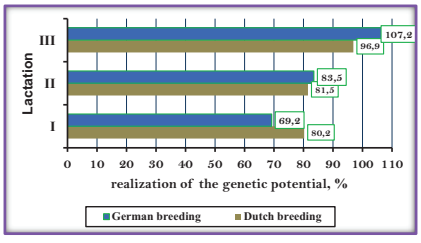

Figure 2. Realization of the genetic potential of milk productivity in the dynamics of lactation of Holstein cows of the Dutch and German breeding

As it can be seen, in the second and third lactations at cows of the German breeding, the implementation of the genetic potential was at the level of 83.5 - 107.2%, which is by 2.4 and 9.6% higher than that of the peers of the Dutch breeding.

For a more complete assessment of the character of the connections of milk production at cows of the III lactation of various breeds, a study was conducted of the presence of correlation between milk yield, fat content in milk and measurements.

Table 2 presents the results of the correlation interrelation of traits of the exterior of cows with milk productivity.

Table 2. The coefficients of correlation of breeding signs - yield – measurements (X ± Sx) of cows for the III lactation, J.S.C. „Aydyn"

Correlated sign	Breeding	
	German	Dutch
Yield of milk and height at withers	-0.244±0.143	0.077±0.226
Yield of milk and height in sacrum	-0.271±0.140	-0.197±0.211
Yield of milk and depth of chest	-0.271±0.140	0.05±0.230
Yield of milk and breast width behind the shoulder blades	0.232±0.144	-0.167±0.215
Yield of milk and width of a croup	*0.336*±0.134	**0.665**±0.136
Yield of milk and width in sciatic tubercles	0.196±0.147	-0.145±0.218
Yield of milk and slanting length of body	0.018±0.163	-0.276±0.200
Yield of milk and girth of chest behind the shoulder blades	*0.364*±0.131	-0.3±0.197
Yield a of milk and girth of the pastern	0.19±0.148	**-0.008**±0.235

Analysis of the results of the interrelation of the milk yield with the measurements of traits of cows exterior revealed a dependence on slightly negative −0.008 (metacarpus girth) to positive - +0.665 (width of a croup) at cows of Dutch breeding

The average interrelation of milk yield was established at cows of German breeding with a chest girth behind the shoulder blades - +0.364 and a width of a croup – +0.336, which indicates a positive dependence of milk production for carrying out a selection according to the size of traits of the exterior. A weak correlation is established between the milk yield and the width of the breast behind the shoulder blades - 0.232; the width in the sciatic tubercles is 0.196 and the girth of the pastern at cows of German breeding, the relationship is positive.

A weak negative correlation was established between the high-altitude measurements at cows of German breeding (height at withers and sacrum).

It should be noted that at cows of German breeding, on the background of higher productivity (11165 kg versus 9636 kg), the interrelation of milk yield with such measures as breast width behind the shoulder blades, width in the sciatic tubercles, girth of chest behind the shoulder blades and girth of the pastern is much higher than those of Dutch origin.

At cows of German breeding is established a weak negative relationship with 8 out of 9 studied measurements with the content of milk fat (Table 3).

Table 3. The coefficients of the relationship of breeding signs (X ± Sx) of cows of III lactation, J.S.C. „Aydyn"

Correlated sign	Selection	
	German	Dutch
Fat content and height at withers	-0.237±0.143	0.031±0.232
Fat content and height in sacrum	-0.201±0.147	0.080±0.226
Fat content and depth of chest	-0.102±0.156	-0.222±0.208
Fat content and breast width behind the shoulder blades	-0.102±0.156	-0.148±0.217
Fat content and width of a croup	-0.176±0.149	0.230±0.207
Fat content and width in sciatic tubercles	0.046±0.160	0.124±0.221
Fat content and slanting length of body	-0.114±0.155	0.274±0.201
Fat content and girth of chest behind the shoulder blades	-0.281±0.139	-0.289±0.198
Fat content and girth of the pastern	-0.017±0.163	-0.248±0.204

The correlation of body measurements with the content of milk fat for these animals varies from -0.017 (girth of the pastern) to 0.046 (width in sciatic tubercles).

It is noteworthy that the cows of the Dutch breeding have a weak positive relationship with the content of milk fat with a width of a croup (0.230) and slanting length of body (0.274).

For the other measurements, there is a slight positive or weak negative relationship in the range -0.148 (breast width behind the shoulder blades) to -0.289 (girth of chest behind the shoulder blades). Similar results received in their studies (Novikov Leshonok, 2014).

Since the samples were not numerous, most of the correlation coefficients were not statistically significant. Comparison of the results did not reveal regularities among the studied signs.

CONCLUSIONS

For 305 days of lactation from the Dutch heifers, was received milk more by 851 kg than from their peers of German breeding, the difference is significant ($P<0.001$). Since the second lactation at cows of German breeding is observed an increase in milk productivity. Therefore, for the next 2 lactations, they exceeded their peers in milk yield by 507 kg of milk (II lactation, $P <0.05$) and 1529 kg of milk (III lactation, $P <0.001$), respectively.

Indicators of the exterior of cows of the third lactation have an interconnection with milk production. It was established the average relationship of the milk yield of cows of German breeding with girth of chest behind the shoulder blades (+0.364) and width of a croup (+0.336). A weak correlation is established between the milk yield and the breast width behind the shoulder blades (+0.232), width in sciatic tubercles (+0.196) and the girth of the pastern at cows of German breeding, the relationship is positive.

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EFFECT OF USE OF PREBIOTICS^{BLS} (*Bacillus licheniformis, Lactobacillus sp.* and *Saccharomyces cerevisiae*) BASED ON SHRIMP WASTE ON PROTEIN EFFICIENCY RATIO IN INDONESIA LOCAL CHICKEN

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Abstract

One of the efforts to improve feed quality is by using BLS (Bacillus licheniformis., Lactobacillus spp. and Saccharomyces cerevisiae) microbial services from the shrimp waste substrate, hereinafter referred to as Prebiotics^{BLS}. BLS microbes are probiotic organisms that can improve the performance of livestock effectively (growth-promoter). Prebiotics^{BLS} is expected to function as an emulsifier in increasing nutrient absorption and metabolism so that it is effective in converting feed proteins into growth in Indonesia local chickens. The aim of the study was to determine the response of Prebiotics^{BLS} in feed to the performance of Indonesia local chickens. The study used 120 local one-day old chickens which were divided into 24 cage units, each cage unit consisted of 5 chickens, and kept for eight weeks. The experimental design used was a completely randomized design, consisting of 6 feed treatments (R0 = basal feed / CP 15%; R1 = basal feed + 1.0% Prebiotics^{BLS}; R2 = basal feed + 1.5% Prebiotics^{BLS}; R3 = basal feed + 2.0% Prebiotics^{BLS}; R4 = basal feed + 2.5% Prebiotics^{BLS}; and RS = standard feed / CP 18%), each treatment was repeated four times. The variables observed were feed consumption, protein consumption, body weight gain, and protein efficiency ratio. Data obtained from the results of the study were analyzed using analysis of variance, and differences between treatments were tested using Duncan's Multiple Distance Test. The experimental results showed that Prebiotics^{BLS} (based on shrimp waste) can be used as a feed supplement in the local chicken feed formula, and the use of Prebiotics^{BLS} at the level of 1.5-2.0% in feed (R4) is equivalent to the standard ration (RS / CP 18%).

Key words*: Prebiotics^{BLS}, shrimp waste; microbes; protein efficiency ratio; local chicken.*

INTRODUCTION

Indonesia Local chicken is one type of local poultry that has the potential to produce meat and eggs which is widely cultivated by the community, especially those who live in rural areas. This is because local chickens adapt well to the environment. Consumer demand for local chicken meat is increasing every year. Based on data from the Direktorat Jenderal Peternakan (2014), the number of local chicken meat production from 2007 to 2014 has seen an increase, namely in 2007 as many as 294,889 tons to 332,095 tons in 2014. Noting the data, farmers must pay attention to the age of harvest from local chickens to meet market demand by paying attention to the efficiency of the ration used to produce high body weight gain.

Most local chickens consume rations to meet their protein and energy needs. The protein content in the diet is very influential in achieving body weight in Indonesia local chickens. The protein content in the ration is needed for tissue growth, tissue repair, and production management as well as part of the structure of the enzyme, so that protein is known as one of the main constituents of body cells and tissues (Degusa, 2002; Close and Menke, 1986). This shows that protein plays an important role in achieving the desired body weight.

Provision of rations with good quality protein can certainly affect the level of growth and development of local chickens. The body weight gain produced is a picture of the quality of rations given. Body weight gain results from the high quality of protein consumption. High protein quality will affect protein intake into meat so that amino acids are fulfilled in the body. Body weight gain is directly caused by

the availability of tissue-forming amino acids, so the consumption of protein rations is directly related to the growth process.

Protein quality is determined by feed ingredients that make up rations, especially feed ingredients that are commonly used in Indonesia, namely fish meal. Fish meal has a high nutrient content, especially in the protein content (by 58%) which affects the quality of protein in chicken rations (Widodo, 2010; Wahju, 1997). However, the price of fish meal is expensive, and its availability is limited, it is necessary to look for alternative protein sources of feed ingredients that are cheap, easy to obtain, abundant availability, and have high protein content. The material is shrimp waste whose quality is expected to match the quality of ration from the use of fish meal.

Shrimp waste is a fishery waste whose numbers are increasing along with the increase in shrimp exports. Shrimp processing businesses in Indonesia have a production capacity of around 500,000 tons per year, out of the total production, 80 - 90% are exported in the form of head and skin frozen shrimp. Head and skin weight reach 60 - 70% of total weight (Direktorat Jenderal Budidaya Departemen Perikanan dan Kelautan, 2005). The volume of waste (head and shrimp skin) produced can reach 203,403 - 325,000 tons per year. This amount is a large enough potential to be used as a feed ingredient for protein in the preparation of local poultry rations.

The specialty of shrimp waste is that it has a good nutrient content, especially protein (42.65%), which almost matches fish meal (Gernat, 2001). Constraints in the utilization of shrimp waste are the limiting factor in chitin. Chitin binds to proteins and minerals in covalent glucosides bonds, making it difficult to digest by the poultry digestive enzymes (Abun et al., 2012; Abun, 2008). Therefore, an effort is needed to improve its quality so that it can be used as feed ingredients in the preparation of poultry rations.

One effort to improve the quality of feed ingredients is by microbiological processing through gradual fermentation techniques using *Bacillus licheniformis, Lactobacillus sp*., and *Saccharomyces cerevisiae*, and supplemented with Cu, Mo, and Se minerals during bio-process (Prebiotics^{BLS}). Bio-process products (Prebiotics^{BLS}), function as emulsifiers in the digestive tract of chickens, thereby increasing absorption and efficiency of nutrients (growth-promoters). *Bacillus licheniformis* bacteria produce chitinase and protease enzymes with deproteination properties which can free up some nitrogen or proteins from chitin bonds (Austin, 1988; Austin et al., 1981). *Lactobacillus sp*. serves to break down glucose, sucrose, maltose, and lactose, and the process of mineralization (Cira et al., 2000; Banwart, 1989). *Saccharomyces cerevisiae* is a yeast that produces the enzymes amylase, lipase, protease, and other enzymes that can help digestion of nutrients in the digestive organs (Bisping, 2005; Lee and Tan 2002). The technology of shrimp waste fermentation is one of the practical processing alternatives, and the results are favored by livestock, the price is low, and the value of nutrients is increased (especially protein), thus affecting the quality of protein rations.

One way to assess the quality of protein rations is to calculate the value of protein efficiency ratio. Protein Eefficiency Ratio (PER) is a method used to determine the quality of protein rations and is interpreted as increasing body weight divided by protein consumed (Leeson and Summers, 2001; Anggorodi, 1994). The protein efficiency ratiodetermines the level of efficiency of a livestock in changing each gram of protein into several body weight growths.

MATERIALS AND METHODS

Making Prebiotics^{BLS}

The materials used in this experiment include: Shrimp waste, Isolate *Bacillus. licheniformis, Lactobacillus sp*. and *Saccharomyces cerevisiae*, aquadest, glucose, yeast extract, tripton, NaCl, NaOH, $CaCO_3$, pH4 buffer, pH7 buffer, pH9 buffer, and Bovin Serum Albumin. The tools used are jars of stenles (reactors), water bath, shaker bath, autoclave, goblets, Bunsen burners, Petri dishes, porcelain dishes, centrifuges, funnels, PH-meters, spectrophotometers, test tubes, kilns, and containment machines. The stages of bio-process are as follows:

Deproteination. Perform fermentation at the Autho-Shaker-Bath (ASB). Shrimp waste is put into a jar of stainless, then inoculated with

Bacillus licheniformis inoculum at a dose of 2% (volume/ weight). Then put into ASB machine for 2 days at 45^0C with a rotation of 120 rpm (Abun et al., 2016).

Demineralization. Deproteination products, then added 2% (volume/weight) *Lactobacillus sp* inoculum, then incubated for 2 days at 35^0C, using ASB machines with 120 rpm rotation (Abun et al., 2016).

Fermentation with *Saccharomyces cerevisiae*. The demineralization products were then fermented using *Saccharomyces cerevisiae* 3% (volume/weight), then incubated for 2 days at 30^0C, using ASB machines with 120 rpm rotation (Abun et al., 2016).

Bindering. Bio-process products are then supplemented with cuprum (Cu) minerals, molybdenum (Mo) and selenium (Se) of 0.15 ppm, and additional energy sources (corn flour). Then the milling with a particle size of 60 mash. The product is Prebiotics^{BLS} (feed supplement) (Abun et al., 2018).

Feeding Trial

The study used 120 Sentul day-old (DOC) type local chickens without straight runes obtained from the Development Centre for Poultry Breeding, Jatiwangi, Majalengka-West Java, Indonesia. DOC has an average coefficient of variation of initial weight of 8%. The cage used is a cage-shaped cage of 24 units with a length of 0.7 m, a width of 0.5 m, and a height of 0.7 m. Each cage unit consists of 5 chicks and is equipped with a feed place in the form of a round feeder and a round water drinking water place made of plastic, and a 15-watt incandescent lamp. Chicken maintenance is carried out from 1 day to 8 weeks, giving rations and drinking water is done in *ad libitum*.

The feed ingredients for the ration consist of yellow corn, fine bran, soybean meal, fish meal (included in RO and RS), CaCO3, and Prebiotics^{BLS}. Basal ration (R0) and standard ration (RS) were prepared based on Indonesia's national standard recommendations (1995) and Zainuddin et al. (2004). The protein and energy content for basal ration (R0) is 15% and 2750 kcal/kg, and the standard ration (RS) is 18% and 2750 kcal/kg. The treatment ration is as follows:

R0 = basal / protein ration 15%;
R1 = basal ration + 1.0% Prebiotics ^{BLS};
R2 = basal ration + 1.5% Prebiotics ^{BLS};
R3 = basal ration + 2.0% Prebiotics ^{BLS};
R4 = basal ration + 2.5% Prebiotics ^{BLS}; and
RS = 18% standard / protein ration.

Prebiotics^{BLS}, treatment rations and local chicken can be seen in Figure 1.

Figure 1. Prebiotics ^{BLS}, Treatment Rations, and Indonesia Local Chickens (Sentul chickens)

The study was carried out using the experimental method and used a completely randomized design (CRD) with 6 types of ration treatment and repeated 4 times. The ration treatment consisted of R0 = basal ration / CP 15%; R1 = basal ration + 1.0% Prebiotics^{BLS}; R2 = basal ration + 1.5% Prebiotics^{BLS}; R3 = basal ration + 2.0% Prebiotics^{BLS}; R4 = basal ration + 2.5% Prebiotics^{BLS}; and RS = standard ration / CP 18%. The data obtained were analyzed Varian (F Test) and the differences between treatments were tested using Duncan's Multiple Distance Test.

RESULTS AND DISCUSSIONS

The average results of the study in the form of feed consumption, protein consumption, body weight gain, and protein efficiency ratio of each local chicken from each treatment during the experiment are presented in Table 1.

Table 1. Average Feed Consumption, Protein Consumption, Weight Gain, and Protein Efficiency Ratio in Indonesia Local Chickens

Variable	Treatment					
	RO	R1	R2	R3	R4	RS
Feed consumption (g/bird)	2892.69 A	2845.04 A	2872.59 A	2849.42 A	2817.76 A	2913.61 A
Protein consumption (g/bird)	433.90 B	426.76 B	430.89 B	427.41 B	422.67 B	524.45 A
Weight gain(g/bird)	1068.08 B	1067.33 B	1130.00 A	1109.39 AB	1073.55 B	1149.26 A
Protein Efficiency Ratio	2.46 B	2.50 B	2.62 A	2.60 A	2.54 AB	2.19 B

Description: R0 = basal ration / CP 15%; R1 = basal ration + 1.0% Prebiotics^{BLS}; R2 = basal ration + 1.5% Prebiotics^{BLS}; R3 = basal ration + 2.0% Prebiotics^{BLS}; R4 = basal ration + 2.5% Prebiotics^{BLS}; and RS = standard ration / CP 18%.

The use of Prebiotics^{BLS} in the ration did not affect the consumption of rations, but it affected the protein consumption, weight gain, and protein efficiency ratio (Table 1). Indonesia Local chicken protein consumption in treatment RS was significant ($P<0.05$) higher than other treatments, but between treatments R0, R1, R2, R3, and R4 did not show significant differences ($P>0.05$). Indonesia Local chicken weight gain in RS was not significantly different ($P>0.05$) with R2 and R3 treatment, but it was significant ($P<0.05$) higher than R0, R1, and R4 treatments. The protein efficiency ratio of Indonesia local chicken in the R2 and R3 treatments showed no significant difference ($P>0.05$), but it was significant ($P<0.05$) higher than the treatment of RS, R0, and R1.

The use of Prebiotics^{BLS} based on shrimp waste up to the level of 2.5% in the ration, did not affect the palatability of Indonesia local chickens, but it gave a positive effect on increasing body weight and protein efficiency ratio. This shows that Prebiotics^{BLS} has better nutritional value, especially organic acids and enzymes produced by microbes *BLS / Bacillus licheniformis, Lactobacillus sp.,* and *Saccharomyces cerevisiae.* (Collins and Gibson, 1999). Organic acids and enzymes are needed by chickens for growth. The use of Prebiotics^{BLS} at the level of 1.5-2.0%, optimal in achieving weight gain and protein efficiency ratio in Indonesia local chickens.

Feed consumption is strongly influenced by the palatability of feed ingredients. As stated by McDonald et al. (1981) and Leeson and Summers (2001) that palatability is an important factor that determines the level of ration consumption, and palatability depends on the odor, taste, color, and texture of feed ingredients. The use of Prebiotics^{BLS} based on shrimp waste did not cause a decrease in feed consumption compared to basal ration or standard ration (R0 and RS). This indicates that the use of Prebiotics^{BLS} to the level of 2.5% in the ration does not cause odor, taste, color, and texture that is not liked by local chickens.

The physical structure of ration constituent feed ingredients also determines the amount of consumption of rations. The chemical structure of chitin is like cellulose, with the bonds that occur between the monomers strung together with glucoside at the position of β (1-4). The difference with cellulose is that the hydroxyl group bound to carbon atom number two is replaced by the acetamide group ($NHCOCH_3$) in chitin so chitin becomes an N-acetyl glucosamine-based polymer (Muzzarelli, 2000; Muzzarelli and Joles, 2000). Chitin monomer units have a molecular formula $(C_8H_{13}NO_5)_n$ with levels of C, H, N and O respectively 47.29%, 6.45%, 6.89% and 39.37%(Zakaria et al., 1995; Williams and Shih 1989; Tsugita, 1990). The chemical structure provides an illustration of the physical form of chitin from tiger shrimp waste. Maynard and Loosely (1978) asserted that the condition of the physical structure of feed rationing ingredients determines the amount of consumption of rations. The same thing was also expressed by Scott et al. (1982) that a large amount of ration consumption is determined by the physical properties of feed ingredients. Other factors that affect ration consumption are digestive tract capacity and digesta movement (Sibbald and Morse, 1983; Sklan and Hurwitz, 1980), gender, daily activities, quality and quantity of rations, and physical form of rations (NRC, 1994), size and nation of chickens, environmental temperature, production stage, and energy in the ration (Wahju, 1997).

A good amino acid balance in the ration is shown by obtaining optimal body weight gain, this illustrates an improvement in the quality of protein rations with the addition of

Prebiotics^{BLS}. Deproteination by *Bacillus licheniformis* which produces the enzyme chitinase and protease enzyme to degrade β (1,4) glyosidic bonds in chitin and free some proteins in the form of N-Acetyl-D-glucosamine and acetyl amino monomers thereby increasing protein digestibility (Rahayu et al., 2004; Kanauchi et al., 1995), *Lactobacilus sp*. bacteria which functions in the demineralization process, and breaks down glucose, sucrose, maltose, and lactose into lactic acid (Lee and Tan, 2002). Fermentation with the help of *Saccharomyces cerevisiae* yeast that produces amylase, lipase, protease, and other enzymes can help digestion of nutrients in the digestive organs (Wagstaff, 1989).

The consumption of protein in the R4 treatment was significantly higher compared to other treatments. This is caused by the protein content of the ration at treatment R4 (protein 18%) higher than the other treatments (protein 15%). High protein consumption does not always have a positive effect on growth and the protein efficiency ratio (Iskandar et al., 2001; Rosenfeld et al., 1997). Tissue protein synthesis is largely determined by the completeness and level of amino acids that come or are transported into the tissue cells. In accordance with the opinion of Maynard and Loosely (1978), that the synthesis process that takes place inside the ribosome is very dependent on the presence of amino acids needed and comes picked up by DNA into the tissue. This causes the ration with the addition of Prebiotics^{BLS} to produce better body weight compared to without Prebiotics^{BLS}, although the protein content of the diet is relatively lower.

Prebiotics^{BLS} based on shrimp waste with *Bacillus licheniformis, Lactobacillus sp.,* and *Saccharomyces cerevisiae* microbes can improve the quality of protein rations by increasing the completeness and balance of essential amino acids contained in it. The resulting protein efficiency ratio is higher than the results of Wiradisastra (1986) study, which states that the protein efficiency ratio for 8 weeks old chickens is 1.72-1.93. This is caused by differences in the types of chicken used and differences in protein content of rations. In accordance with Wahju (1997) opinion, the protein efficiency ratio is influenced by age, type of chicken, sex, duration of experimentation, and protein ration level. The difference in protein efficiency ratio values is caused by the presence of shrimp-based Prebiotics^{BLS}.

Bio-process shrimp waste by *Bacillus licheniformis, Lactobacillus sp.,* and *Saccharomyces cerevisiae* can improve the quality of protein ration by increasing the completeness and balance of essential amino acids contained (Rao et al., 1998; Reddy and Quddratullah, 1996), so Prebiotics^{BLS} can be used as a feed supplement in Indonesia local poultry feed formulas. The balance of amino acids, especially methionine and lysine in the treatment ration with the addition of Prebiotics^{BAS} at the level of 1.5-2.0%, is in the ideal balance (methionine:lysine = 0.49-0.52:1) In line with Widodo (2010), that the balance of the amino acids methionine and lysine in the ration formula is between 0.48-0.52:1. This illustrates that the optimal level of use of Prebiotics^{BLS} is 1.5-2.0% in the Indonesia local chicken feed formula, which results in the highest growth and protein efficiency ratio.

CONCLUSIONS

Based on the results of the research and discussion it can be concluded that the use of shrimp waste-based Prebiotics^{BLS} at the level of 1.5% is optimal in increasing growth and protein efficiency ratio in Indonesian local chicken.

Prebiotics^{BLS} based on shrimp waste can be used as feed supplement in Indonesian local chicken feed formulas, and its use is between 1.5-2.0%.

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EFFECTIVENESS OF JAPANESE ANTS (*Ulomoides dermestoides*) AS ANTI-DIABETIC ON WHITE RATS (*Rattus norvegicus*)

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Abstract

Diabetes mellitus is a chronic disease characterized by hyperglycemia due to a disruption of carbohydrate, fat and protein metabolism, caused by inherited or acquired deficiency in production of insulin by the pancreas. This research was conducted to know the effectiveness anti-diabetic activity of Ulomoides dermestoides. Thirty-five rats were divided into 7 treatment groups with five in each group. Rats were made diabetic by single intraperitoneal alloxan. The result of experiment for 14 days showed that distribution of ½ and 1 part of larvae reduced blood glucose levels by 45.51 and 59.92%, respectively. While giving½and 1 part of imago reduced blood glucose level by 65.81 and 76.46%, respectively. This research showed that Ulomoides dermestoides has anti-diabetic potential in the diabetic rats.

Key words*: anti-diabetic activity, Ulomoides dermestoides, Rattus norvegicus*.

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease characterized by hyperglycemia due to a disruption of carbohydrate, fat and protein metabolism which is associated with absolute or relative deficiencies of work or insulin secretion. (Suryono, 2007).

According to WHO in 2007 the number of DM patients in the world reached 246 million people. In Indonesia according statistics on DM patients in 2003 as many as 11.3 million people and is estimated to reach 21.3 million people in 2030. At present day, Indonesia has ranked fourth in the world after the United States, China and India. Awad et al. (2013), reported that in Manado city the patients of DM were more likely to be women than men and the highest age at 51-60 years.

Around the world many cultures use insect and their products as nutraceutical (Rumokoy et al., 2016; Toar et al., 2017). *Ulomoides dermestoides* is eaten a live as an alternative therapy for branchial asthma, psoriasis, vitiligo, chronic skin deseaes, inflamation and diabetes mellitus (Costa-Neto, 2002; Flores et al., 2002).

To determine the effectiveness of *Ulomoides dermestoides* in reducing blood sugar levels in DM disease, this study was conducted by using white rat *(Rattus norvegicus*) as animals experimental.

MATERIALS AND METHODS

This experiment used larvae and imago of *Ulomoides dermestoides*, making preparation by grinding until smooth, then dissolved with distilled water.

Thirty five white rats (*Rattus norvegicus*) with 150-300 g were used in this experiment. Animals were kept in cages 40 x 30 x 20 cm individually, and feeding and drinking water were carried out by *ad libitum*. Before initiation of experiment, the rats were acclimatized for 7 days period.

All the animals were randomly divided into 7 group with five in each group. Group I as control (P0), II (P1) diabetic (giving alloksan), III (P2) standard drug (glibenclamide 5 mg/kg BW), IV (P3) and V (P4) were treated with ½ and 1 part of larvae *U. dermestoides*, respectively. Group VI (P5) and VII (P6) were treated with ½ and 1 part of imago *U. dermestoides*, respectively. Treatment of glibenclamide, larvae and imago *U. dermestoides* are given daily for 14 day. The preparation by grinding until smooth mixed with distilled water 1,8 ml in each rat and given orally.

Rat were made diabetic by single intraperitoneal injection of alloxan monohydrate (TCL Tokyo Japan) 90 mg/kg body weight (BW) and solubilized with 0.2% saline before injection (Ahmed*, et.al*. 2005). Three days after alloxan injection, rat with plasma glucose level more

than 140 mg/dl, were included in experiment. Blood samples were collected from tip of tail and blood glucose levels were estimated using electronic glucometer (Accu-Check Performance). Treatment with glibenclamide and material *Ulomoides dermestoides* was started three days after alloksan injection. Blood sugar levels were measured on day 0, 7th and 14th.

RESULTS AND DISCUSSIONS

The value of blood glucose levels of white rats for 14 days of treatment are presented in Table 1.

In treatment (P1) it was seen on day 0 that blood glucose levels were 312.00 ± 168.22 mg/dl, then increased significantly on day 7 (417.00 ± 35.34 mg/dl) and on day 14 (446.00 ± 94.04 mg/dl).

Tabel 1. Analysis of blood glucose level in white rats for 14 days of treatment

Treatment	Blood Glucosa (mg/dl)		
	Day 0	Day7	Day14
Normal Control (PO)	89.33 ± 8.50	85.00 ± 9.53	86.33± 10.11
Negative Control (P1)	312.00± 168.22	417.00± 35.34	446.00± 94.04
Positive Control (P2)	466.60± 188.24	176.00± 85.28	188.60± 10.98
½ larva *U. dermestoides* (P3)	294.00± 181.04	213.60± 154.80	160.20± 115.16
1 larva *U. dermestoides* (P4)	428.60± 98.00	272.40± 142.66	171.80± 93.43
½ imago *U. dermestoides* (P5)	343.40± 179.75	336.80± 184.88	117.40± 38.14
1 imago *U dermestoides* (P6)	401.00 ± 144.51	263.60± 167.00	94.40± 447.99

Values are present in Mean and SEM

Treatment glibenclamide after administration of alloxan (P2) showed a decrease in blood glucosa level from 466.60 ± 188.24 mg/dl on day 0 to176.00 ± 85.28 mg/dl on day 7 and decreased again to 188.60 ± 100.98 mg/dl on day 14. In the treatment of ½ part larvae (P3), blood glucose level from 0 294.00 ± 181.04 mg/dl on day 0 to 213.60 ± 154.80 mg/dl on day 7 and decreased again to 160.20 ± 115.16 mg/dl on day 14. In the treatment of 1 part larvae (P4), blood glucose levels were showed a decrease from 428.60 ± 98.00 mg/dl on day 0 to 272.40 ± 142.66 mg/dl on day 7 and decreased again 117.40 ± 38.14 mg/dl on day 14. In the treatment of the ½ part imago (P5), blood glucose levels were showed a decrease from 343.40 ± 179.75 mg/dl on day 0 to 336.80 ± 184.88 mg/dl on day 7 and decreased again to 117.40 ± 38.14 mg/dl on day 14. In the treatment of one part imago (P6) it were showed that blood glucose levels from 401.00 ± 144.51 mg/dl on day 0 to 263.60 ± 167.00 on day 7 and decreased again 94.40 ± 47.99 mg/dl on day 14. The blood glucose levels in rats during treatment are presented in Figure 1.

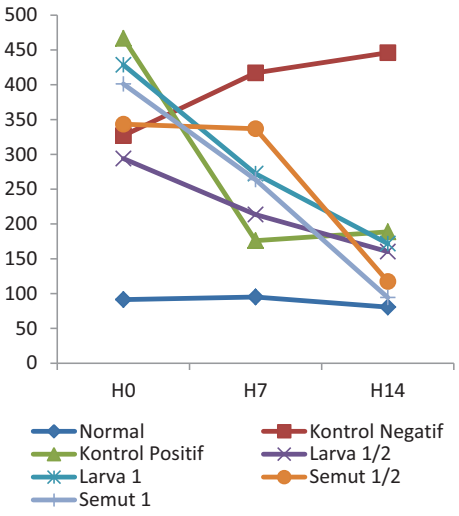

Figure 1. Graph of mean reduction in blood glucose levels in rats during treatment with *U. dermestoides*

The results of the initial experiment (0 or 3rd days after injection of alloxan) showed that the differences in rat blood glucose levels varied greatly. According to Suarsana et al. (2010), one of the causal factors for the existence of very large variations in blood glucose profiles of rate induced by alloxan was the resistance of individual rats to alloxan which caused the initial condition of diabetes to be uneven.

From Table 1, it can be seen that compared to control rat, giving alloxan was significantly able to increase blood glucose levels until the end of the experiment. This is because alloxan is one of the diabetogenic agents that is toxic, especially for pancreatic beta cells which, when given to test animals such as rat, will cause test animals to become diabetic (Prameswari and Widjanarko, 2014). Alloxan reacts by damaging essential substances in the pancreatic

beta cells, causing reduced insulin-bearing granules in pancreatic beta cells (Chandra, 2012).

Treatment with glibenclamide was able to reduce blood glucose levels by 59.58% of rat previously diabetogenic due to administration of alloxan. Glibenclamide is an oral hypoglycemic drug in the sulfonylurea group which has therapeutic effects to reduce blood glucose levels so that it is chosen as a comparative compound in research (Tanu, 2007). This is because glibenclamide works primarily in increasing insulin secretion (Bhowmik et al., 2009). The mechanism of action of glibenclamide is to stimulate the secretion of the hormone insulin from the granules of β cells of the islands of Langerhans pancreas. The interaction with ATP - sensitive K channel on the membrane of β cells causes membrane depolarization and this condition will open the Ca channel. After opening the Ca channel, the Ca2 + ion will enter the β cell and then stimulate the granule containing insulin and insulin secretion will occur (Suherman, 2007). The effective dose of glibenclamide in humans is 5 mg/kg body weight. This dose is then converted to dosage for test animals, namely white rat.In this experiment, all of the treatment both *U. dermestoides* larvae and imago were able to reduce blood glucose levels in diabetogenic rats. Treatment of ½ and 1 part larvae decreased blood glucose levels by 45.51% and 59.92%, respectively. While giving ½ and 1 parts of imago in the body reduced blood glucose levels by 65.81% and 76.46%, respectively. This shows the potential of *U. dermestoides* imago in reducing blood glucose levels in diabetogenic rats are greater than those of larvae. The same thing with the given dose seen both larvae and imago 1 part showed a greater decrease compared to only ½ part.

The results of the previous study showed that *U. dermestoides* containing various amino acids, saturated fatty acids and unsaturated fatty acids (Tables 2 and 3). Arginine is associated with wound healing, especially in people with diabetes mellitus. The mechanism of the influence of arginine in wound healing that arginine is one of the nitric oxide (NO) forming materials that will help the synthesis of collagen in the injured area (Abumrad and Barbul, 2005). Other studies reported that NO synthesized from arginine will regulate glucose metabolism, fatty acids and amino acids, so consumption of arginine will reduce fat mass in obese and diabetic mice (Shi et al., 2006). Nitric oxide also increases glucose transport, decreases the synthesis of glucose and glycogen and stimulates insulin release.

Table 2. Amino acid content - *U. dermestoides*

No	Amino acid element	Larva (%)	Imago (%)
1	Aspartic acid	2.42	1.86
2	Glutamic acid	3.26	2.91
3	Serin	0.51	0.42
4	Glycine	0.70	0.31
5	Histidine	0.53	0.54
6	Arginine	0.65	0.42
7	Treonine	0.38	0.63
8	Alanine	0.76	0.60
9	Proline	0.54	0.62
10	Tyrosine	0.26	0.31
11	Valine	0.50	0.46
12	Methionine	0.38	0.40
13	Cysteine	0.54	0.59
14	Isoleusi	0.31	0.30
15	Leusin	0.84	0.54
16	Phenyl-alanine	0.28	0.75
17	Lysine	0.63	0.48

Table 3. The content of saturated fatty acids and unsaturated fatty acids - *U. dermestoides*

No	Tipe Analysis	Larva (%)	Imago (%)
1	Kaprat	Undetected	Undetected
2	Laurat	Undetected	Undetected
3	Miristat	0.72	0.37
4	Palmitat	33.02	32.79
5	Stearic	0.67	0.49
Saturated fatty acid		34.11	33.65
6	Oleat	49.39	47.51
7	Linoleat	15.94	16.03
8	Linolenat	0.33	0.30
Unsaturated fatty acid		65.66	63.84

Hypoglycemic power is caused by the inhibition of the enzyme α glucosidasein the intestine so that it will slow down the breakdown of carbohydrates into a simple form and consequently the release of glucose and its absorption will be slowed in the intestinal brush border.

Besides amino acids, *U. dermestoide*s contain saturated fatty acids and unsaturated fatty acids. *U.* dermestoides extracts contain secondary metabolites which have antioxidant activity

(Mendoza et al., 2013) and antioxidant enzymes such as superoxide dismutase (Long et al., 2009). Antioxidants are known to have a function in counteracting free radicals that cause cell or tissue damage and can cause degenerative diseases.

Glutamic acid has an important role in sugar and fat metabolism. Fatty acids in animals and plants can be used as a treatment ingredient in treating epilepsy, mental retardation, muscular dystrophy, hypoglycemic ulcers and coma and side effects of insulin drugs for diabetes.

CONCLUSIONS

Base on the results and on the discussion we concluded that *U. dermestoides* were significantly reduced blood glucose levels in diabetic rats. Potential of *U. dermestoides* imago in reducing blood glucose levels in diabetogenic rats are greater than those of larvae.

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THE INFLUENCE OF OREGANO ESSENTIAL OIL ON EGG QUALITY AND EGG SHELL CONTAMINATION OF LAYING HENS KEPT IN FURNISHED CAGES

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Abstract

The aim of this study was to determine the effects of adding oregano essential oils (OEO) to diets on egg quality and egg shell contamination of laying hens kept in furnished cages. For this aim 300 Atak-S at 31 weeks old laying hens were randomly divided into two groups negative control (NC; basal diet) and oregano essential oils (OEO; basal diets plus 150 mg/kg oregano essential oils) for 6 months. Dietary inclusion of OEO significantly decreased E.coli and Coliform contamination on egg shell ($P<0.05$). In addition, including diet with OEO improved means of egg weight comparing to the control ($P<0.05$). However, no significant differences in the internal quality characteristics of eggs were observed between two groups during the all experimental period ($P>0.05$). Results of the study indicated that the adding OEO to diets plays an important role in decreasing egg shell bacterial contamination.

Key words*: furnished cage, egg shell contamination, egg quality, oregano essential oil, laying hens.*

INTRODUCTION

The housing system is an external factor that influences both the performance of hens and the egg quality characteristics. Conventional cages have been banned in the European Union since 2012. The free range system for laying hens is the most widely accepted alternative (cages and non-cages) to animal welfare. In this rearing system, hens can access the outdoor range area at any time during the day. In the free range system tend to require more feed and land to produce eggs or meat. Other benefits of free-range farming are greater comfort for the hens resulting in quality products with lower possibility of egg shell contamination of pathogens (Hammershøj and Steenfeldt, 2015; Pesavento et al., 2017). In addition, the environmental impacts of this system can be higher than that of cage system production. Injury and mortality rates were found to be higher in the free range system than cage system (Michel and Huonnic, 2003).

De Vylder et al. (2009) reported that eggs obtained from chickens housed in welfare-friendly housing systems do not pose more risks of layers being colonized with Salmonella compared to conventional battery cage. On the other hand there are many scientific studies showing that the total bacterial levels is higher in the shells surface of the eggs obtained from the hens housed in the free range system. *Escherichia coli* were the most frequently isolated *Enterobacteriaceae* species (Singh et al., 2009; Jones and Anderson, 2013).

Many kinds of herbs, plant extracts, spices and essential oils, have received great attention being used as feed supplements in poultry feeds for improving their performance and also egg quality (Akyildiz and Denli, 2016). In addition, many synthetic antioxidants including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) have been used widely in the food industry for prevention the deteriorative changes (Al-Hijazeen et al., 2006). However the most of them have not been received consumer concern due to their negative effects on human health. Therefore, the use of natural organic plant

extracts and some essential oils derived from them received attention.

Oregano *(Origanum vulgare L.)* essential oils, a phytogenic additive, are an aromaticplant that is indigenous to the Mediterranean region (He et al., 2017). Essential oils of thyme is derived from thyme (*Thymus vulgaris*), a medicinal plant with several therapeutic properties. Ghasemi et al. (2010) reported that dietary inclusion of garlic and thyme can have beneficial effects on performance of laying hens in terms of improving egg weight and yolk color. Bolukbasi (2008) reported that supplementation with 200 mg/kg EO of sage, thyme or rosemary reduced total coliform and *E. coli* counts in feces of 24-week old laying hens after a 12-week administration period. However, there is limited published data concerning with using thyme essential oil to improve egg quality and reduce the bacterial contamination on egg shell surface obtained in free-range systems.

MATERIALS AND METHODS

A total number of 300, 31 week old Atak-S laying hens with an average body weight of 1650±20 g were housed in furnished cages (750 cm^2/hen, 10 hens per cage) to 54 week of age. Hens were fed the same diet formulated was based on National Research Council (NRC) (1994). The composition of basal diet is shown in Table 1. Water and feed were provided as *ad libitum*. The daily photoperiod consisted of 16 h of light and 8 h of darkness (16L: 8D) throughout the experimental period. Temperature was maintained at 21 ± 1°C during the experimental period. Feeders were filled manually every day and eggs were collected daily during the morning hours. Laying hens were randomly divided into two groups; negative control (NC; basal diet) and oregano essential oils (OEO; basal diets plus 150 mg/kg oregano essential oils) from weeks to 54 weeks of age. Eggs were collected using sterile shears then placed in sterile bags and transported to the laboratory for eggshell bacterial contamination analysis. Egg samples were stored at 4°C overnight before analysis. Thirteen eggs were collected from each group approximately every 4 week from 31 to 54 week of hen age (6 sampling periods). Total aerobic populations were determined by duplicate spread plating 100 µL of the serial dilutions made from the rinse solution on to plate count agar. Plates were incubated at 35°C for 48 h before enumeration. Coliforms were enumerated by dispensing 1 mL of appropriate dilutions from shell emulsions into violet red bile agar pour plates with overlay. Duplicate plates per sample were incubated at 37°C for 18 to 20 h before typical colonies were counted.

Table 1. Composition of the basal hen diet

Components	**(%)**
Maize	45.0
Soybean meal (44%, CP)	10.0
Full-fat soybean meal	16.5
Sunflower meal (32%, CP)	9.50
Wheat	7.50
Limestone	8.80
Dicalcium phosphate[a]	1.85
Vitamin-mineral premix[b]	0.25
NaCl	0.30
DL-methionine	0.15
Calculated analysis	
Dry Matter	89.1
Crude Protein	18.0
Metabolic energy (kcal/kg feed)	2750
Ca	3.6
Available P	0.45
Methionine + Cysteine	0.63

[a] Composition (for each kg premix): Ca 24.5%; P 18%

[b] Composition (for each kg premix): vitamin A 12,000,000 IU; vitamin D_3 2,500,000 IU; vitamin E 30,000 mg, vitamin K_3 4,000 mg; Vitamin B_1 3,000 mg; Vitamin B_2 7,000 mg; Vitamin B_{12} 5,000 mg; Vitamin B_6 5,000 mg; Vitamin C 50,000 mg; Niacine 30,000 mg; Cal-D-Pantotenat 10.000 mg; Biotine 45 mg; Folicasit 1,000 mg; Cholincloride 200,000 mg; Canstatin 1,500 mg; Mn 80,000 mg; Fe 60,000 mg; Zn 60,000 mg; Cu 5,000 mg; I 1,000 mg; Co 200 mg; Se150 mg

Statistical analysis was performed using the mixed model and t-test procedure of SPSS 18.0. Tukey's test was used to separate group means. A significant difference was at $P<0.05$

RESULTS AND DISCUSSIONS

There are two basic food safety concerns that are microbiological safety and chemical contamination. Contamination of egg and egg products with pathogenic microorganisms (*Salmonella, Coliforms*, *Escherichia coli* etc.) can turn the egg into a poisonous food.

Dust samples were found to more readily detect the presence of *Salmonella* than fecal samples (EFSA, 2006).

The housing system has significant effects on the total count of bacteria on the egg surface and the microbial contamination of *Enterococcus* and *Escherichia coli* (Englmaierová et al., 2014). It has been reported that the rate of dirty and cracked eggs was increased in the eggs obtained from chickens grown in furnished cages (Tauson, 2002). Microbial contamination is generally higher in eggs or dirty eggs obtained from sick animals.

On the other hand, oregano essential oils have been used in poultry feeds as alternative antibiotics due to its antimicrobial, antioxidant, antiseptic and antiparasitic activities.

Eggs collected from alternative housing systems may have higher bacterial contamination probability of eggs due to contact with faeces or bedding material (Englmaierová et al., 2014). Similarly, Singh et al. (2009) reported that eggs from cages had lower *Escherichia coli* and coliform contamination than those from nest-boxes and the floor. In a recent review, DeReu et al. (2008), in observed that the aerobic bacterial counts on the egg shells were lower than the flocks (cage and ground) with cages (bird and cage), and the difference was that the eggs placed outside the nest boxes in the cage were very pronounced.

Effects of dietary supplementation of OEO on the percentage of shells positive for bacterial contamination by enrichment are presented in Table 2.

He et al, (2017) found that the addition of oregano essential oils decreased the number of intestinal *Escherichia coli*. Furthermore, Roofchaee et al. (2011) discovered that feed supplementation with OEO displayed potent antibacterial effects against cecal *Escherichia coli.*

In our study, the percentage of eggshells positive for *Enterobacteri, Coliform* and *Escherichia coli* were significantly decreased by dietary supplementation of OEO ($P<0.05$). This decrease of the percentage of the bacterial contamination on eggshells in was probably caused by the antimicrobial effects of OEO phenolic compounds such as thymol and carvacrol.

The results of eggshell bacterial contamination analysis are summarized in Table 3. Eggs from OEO treatment group had significantly ($P<0.05$) lower values of egg shell contamination for both the average of total *Coliform* and *Escherichia coli*; had 2.35 and 1.96 log CFU/egg, respectively, and control group had 2.35 and 3.02 log CFU/egg, respectively. These results are in agreement with Turcu et al. (2014), who observed the inclusion of OEO in to broiler diets significantly reduced *Enterobacteriaceae*, *E. coli* and staphylococci in the intestinal microflora compared to the control group. In addition, Criste et al. (2017) reported a significant decrease of *Escherichia coli* colony in the intestinal microflora of broilers reared under heat stress (32^{o}C) and fed with diets that included 2% oregano powder ($P\leq0.05$).These results may be due to the antimicrobial effect of thyme essential oils in the intestinal system of poultry.

Table 2. Effects of dietary inclusion OEO on contaminated eggshell ratio in laying hens housed in free-range system for 6 months

Period (months)	*Enterobacteria* (positive/total, %)		*Coliform* (positive/total, %)		*E.coli* (positive/total, %)	
	Control	OEO	Control	OEO	Control	OEO
1	60	10	30	ND	10	ND
2	ND	ND	ND	ND	ND	ND
3	50	20	50	20	50	20
4	70	50	70	50	40	ND
5	20	30	20	20	20	10
6	70	70	70	20	ND	20
Periods Average (1 to 6)	45	30	40	18	20	8

Table 3. Effects of dietary inclusion OEO on average of bacterial colony count of eggshell in laying hens housed in free-range system for 6 months

Period (months)	Average of total aerobes (log CFU/mL)		Average of total coliforms (log CFU/mL)		Average of total *E. coli* (log CFU/mL)	
	Control	OEO	Control	OEO	Control	OEO
1	3.38±0.14	3.57±0.11	4.32	ND	2.48	ND
2	2.03±0.18	1.99±0.01	ND	ND	ND	ND
3	3.01±0.11	2.60±0.21	2.07	2.15	1.95	1.93
4	3.71±0.22	4.13±0.17	3.65	2.24	3.23	ND
5	3.41±0.33	3.64±0.13	2.23	4.31	3.54	ND
6	6.10±0.19	5.52±0.15	4.38	3.30	3.88	ND
Periods Average (1 to 6)	3.60±0.18	3.35±0.15	2.96^{a}±0.2	2.35^{b}±0.2	3.02^{a}±03	1.93^{b}±0.0

Our results are in agreement with the previous reports who found that oregano had antibacterial activity against *Escherichia coli.* The antimicrobial effects of oregano essential oils may be attributed to its phenolic compounds (Bozin et al., 2006).

CONCLUSIONS

Results of our study indicated that dietary inclusion oregano essential oils play an important role in decreasing egg shell bacterial contamination. In addition, no adverse effect were observed on any parameters checked in this study, therefore this kind of feed additive would be beneficial in egg safety in furnished cages system.

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PROTEOLITIC POTENTIAL OF *Bacillus sp*. FROM FISH GUT AND NUTRIENT CONTENT OF SUBSTRATE

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Abstract

Proteolytic bacteria are bacteria that can hydrolyze proteins into smaller peptides or amino acid units. The existence of extracellular protease-producing bacteria is very important for life because it provides the need for nitrogenous compounds and can be used as a probiotic agent. This study was conducted to determine the proteolytic activity of Bacillus sp. Isolates. From the results of the collection and isolation of indigenous bacteria, the gut of carp. The method used is purposive sampling method, the data is analyzed quantitatively descriptive. The material used is the bacterium Bacillus sp. selected from the digestive tract of omnivore fish. Proteolytic test using clear zone method in skim milk agar (SMA) culture was measured at 12th, 24th, and 48th hours. The results showed that nine isolates of Bacillus sp. selected has proteolytic activity. The diameter of the clear zone (14.33-18.33.mm) Bacillus isolates from the 12th hour showed qualitatively the high proteolytic ability of the protease enzyme produced or also the high number of enzymes produced and released out. The optimum pattern of production time is in line with the general bacterial growth curve pattern. The results showed that at the 48th hour, bacterial isolates CP013984_s Bacillus sp. produce the highest protease activity, which is equal to 46.84 mm. The use of bacillus isolates in making probiotics can increase protein content and reduce crude fiber substrate, with a crude protein composition of 31.23%, extract ether 4.38% and crude fiber 8.64%.

Key words*: Bacillus sp., proteolytic activity, fishgut, nutrient content of substrate.*

INTRODUCTION

The digestive tract, especially the intestine, is an important part of the habitat of the bacteria that live in it. According to Fidyandini (2015) compared to gills, mucus in scales and the surrounding aquatic environment, the highest number of microorganisms is found in fish intestines. Interactions that occur between bacteria in the intestine can be negative and positive. These negative interactions can cause fish health problems but can also produce positive interactions that affect the increase in digestibility and the fish's immune system (as immunostimulants). Therefore, microbial communities of each aquatic species need to be identified to provide a more effective development opportunity for probiotics or immunostimulants. One type of bacteria in many probiotic products used today in the field of aquaculture is the *Bacillus* genus. Gupta et al. (2002) state that the genus *Bacillus* is suitable as a probiotic for aquaculture as it is commonly found as part of microbiota in fresh and marine water, and in the digestive tract of animals. *Bacillus* inhibition activity on the growth of *A. hydrophila* due to this bacterium produces enzymes including esterase lipase, leucine aryl amidase, acid phosphatase, lipase, and Naphthol-AS-BI-phospholipase. Whereas according to Susanti et al. (2002) that probiotics from the *Bacillus* group are widely applied for biotechnological purposes including the types of enzymes and amino acids produced and the production of antibiotics for fermentation and pathogen control.

Microorganisms are the most potential source of enzymes compared to plants and animals. The use of microorganisms is more beneficial because of their fast growth, can grow on a cheap substrate, the results are easier to increase through regulation of growth conditions and genetic engineering. Some genera of bacteria known to produce proteases

include *Bacillus, Lactococcus, Streptomyces*, and *Pseudomonas* (Rao et al., 1998).

The results of the research by Susanti et al. (2003) stated that four *Bacillus* strains isolated from the digestive tract of healthy white shrimp and applied through water at a concentration of 10^5 CFU/mL could improve the health of white shrimp larvae (*Litopenaeus vannamei*). Wijaya (2011) stated that probiotics *Bacillus* P4I1 applied to tilapia (*Oreochromis niloticus*) culture media at a concentration of 10^9 CFU/mL showed a survival value of 51.67% higher than control (21.67%) after being infected with Streptococcus agalactiae. The application of the superiority of *Bacillus* probiotics has emerged in the market especially in the form of drugs and food.

Based on the background explanation, a problem can be formulated regarding the extent to which bacteria originating from the genus *Bacillus* isolated from the gut of carp (*Cyprinus carpio*) can potentially be bacteria that have proteolytic activity. Based on the research background described, the objectives of this study are:Identify bacteria from the genus *Bacillus* that are found and Knowing proteolytic activity through the measurement of clear zones and change in the composition of the bioprocess substrate in making probiotic products. The usefulness of this study is to provide scientific information for farmers regarding indigenous bacteria species from the intestine that have proteolytic abilities so that they can function as a source of microbial and probiotic enzymes.

MATERIALS AND METHODS

Collecting Intestine Samples

- Incubator for incubating bacteria.
- Ruler to measure the diameter of the clear zone
- Disc paper to be dipped in the supernatant isolate

Isolation and Purification of Bacteria

- Fish gut
- Physiological NaCl and H_2O
- Cotton and gauze
- Plastic and plastic wrap
- foil alumina
- Medium skim milk

Proteolytic Characterization of Intestinal Bacteria with a Clear Zone

- Isolates pure bacteria of the genus *Bacillus*
- Medium SIM
- H_2O_2 solution 3%
- Erich reagent
- Skim medium for agar milk (SMA)
- Laminar as a place to conduct test activities
- Petri dishes as a medium place and testing
- Bunsen to avoid contamination when testing
- Incubator for incubating bacteria.
- Ruler to measure the diameter of the clear zone
- Paper discs to be dipped in supernatant isolates

Research methods

This research was conducted using qualitative and quantitative descriptive analysis. Sampling of fish and bacteria was carried out using purposive sampling method, is a technique of determining samples with certain considerations. The research was conducted at the Aquaculture Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University.

Carp Intestine Sample Preparation

The intestine of goldfish as the research sample is derived from carp cultivated in the floating net cage of the Cirata Reservoir in Gandasoli Village, West Bandung. The fish is brought to life as an aseptic intestine in the laboratory for laminar. Goldfish intestine is taken through several stages, namely: (a) Turn off the fish by piercing the brain using a sonde needle; (b) Washing the outside of the fish with sterile distilled water, then spraying alcohol on the entire surface of the fish's body and on a cloth using sterile cotton; (c) Place the fish in the tray and make an incision in the belly of the fish until the internal organs are visible; (d) Separating the digestive organs of the fish and cutting the intestines of the fish from the stomach to the anus; (e) Measuring the length of the intestine using a ruler, then divided into three parts (front, middle, and back of the intestine), each in a separate analysis; (f) Taking the contents of the intestine as much as 0.5-1 grams by splitting the fish intestine using scissors then scraping the intestinal contents; (g) Store the contents of the intestine in a sterile vial bottle that has added 10 ml of physio-

logical NaCl; (h) Take 1.0 ml of the solution using a micropipette and put it in the second test tube. And so on until 10-7 dilutions and obtained a single isolate.

As much as 1 ml of each dilution 10-2-10-10 by taking 1 ml of each dilution, put on the Skim Milk Agar media using the pour plate method.

The isolates of protease producing bacteria from the genus *Bacillus*, are characterized and biochemical identified by reference to the Manual of Determinative Bacteriology, which includes microscopic and macroscopic observations.

The results of bacterial isolates that have been isolated from goldfish intestine, then identified on agar culture, then carried out qualitatively proteolytic tests. Using a needle in the SMA media center on the petri dish. The media is first incubated at 50^{o}C for 48 hours. Qualitative protease activity is indicated by the formation of clear zones around the colonies. Diameter of colonies and clear zones using a ruler. The greater the clear zone formed, the greater the protease activity produced.

Proteolytic measurement through a clear zone

After obtaining a single isolate a proteolytic activity test was conducted. This screening test is carried out on the agar media with the addition of skim milk from the agar volume. In this test, the clear zone is produced. This test is carried out through several stages, namely: a) Taking bacteria that are present in a single isolate using an ose needle; b) Move the bacteria using an osseous needle into another petri dish containing medium so that it is skimmed; c) Perform bacterial cultivation for 2×24 hours; d) Observe and measure the distance of the diameter of the clear zone produced.

Proteolytic activity was determined by the size of the clear zone formed. Clear zone is a response from bacteria to TSB and skim milk and soy flour added to the medium. Clear zone formation activity was calculated from the difference in the diameter of the clear zone with the diameter of the bacterial colonies (Isnansetyo and Kamei, 2009).

A total of 20 µl of *Bacillus* probiotic inoculum was inserted into a well on NA media which was inoculated with Aeromonas bacteria which was planted on a pour plate with 0.5 McFarland concentration. Then incubated for 24 hours at 37^{0}C. The inhibitory zone (clear) formed on the test media was measured using a caliper. The variable measured is the diameter of the inhibitory zone (clear) formed in each well hole in mm.

RESULTS AND DISCUSSIONS

Isolation of *Bacillus sp*. on the digestive tract

The initial stage of the bacterial isolation process is to do the grinding of the mid gut, dilution, planting bacteria on agar media and purification of bacteria to obtain a single colony (Figure 1). Average of weight common carp fish was 76.95 g with a length of 18.5 cm. The intestinal weight of the fish is 1.3 g with a length of 20 cm and the degree of acidity (pH) of the intestine 6.5. The contents of the intestine were taken as much as 0.5-1 grams.

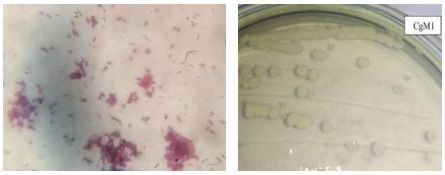

Figure 1.Colony morphology of isolated bacteria

In Figure 1 it appears that the main key character of the *Bacillus* genus is basil-shaped cells, gram-positive and forming endospores. These purified bacteria were then used as a single isolate and were used to test proteolytic activity. The morphological identification results obtained nine types of *Bacillus* bacterial isolates in the digestive tract (gut) (Table 1).

Table 1. Purified *Bacillus* isolates in nutrient agar and MRS cultures

No.	Code	Genus Bacillus
1.	I_1 (CgN2)	*Bacillus flexus*
2.	I_2 (CgN3)	*Bacillus flexus*
3.	I_3 (CgN4)	*Bacillus cereus*
4.	I_4 (CgN6)	*Bacillus carboniphilus*
5.	I_5 (CgM1)	*Bacillus haynesii*
6.	I_6 (CgM8)	CP013984_s *Bacillus* sp.
7.	I_7 (CgM18)	*Bacillus zhangzhouensis*
8.	I_8 (CgM22)	CP013984_s *Bacillus* sp.
9.	I_9 (CgM38)	CP013984_s *Bacillus* sp.

Genus *Bacillus* is a bacterium that has a large stem shape, gram-positive cell type, can grow in aerobic conditions that form a chain. Most

members of this genus are saprophytic organisms commonly found in soil, water, air, and plants. Fuller (1992) states that the genus *Bacillus* can grow at temperatures over 50^0C, is able to grow at high salt concentrations (>10%) and can produce spores. *Bacillus* bacteria can grow at high salt concentrations above 10 ppt, and still work well at pH fluctuations between 7.3-10.5. Some species are even able to live at very high pH conditions up to >11. Seeing the properties possessed by *Bacillus*, microbial cultures can be used both inside and outside the digestive tract by growing the right number of microbial populations so that it can be an alternative because used to suppress the growth of pathogenic bacteria.

Selected single isolates were as many as nine genera of *Bacillus* bacteria, then screening was performed on skim milk medium (SMA) with additional TSB, and soy flour from agar volume. In this test, the clear zone is produced. The bacteria that were successfully isolated were grown on media suitable for microbial growth because they were rich in nutrients. The results of isolation have been carried out, obtained nine isolates who have morphological characteristics of colonies that are different from each other, and able to grow in high school media (Figure 2).

Figure 2 shows that the *Bacillus* genus has a qualitative proteolytic ability isolates were obtained which had proteolytic activity. Skim milk contains casein which is included in the bacterial growth medium which functions as an enzyme substrate. Casein hydrolyses was used to show the hydrolytic activity of proteases. Proteases catalyze the degradation of casein, i.e. by breaking the CO-NH peptide bond with the entry of water into the molecule (Susanti, 2002).

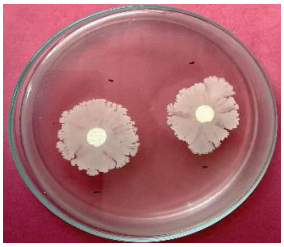

Figure 2. Qualitative Proteolytic Bacteria (a. clear zone b. colony)

Proteolytic Activity Test

The magnitude of proteolytic enzyme activity is shown by the increasing width of the clear zone, but the magnitude of proteolytic enzyme activity that plays a role in the solid medium cannot be quantified and measured quantitatively. The results of polymer protein reformation are only indicated by the presence of a clear zone which indicates that the protein has been overhauled into peptide compounds and amino acids which are dissolved in the medium. Qualitative hydrolysis activity is an illustration of the ability of proteolytic bacterial isolates to overhaul proteins by comparing the size of the clear zone around the colony with the size of the colony diameter.

The results of the proteolytic activity test showed that of the nine bacterial isolates of the bacillus genus that were obtained all produced clear zones which showed proteolytic activity at 12^{th} hour observation, 24^{th} hour, and 48^{th} hour (Table 2).

Table 2. Duncan's Multiple Distance Test Clear Zone *Bacillus* Isolate at 12^{th}, 24^{th}, 48^{th}hours.

No. Isolate (Code)	Clear Zona at Hour-		
	12	24	48
	(mm)		
I_1 (CgN2)	17.67^{A}	25.03^{A}	40.72^{BCD}
I_2 (CgN3)	16.67^{AB}	25.88^{A}	43.02^{ABC}
I_3 (CgN4)	17.00^{AB}	22.64^{B}	44.73^{AB}
I_4 (CgN6)	14.33^{C}	21.01^{B}	42.57^{ABC}
I_5 (CgM1)	16.63^{B}	19.25^{B}	36.52^{CD}
I_6 (CgM8)	17.33^{AB}	23.01^{AB}	46.84^{A}
I_7 (CgM18)	18.33^{A}	20.57^{B}	42.33^{ABC}
I_8 (CgM22)	$17,67^{AB}$	$20,87^{B}$	34.27^{D}
I_9 (CgM38)	16.00^{BC}	20.62^{B}	37.03^{CD}

Description: the same letter towards the column, shows no significant difference ($P<0.05$)

Based on the results of the study, all bacteria from the genus *Bacillus* identified from the digestion of carp can produce extracellular proteolytic enzymes using casein which functions as a substrate for the protease enzyme. Casein is the main protein of milk, a micro-molecule composed of subunits of amino acids that are connected by peptide bonds.

Table 2 shows that after incubation in a high school medium for one day (24 hours), bacterial isolates I2 (CgN3) had the highest proteolytic activity (by 25.88 mm) but did not show a significant difference with isolates CgN2(25.03 mm) and CgM8 (23.01 mm). After 48 hours, isolate I6 (CgM8), showed the highest clear zone diameter, almost covered the surface of the petri dish (46.84 mm), and did not show significant differences with Isolates I2, I3, and I7. The Isolates I5 (CgM1) and I8 and isolates I8 (CgM22), both at 24th and 48th hours, yielded the clearest clear zone ($P<0.05$) at the lowest.

The diameter of the clear zone formed can quantitatively indicate the proteolytic ability of the protease enzyme produced or also the high number of enzymes produced and released out. Proteolytic bacteria are bacteria that can degrade proteins, because they produce extracellular protease enzymes.

Proteases are proteolytic enzymes that catalyze the breakdown of peptide bonds in proteins. The ability of microorganisms to secrete proteases shows a change or protein degradation in skim milk containing medium.

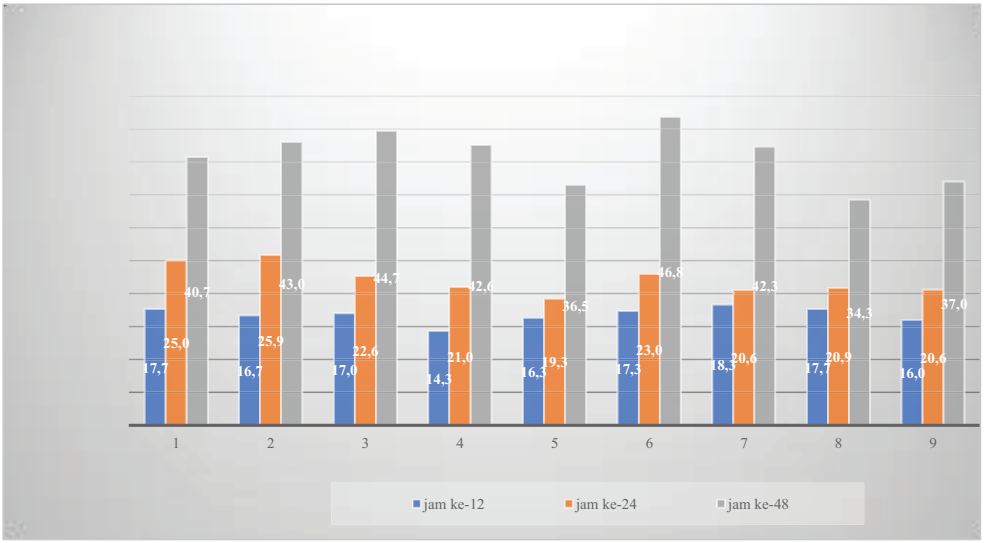

Figure 3. Proteolytic activity (Clear Zone) of ninetypes digestive isolates of fish gut

Proteolytic Index

The magnitude of the proteolytic index is related to the increase in the diameter of the inhibitory zone which is proportionally related to the increase in the diameter of the bacterial colonies, for example CgN6 and CgM8 isolates have a large bacterial diameter which shows a clear zone and high proteolytic index. The results of testing of proteolytic activity of bacterial isolates of carp samples are presented in Table 3

In Table 2 it appears that quantitatively-qualitative CgN3, CgM8 and CgM18 bacterial isolates were the largest isolates of bacteria with a proteolytic index value because almost all surfaces of the petri dishes had formed clear zones, whereas bacterial isolates I8 (CgM22) (8.70 mm) were bacterial isolates which have the smallest proteolytic index value.

Table 2 shows that *Bacillus sp*. and *Bacillus zhangzhouensis* has the best proteolytic index, as indigenous bacteria in the digestive tract of carp. The diameter of the clear zone formed can

qualitatively indicate the proteolytic ability of the protease enzyme produced or also the high number of enzymes produced and released out to carry out degradation activities. Indigenous bacteria are microbial bacteria obtained from the habitat of the digestive tract of fish. As for the research results of Affandi (2017), there were found the types of Bacillus subtilis proteolytic microbes which can produce extracellular proteolytic enzymes.

The proteolytic properties of microbes can be applied as probiotics in various interests. *Bacillus* in inhibiting microbial growth is a probiotic *Bacillus* with its metabolite binding to a negative carboxylic group on the surface of bacterial cells (Rabea et al., 2003). Some beneficial bacteria have proteolytic properties causing weakening or damage to membranes and other microbial cell components.

Bacillus probiotics also contain the lysozyme enzyme and amino-polysaccharide group which can inhibit microbial growth. Several mechanisms of inhibition of microbes by probiotics *Bacillus* have been proposed by several researchers, but the exact mechanism is not yet known. The most accepted mechanism is the interaction between probiotics *Bacillus* and the surface of bacteria, which causes changes in cell surface permeability.

Number of Microbial Colonies Results of Application in Feed Substrate

Table 3. Nutrient Content of Substrate and Products *Bacillus* sp.

		CFUCP	EE	CF	Ca	P
$\times 10^9$CFU				(%)		
Initial Substrate	4.01-4.42	22.19	5.91	12.82	3.41	1.44
Prebiotic *Bacillus*	15.22	31.23	4.38	8.64	4.22	2.05

CFU: colony forming units, CP: Crude Protein, EE: Ether Extracts; CF: Crude Fiber

In Table 3, it appears that there is a change in the composition of the bioprocess substrate in making probiotic products. This is in line with the opinion of Shurtleff and Aoyagi (1979), which states that in bioprocess there will be changes in complex molecules or organic compounds such as proteins, carbohydrates and fats into simpler molecules. Addition of *Bacillus* sp. and *Staphylococcus* sp. 10^3 CFU/ml each can increase the body's endurance in public waters which usually has *Aeromonas hydrophila* of 10^3 CFU/ml (Fidyandini, 2015). Feliatra et al. (2004) have also examined that in the digestive tract of carnivorous fish there are at least nine bacteria that function to help increase feed digestibility. The types of bacteria are *Lactococcus sp., Carnobacterium* sp*., Staphylococcus* sp., *Bacillus* sp., *Eubacterium* sp., *Pseudomonas* sp., *Lactobacillus* sp., *Micrococcus* sp., and *Bifidobacterium* sp. These bacteria are often used as probiotic candidates. Based on Aslamyah's research (2006), it was found that bacteria in the digestive tract of milkfish were: (i) amylolytic (*Citrobacter* sp*., Aeromonas hydrophila, Staphylococcus* sp*., Flavobacterium* sp*., Carnobacterium* sp*., Moraxella* sp., and *Vibrio* sp.); (ii) proteolytic (*Vibrio alginoliticus, Streptococcus* sp*., Micrococcus* sp*., Proteus sp., Pseudomonas sp*., and *Bacillus* sp.); and (iii) lipolytics (*Planococcus* sp., *Kurthia* sp., *Serratia* sp., and *Plesiomonas* sp.). Amylolytic bacteria isolated from milkfish can increase the availability of carbohydrate feed, thereby reducing the use of energy sources from protein.

CONCLUSIONS

The results showed that nine isolates of *Bacillus* sp. selected has proteolytic activity and bacterial isolates CP013984_s *Bacillus sp.* produce the highest protease activity, which is diameter of clear zone equal to 46.84 mm. The use of bacillus isolates in making probiotics can increase protein content and reduce crude fiber substrate, with a crude protein composition of 31.23%, extract ether 4.38% and crude fiber 8.64%.

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PHYTOCHEMICAL POTENCY AND ANTIMICROBIAL ACTIVITY OF ARECA VESTIARIA GISEKE AS A CANDIDATE FEED ADDITIVES IN BROILER

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Abstract

Abstract o protect themselves, plants accumulate the secondary metabolites as a source of antioxidant and antimicrobial. The objectives of the present study were to investigate the potency of Areca vestiaria Giseke as a candidate for broiler feed additives. The screening methods were performed using phytochemical screening, antibacterial assay against Escherichia coli and Staphylococcus aureus, antioxidant assay, and proximate analysis. The result showed that plant material extracted with 50% ethanol in water, contain flavonoid, tannin, saponin, and quinone. This compound can play a role as an antioxidant agent. An antioxidant activity (% inhibition) of Areca vestiaria is 32.58 ppm. There is antimicrobial activity either by microdilution or diffusion method. Proximate analysis showed, Areca vestiaria contain 6.59% moisture, 3.71% ash, 5.33% crude protein, 4.7% extract ether, 1.1% crude fiber, 47.16% nitrogen free extract, and 4045 kcal kg^{-1} metabolizable energy. From this study can be concluded that based on content of nutrition, antioxidant activity, and antibacterial activity, Areca vestiaria Giseke can be used as feed additives in broiler ration.

Key words*: phytochemical, antimicrobial activity, feed additives, Areca vestiaria.*

INTRODUCTION

In Indonesia since January 2018, antibiotic growth promoter (AGP) is prohibited its use in chicken feed. AGP is an antibiotic that is added in the diet in small concentrations to help suppress the adverse microbes present in the gastrointestinal tract. Prohibition of use of AGP provides consequences of possible nutrient absorption disorders that will eventually interrupt the productivity of chickens. Exploration of natural materials that have double biological activity, both as antibiotics and antioxidants become one of the target researchers. Based on several studies that have been developed, the compounds that have potential as antimicrobial and antioxidants are generally tannins, saponins and alkaloids as well as phenol compounds such as flavonoids. One of the plants that potentially contain these compounds is *Areca vestiaria* (yaki betel nuts). Yaki betel nuts are a kind of wild palm, one of the ornamental plants, endemic to the eastern part of Indonesia. In the area of origin of North Sulawesi, this betel nut called "*pinang yaki*" (monkey nut) because it is a typical monkey of Sulawesi *Macaca nigra* (black monkey) like to dwell on the stem of betel nut tree and eating the fruit. Yaki betel nuts are known to contain flavonoids as quercetin, catechins and tannins in seeds that have antioxidant activity against 2.2-diphenil-1-picrylhydrazyl.The amount of antioxidant figures closely related to the content of flavonoids. The more flavonoids contained the greater the total antioxidant activity. Utilization of yaki betel nut, especially the seeds as one source of antioxidants and antimicrobial need to be studied for livestock.This study aims to explore and test the potential of *Areca vestiaria* Giseke as a broiler feed additive.

MATERIALS AND METHODS

The research material consisted of yaki betel nut (AV) flour obtained during March to August 2015 from Tomohon area of North Sulawesi. The preparation of AV flour sample begins by separating the flesh from the fruit in a fresh state. Seed part was dried, after dry

separated from seed leather. The seeds were then dried again using a 40°C oven, resulting in a water content of less than 10%. The dried seed sample was smoothed with a JZ7114 1400 rpm type milling machine to obtain a size of 65 mesh (Satolom et al., 2015). Proximate analysis of yaki betel nut flour followed AOAC method 2003.6 (2005). Qualitative analysis (phytochemical screen) and quantitative flavonoids, catechins, tannins, and saponins were performed according to Harborne (2006).

Quantitative antioxidant activity test of the sample was performed following the procedure applied by Laboratory of Research Center for Medicinal Plants and Herbs, Bogor, Indonesia. Determination of antioxidant activity was done by α method, α-diphenyl-β picrylhydrazil (DPPH) (Li *et al*., 2011). The process for obtaining resistance values in DPPH was used a series of residual volumes that have been dissolved in methanol p.a. into a test tube containing a volume of test sample added 1 mL of methanol and 1 mL of 0.002% DPPH solution (time recorded at the time of addition of DPPH solution). The solution was shaken and allowed to stand for 30 minutes in a dark room and measured uptake at a wavelength of 517 nm. The calibration curve is made by making a series of butylated hydroxyl toluene (BHT) solution as standard. The inhibitory strength is expressed in% inhibition (IC50) which can be determined by making the concentration graph as the x-axis and % inhibition as the y-axis to obtain linear linear equations y = ax + b. Then this value is converted into μmol-ek unit BHT.100^{-1} g fresh weight. Manufacture of BHT calibration curve. BHT stock solution was made with 1000 μg mL^{-1} in methanol. Then the stock solution was diluted to a working solution of 10 μg mL^{-1}. The standard series solution was made by piping the working solution so that the standard end result of the standard series was 2.27-227.27 μg mL^{-1}. The percentage of inhibition can be determined by drawing concentration graph as x-axis and% inhibition as y-axis, so that linear line equation y = ax + b. Then this value is converted into μmol-ek unit BHTx 100^{-1} g fresh weight. Percentage of DPPH radical inhibition was calculated by the formula:

% inhibition = (Abs. control - Abs. Sample)/(Control Abs) × 100

The percentage of inhibition states the antioxidant activity for each sample. All the analysis was carried out twice, and the taken is the average value. BHT (Sigma Chemical Co., St. Louis, MO), was used as an antioxidant reference.

Determination of water content of sample is done by heating method (AOAC 2005) using oven. Dry the empty cup covered in oven 105°C for 3 hours then cooled in desiccator, then weighed (W1). The sample is weighed as much as ± 3 grams and fed into the porcelain plate evenly, place the cup containing the sample and closed into the oven and dry at 105°C for 3 hours then cooled in desiccator, then the sample weighed. Then dried again with oven and cooled in a desiccator until it reaches a constant weight (W2).Calculation of water content as follows:

Water content (%) = $(W_1-W_2)/(W_1) \times 100$

where:

W_1 = weight (g) sample before being dried

W_2 = weight (g) of sample after being dried

The antibacterial test performed on this experiment was by agar diffusion method. Preparation of test solution with various dilution of ethanol extract 70% of yaki betel nut was 10%, 15%, 20%, 25% and 30%. The medium used is NA (nutrient agar) and TSA (tryptone soya agar). The sterilized NA and TSA media were added each with *S. aureus* bacteria for NA and E. coli for TSA of 0.1 mL 100 mL^{-1} and then the media poured into different petri dishes already marked using markers for each extract concentration of 20 ml. Each bacterium was made of 5 Petri each. After the NA and TSA media hardened then the paper discs were placed on the already labelled medium and dripped with ethanol extract 70% *Kalanchoe pinnata* (Lam.) leaf as much as 10 μL with concentration of 10%; 15%; 20%; 25% and 30%. For positive control, on other paper discs dripped with 10μL of chloramphenicol solution and for negative control only containing DMSO-treated dessy paper (dimethyl sufoxide). The Petri dish was then wrapped in wrap paper and incubated at 37°C for 24 hours in an upside position. The resistor zone formed on each disc was measured using a sliding range.

The other antibacterial test performed on this experiment was by microdilution method on 96

well plates. The media used are TSB (Typtic Soy Broth) and NB (Nutrient Broth). A number of *S. aureus* and *E. coli* made and determined its optical density. The extract was diluted in DMSO with a concentration of 10000-78.12 µg mL^{-1} in the well. A total of 100 µL TSB and 10 µL inoculant solutions of *S. aureus* bacteria were added, as did 100 µL NB and 10 µL inoculant solution of E. coli bacteria. The process was continued with incubation at 37°C for 24 hours. MIC (minimum inhibitory concentration) is the lowest concentration of the well clear after incubation. The clear wells were piped 100 µL to 96 new well plates and TSB media added to *S. aureus* and NB media for *E. coli* of 100 µL. Incubation was continued for 24 hours at 37°C. Continued with incubation for 24 hours at 37°C. The clear well after incubation is MKC (minimum kill concentration). DMSO 20% was used as a negative control whereas positive control was used chloramphenicol.

RESULTS AND DISCUSSIONS

A qualitative test of phytochemical AV flour is conducted to determine the chemical compounds contained. Determination of this class of chemical compounds by looking at the presence or absence of color changes by reagents used. Furthermore, a quantitative test of flavonoids, catechins, tannins and saponins was performed. Phytochemical test results obtained from this study can be seen in Tables 1 and 2.

The results of qualitative phytochemical tests show that yaki nut seeds contain saponin, tannin, alkaloid, phenolic, flavonoid, triterpenoid and glycoside compounds which are likely to be bioactive potential compounds. It is known that phytochemical compounds are produced with the ability to protect these plants against environmental attacks (Gonzales-Lamothe et al., 2009).

Tannins, and flavonoids have antitumor, antiallergic, antihepatotoxic, and antioxidant activity. The triterpenoid group can be used as an antibacterial, anticancer, and to treat wounds and inflammation (Simbala and Tallei, 2010).

Feed ingredients generally contain anti-nutritional substances that can inhibit the efficiency of its nutrient utilization (Farrel, 2005).

Table 1. Phytochemical screening of ethanol extracts of *Areca vestiaria* Giseke[1]

Sample code	Secondary metabolites	Result[2]	Note
AVB red	Saponins	+	Produce stable foam after shaking
	Tannins	+	Produces a greenish black after droping 1% FeCl
	Alkaloids	+	Produces red sediment color after added Dragendrof reagent, and brownish color after added Wagner reagent
	Phenolic	+	There is a change in color to blackish blue after dropping FeCl3 5%
	Flavonoids	+	Produces orange in the amylalchohol layer
	Triterpenoid	+	Does not produce red colour after adding anhydrous acetic acid and concentrated sulphuric acid
	Steroid	-	Does not produce light blue after adding anhydrous acid and concentrated sulphuric acid

Note: [1]Result of laboratory Analisis of Research Center for Medicinal Plants and Herbs. Bogor (2015); [2]+ = present; - = not present

Table 2. Secondary metabolites content of *Areca vestiaria* Giseke

Secondary metabolites	%
Flavonoids as quercetine	0.33
Catekin	3.5
Tannins	8.41
Saponins	0.92

Note: [1]Result of laboratory Analisis of Research Center for Medicinal Plants and Herbs. Bogor (2015).

To anticipate that we would analyzed the nutritional and anti-nutritional content of AV (Table 2 and 3). AV seeds contain flavonoids, catechins, tannins, and saponins. The compounds are classified as compounds of bioactive potential or secondary metabolites. Secondary metabolites are non-nutritional chemicals that play an important role in the process of joint existence and evaluation among the different types in the environment (Mursyidi, 1989). It is known that these secondary metabolic compounds are produced by the ability to protect the plants against environmental attacks. It also has antioxidant activity that can neutralize the instability that occurs due to the existence of reactive molecules called free radicals. These compounds classified as secondary antioxidants (Winarsi, 2007), which serves to capture the oxidant compounds and prevent the occurrence of chain reactions.

In several studies that have been conducted on the activity of the three types of flavonoids it

turns out that quersetin studied in broiler to see its activity as immunostimulan with a dose up to 100 mg kg^{-1} BW (Zulnaidi, 2000). Another study of quercetin extracted with methanol from guava leaf, to a dose of 21.0 mg has not demonstrated contraceptive antifertility activity in white rats (Ariani et al., 2008). Catechins have inhibitory activity of converting histidine into histamine in the presence of histidine decarboxylase enzyme (associated with immune response), inhibiting cytokines TNF-α and IL-1β proteins. Catechins of green tea at doses of 800 mg kg^{-1} BW per day administered to mice can inhibit the growth of mammary gland tumors by 57.14% (Gunawijaya et al., 1999). The antioxidant activity of catechins and epicatechins was studied on the *Acacia catechu* tree and the leaves and stems of pale catechu (Duangyod et al., 2014). Antibacterial activity of Gambir has performed by Amos (2009) with catechin content of 25-35% and tannin of 60-65%. Inhibition of the action of digestive enzymes by tannins was generally indicated by the ability to binding proteins. Tannin inhibits pectinase, cellulase, amylase, protease, β-galatosidase, lipase and other enzymes that play a role in microbial fermentation (Fahey and Jung, 2000). The greatest inhibitory affinity of tannins was greater in protein than carbohydrates, due to the strength of the affinity of hydrogen binding to carboxyl oxygen in the peptide group. The use of tannins in the feed is limited to 2.6 g per kilogram of ration (Pour-Reza and Edriss, 1997).

Table 3. Nutrient composition of *Areca vestiaria* (as fed)[1]

Chemical composition	
Dry mater (%)	93.41
Ash (%)	3.71
Crude Protein (%)	5.33
Crude Fat (%)	4.7
Crude Fiber (%)	1.1
Nitrogen Free Extract (%)	47.16
[2]ME (kcal/kg)	4045

Note: [1]The results of the analysis of the Feed Science and Technology Laboratory, Fapet IPB, Bogor; [2]ME - metabolisable energy.

AV seeds contain dietary substances in the form of fat, protein, crude fiber and energy as listed in Table 3. Carbohydrates are components according to proximate analysis i.e. nitrogen free extract (NFE) and crude fiber. The easily digestible fraction and used as an energy source are NFE, whereas the hard-to-digest fractions are classified as crude fibers (Tilman et al., 1994). NFE contains soluble compounds in acidic and alkaline solutions and has high digestibility such as mono, di, tri and polysaccharide substances, especially starch. The content of food substances in the AV shows that this material can be used as a source of feed.

AV antioxidant activity compared with vitamin E can be seen in Table 4. This antioxidant activity test to know the value of resistance activity against free radical by DPPH method (2,2-diphenyl-1-picrylhydrazil).The parameters used are IC50 which was defined as the concentration of antioxidant compounds which causes 50% loss of DPPH activity (Molyneux, 2004). A substance has antioxidant properties if the IC_{50} value was less than 200 ppm. The smaller value of IC_{50} was then the compound effectiveness as a better radical catcher. The results of this study indicate that vitamin E antioxidant activity was better than AV.

Table 4. Antioxidant activity of *Areca vestiaria* and vitamin E

Test material	% inhibition (ppm)
Biji *Areca vestiaria*[1]	32.58
Vitamin-E[2]	10.43

Note:[1]Result of laboratory Analisis of Research Center for Medicinal Plants and Herbs. Bogor (2015);[2]Kurniawati (2011)

CONCLUSIONS

Areva vestiaria Giseke (AV) seed meal can be used as a feed additivesin broiler chickens ration in terms of phytochemical and antimicrobial activity.

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BENEFICIAL USES OF CHROMIUM IN LAYING HENS NUTRITION: A REVIEW

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Abstract:

Research on various avian species has shown that supplementation of diets with various mineral elements can have favourable effects on production indices, production quality and bird health. In particular, chromium has proven to be an essential mineral, active biologically as a component of glucose tolerance factor, which enhances tissue sensitivity to insulin and glucose utilization. Clinical trials conducted in human patients diagnosed with Type II diabetes along with studies on production animals have led to the conclusion that chromium is beneficial to animals and people undergoing physical or metabolic stress. A brief analysis of chromium supplementation of avian species feed revealed more results of chromium supplementation in broiler nutrition compared to laying hens, which is why the present review was conducted to evaluate the results of using different chromium sources in laying hens. Summarizing the information from the scientific literature on the use of chromium in laying hens nutrition can highlight whether this nutritional strategy is useful or not for laying hens farmers.

Key words*: laying hens, chromium supplementation, chromium sources, beneficial uses.*

INTRODUCTION

A plenty of studies supported the use of chromium supplementation in laying hens nutrition to improve the feed intake, growth performances, carcass quality, meat lipid profile and immune response (Sahin et al., 2001; Torki et al., 2014; Pogurschi, 2007; Uyanik et al., 2002; Ma et al., 2014; Du et al., 2005; Hanafy, 2011).

Plant feed ingredients commonly used in poultry diet contain small amounts of chromium and addition of this mineral may, therefore become a common micronutrient for animals in the future (Dikeman and Devine, 2004). Chromium deficiency cases in poultry are rare, the diet without chromium addition seem to be meeting their daily requirements. The NRC (1995, 1997) did not specify any recommendation of chromium in poultry diet. Chromium supplementation is required in difficult situations such heat stress, metabolic stress or fatigue. The first experimental data on chromium supplementation in the laying hens feed have been reported in 1970 by Hill and Matrone. Their studies support Cr supplementation in the laying hens diet to decreased the toxicity of dietary vanadium. The interest in the study of chromium and its supplementation in laying hens diets has increased considerably with the demonstration of its positive effect on egg quality. The first results for this purpose were reported by Jensen et al., in 1978. Data from different studies since then have been mixed. The influence of chromium supplementation on egg quality obtained from laying hens feed with different chromium sources have varied. The present review is a synthesis of the results reported in the literature on the forms of chromium administered, the doses and the optimal experimental periods for obtaining considerable effects in the growth of the laying hens. Several sources of chromium have been studied in poultry nutrition, but organic forms have proven to be more effective.

MATERIALS AND METHODS

This review consisted of a compressive analysis of the studies published in PUB Med between 1979 and present.
The search terms were: chromium and laying hens. For the two criteria used, the PUB Med database published a total of 21 published studies. The studies which included chickens (1) or quails (1) were excluded. Another study that did not refer to the proposed criteria but was listed in the database was also ruled out (this is the study of Choi et al., 1979, which used chromium oxide as marker to assess the phosphorus excretion of laying hens). Figure 1 shows the chromium sources used in feeding the laying hens in the published studies analysed.

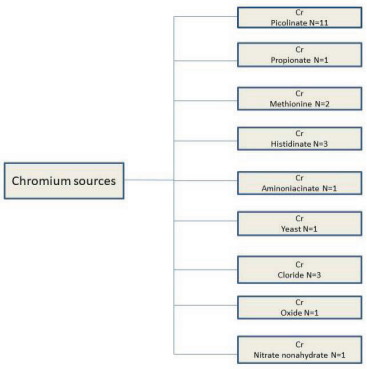

Figure 1. Chromium sources cited
Abbreviations: Cr-Chromium; N-number of studies cited chromium sources

Chromium doses, duration of the experimental periods, and the results obtained were also evaluated and analyzed (Table 1).

RESULTS AND DISCUSSIONS

Most studies used chromium picolinate (N = 11) while studies using inorganic chromium were very few and performed more than 2 decades ago (3-chromium cloride, 1- chromium oxide). Chromium histidinate (CrHis) was only used together with chromium picolinate (CrPic), the purpose of the research being to highlight the chromium source that led to the best results in production. Orhan et al. (2018) reported that the efficacy of Cr as Cr His was more notable than Cr as CrPic. At the same doses (1600 mg CrPic/kg of diet and 0,788 mg CrHis/kg of diet), at the same ages but when the laying hens were exposed to heat stress, both chromium sources were equally effective in alleviating performance variables under heat stress condition (Sahin et al., 2018). Moreover, Ozdemir et al. (2017) reported that the same doses and sources of chromium supported the relief and treatment of stress complications.
Comparing the bioavailability of organic and inorganic chromium forms associated with other mineral elements such asmanganese, zinc and copper, Yenice et al. (2015) reported a significant increase of Mn, Zn, Cu and Ca in the laying hens serum, when the minerals were administered in organic form. When the level of these elements was double in diet the Cr and Ca excretion was not affected. A combination of Mn (80 mg/kg diet), Zn (60 mg/kg diet), Cu (5 mg/kg diet) and Cr (0.15 mg/kgdiet), administrated in organic form showed a significant increase of egg content in Mn, Zn, Cu and Cr. In the eggshell only Zn and Cr concentration was notable.
The diet supplemented with the combination of chromium and vitamin C was also studied extensively by Mirfendereski and Jahanian (2015) and Torki et al. (2014). A combination of CrMet and acid ascorbic revealed beneficial effectsin terms of egg production and egg mass but when the CrMet was administered separately in the diet the egg production and feed conversion was improved (Mirfendereski and Jahanian, 2015).
The addition ofchromium propionate at 400 µg dose/kg diet has been found to improve egg production during the latter 4 weeks as reported Ma et al., 2014. At this dose, however, there was a decrease in the height of albumen, yolk color score and Haug unit. The addition of 600 µg chromium/kg diet as chromium propionate improve the shell thickness. The dose of 200 µg/kg diet only led to a 31% decrease in uric acid. A combination of CrPic and copper did not significantly affect egg production, egg weight, eggshell thickness and eggshell strength as Lien et al. reported in 2004. In previous investigation the same authors also showed the copper and chromium supplementation did not influence egg production (Lien et al., 1996; Chiou et al.,

1997). It was also reported a significant interaction between copper and chromium. Egg cholesterol was significant affected by copper in the sense of reducing that, while chromium supplements had no effect on this parameter. Very Low-Density Lipoprotein was significantly reduced when the laying hens were fed with diet supplemented with copper and chromium while High Density Lipoprotein was increased. The interaction between chromium and a microelement, this time zinc, has been studied by Onderci et al. in 2003. The reported results showed that diet supplemented with chromium or supplemented simultaneously with chromium and zinc increased significantly the digestibility of nutrients alleviating the negative effect of cold in laying hens. Reducing the effect of low temperature on the hens can also be done by supplementing the diet with chromium and vitamin C, as Sahin et al., reported in 2002a. The chromium source was chromium picolinate and the dose was 400 micrograms of Cr/kg diet. The authors did not specify the duration of the experimental period. Supplemental chromium and ascorbic acid increased serum vitamin C and E bud decreased malondialdehyde concentration (Sahin et al., 2002b).

Because of the growing interest in the use of chromium as a supplemental micronutrient in laying hens, Guerra et al., 2002 investigated whether high Cr(III) levels, fed in the form of chromium yeast (Cr-Y), chromium aminoniacinate (Cr-AN) or chromium chloride ($CrCl_3$), produces changes in the hepatic microsomal metabolism.

The activity of ECOD (anspecific marker of a number of CYPs-Cytochrome P-450) was significantly ($P<0.01$) reduced by $CrCl_3$ (~63% loss), Cr-Y (~35% loss) and Cr-AN (~54% loss). The diets supplemented with 50 ppm Cr/kg diet (different forms) administered to hens for a period of 28 days led to changes in the microsomal metabolism. The addition of half dose of chromium in diet (25 ppm/kg diet) did not change noticeably the microsomal metabolism. The doses of 50 ppm/kg diet (whatever the chromium source was) did not affect the egg production or egg quality.

Supplementing the diet with chromium and zinc, when the chromium dose was 400 micrograms CrPic/kg diet, has led to improved feed efficiency, egg production and even live weight of laying hens treated (Sahin et al., 2002). According to the authors serum glucose and cholesterol concentration decreased while protein concentration increased.

Table1. Doses, sources of chromium and duration of experimental period

Reference	Dietary Cr concentration/kg (source)	Duration of experimental period/Age
Witkowska et al., 2019	0,1g Enriched Soybean meal-Cr nitrate nonahydrate	12 wk/30 wk old hens
Orhan et al., 2018	1600 mg CrPic& 0,788 mg CrHis	12 wk
Zhang et al., 2018	0,4 mg and 0,6 mg CrPic	10 wk/23 wk old hens
Sahin N. et al., 2018	1600 mg CrPic& 0,788 mg CrHis	12 wk
Ozdemir et al., 2017	1600 mg CrPic& 0,788 mg CrHis	12 wk
Yenice et al., 2015	0,15 mg Cr oxide & 0,07 CrMet	16 wk
Mirfendereski et al., 2015	0, 500, 1000 ppb CrMet	12 wk
Torki et al., 2014	0, 200, 400 µg CrPic	8 wk
Ma et al., 2014	0, 200, 400, 600 µg CrProp	8 wk
Lien et al., 2004	0, 800, 1600 mc CrPic	28 days
Onderci et. al, 2003	0,4 mg CrPic	108 days
SahinK. et al., 2002a	400 µg CrPic	32 wk old hens
Sahin K. et al., 2002b	400 µg CrPic	32 wk old hens
Guerra et al,, 2002	25, 50 ppm CrY/25,50 ppm CrAN /25, 50 ppm $CrCl_3$	28 days
Sahin N. et al., 2002	400 µg CrPic	32 wk old hens
Sahin K. et al., 2001	100, 200, 400 µg CrPic	120 days/46 wk old hens
Ousterhout L.E. et al., 1981	20 ppm $CrCl_3$	10 days/50 wk
Maurice DV et al., 1979	10 µg/g $CrCl_3$	12 wk/40 wk old hens

Definitions of abbreviations used:CrProp-chromium propionate; CrMet-chromium methionine; CrPic-chromiumpicolinate; CrAN-chromium aminoniacinate; $CrCl_3$-chromium chloride; CrY-chromium yeast; CrHis-chromium histidinateWk-week

In the experiment conducted by Sahin et al., in 2001, 3 doses of chromium were used: 100, 200 and 400 µg CrPic respectively, but only the 200 µg CrPic dose has been shown to have

positive influence on egg production. In this case the increase in chromium dose of diet also led to a linear increase in live weight.

The authors also reported a linear increase in insulin concentration in plasma while corticosterone concentration decreased linearly. Zhang et al. (2018) stated that brown-egg laying hens diet supplemented for 10 weeks with 0.4 or 0.6 mg Cr/kg led to a significant reduction of serum glucose concentration and increased serum antibody titer against Newcastle disease. These doses of chromium (0.4 and 0.6 mg Cr/kg diet) have not been shown to have a positive influence on egg production performance and egg quality.

Researches to obtain eggs fortified with chromium but also with other microelements were carried out by Witkowska et al., in 2019. Eggs fortified with chromium could be used as a supplement for humans as an alternative for the currently used chromium picolinate (Witkowska et al., 2014).In the context in which the consumer has a skeptical attitude towards food additives (Zugravu et al., 2017), fortified products are a perfect alternative to reaching optimal levels of the various micronutrients essential for certain categories of population. The authors compared the results of basal diet to the results of a diet which include soybean meal enriched with Cr(III). The raw soybean meal from the basal diet had a chromium content of 0,022 mg/g and the soybean meal after biosorption had 20,588 mg/g. The soybean meal enriched with chromium did not significantly affect the weight of eggs. Statistically significant differences were observed between the control group and group with soybean meal enriched with chromium, where the egg shell strength was higher by 11.3% ($p=0.045$). Regarding the eggshell thickness, the authors reported a decrease after the first two series of the experiment but the differences were not statistically significant, however, compare to the control group the eggshell thickness was higher. A significant decrease in feed intake and feed conversion rate was observed after every series compare to the control group. These results may indicate that a biological form of chromium improved hens performances due to better feed conversion. After eating eggs obtained from laying hens fed with soybean meal enriched with chromium, the consumers indicated that eggs smelled more pleasantly.

Concluding, soybean meal enriched with chromium, influenced the content of chromium in the albumen, particularly due to the increased dosing. It is worth underlining that the chromium was accumulated primarily in the albumen, probably because chromium was supplemented in a form bound with protein. Michalak et al. (2011) documented that the diet of laying hens supplemented with two marine macro algae enriched with chromium compare to the diet where chromium was included in inorganic salt favoured the increase in the content of chromium in yolk and albumen.

The study of Ousterhout et al. (1981) revealed that addition of 20 ppm chromium to laying hens diet had no detectible effect in preventing the albumen quality deterioration caused by vanadium. The effect of chromium supplementation on albumen quality is contrary to other report (Jensen and Maurice, 1980). The differences between the obtained results are caused by the experimental periods that had different duration (4 to 6 weeks at Jensen and Maurice's experiences and no 2 weeks at Ousterhout's experience). In order to show a protective effect, chromium should be administered for more than 10 days in the laying hen’s diet.

Contrary to recent studies (Du et al., 2005; Sahin et al., 2002a) where the hypolipidemic effect of diet supplemented with chromium has been shown in laying hens, Maurice and Jensen (1979) reported no significant effect of chromium on liver fat and incidence of liver hemorrhage. The data on egg production, egg weight or body weight reported in the mentioned study do not provide evidence to demonstrate the positive effect of dietary chromium supplementation for these parameters.

CONCLUSIONS

In conclusion, after a thorough review of trials relevant to the issue, there are many reasons to recommend supplementation of chromium in laying hens diet. The organic chromium sources have proven to be more effective than the inorganic forms. CrPic has been used in

more than 10 researches. CrHis has been shown to be much more efficient compare to CrPic due to its higher bioavailability. Under heat stress, both sources had the same efficiency. When chromium was associated with various mineral elements, the production proved to be superior. The minerals in the organic forms associated with a chromium supplement in the laying hens diet led to significantly increased concentrations of these minerals in the egg. The chromium/vitamin C combination revealed beneficial effects in terms of egg production and egg mass. The egg shell thickness could be improved by adding 600 µg chromium propionate to laying hen's diet while 400 µg chromium propionate improve egg production. The chromium/copper combination in the laying hen's diet has been shown to have significant results on the cholesterol content of the egg. Changes in microsomal metabolism were observed in laying hens fed with chromium. The analysed studies have shown the beneficial effect of chromium on the feed efficiency, egg production and live weight. Recent research with laying hens has shown that supplemental chromium decreased serum glucose and cholesterol concentration. The evidences available indicates that supplemental dietary chromium can affect egg production, egg quality, well-being of poultry and even their metabolism. In order to be able to show the beneficial effects. The chromium should be introduced in the diet of laying hens for an experimental period of at least 8 weeks.

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ANTIBACTERIAL ACTIVITY OF NONI JUICE FRUIT (*Morinda citrifolia* L) ON PERFORMANCE AND HEMATOLOGIC INDICATOR ON SENTUL CHICKEN

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Abstract

Noni juice fruit is a natural antibiotic from herbal plants, and has an anthraquinone active as an antibacterial. The research was held to find out the effect of Noni juice fruit in the drink water on the performance and hematologic indicator on Sentul Chicken. The experimental used 120 day old chick Sentul chicken, with a Completely Randomized Design (CRD), with six treatments and four replications. The ration treatments were P0: control ration without Noni juice fruit, P1: Ration + 1 ml Noni juice fruit, P2: Ration + 2 ml, P3: Ration + 3 ml, P4: Ration + 4 ml and P5: Ration + 5 ml. Variables observed were water consumption, feed consumption, body weight, feed efficiency, and hematologic indicator of Sentul chicken. Statistical analysis showed that the treatment significantly affect (P<0.05) on body weight, feed conversion and hematologic indicator, but not significant effect on feed consumption, and water consumption. It can be concluded that treatment Noni juice fruit 3 ml/litre drinking water produced good performance of Sentul chickens.

Key words*: Noni juice fruit, performance, hematologic indicator, Sentul chicken.*

INTRODUCTION

Sentul Chicken is a local chicken found in West Java, especially in Ciamis Regency, which has the characteristic of gray and white feathers in general in addition to the combination of gray and yellowish brown color variations. The fur is arranged neatly on its chest like dragon scales, and the color of its scales is gray, white or yellow (Sartika and Iskandar, 2008; Widjastuti et al., 2017). Sentul chicken is susceptible to attack by pathogenic bacteria that often attack poultry including *Escherichia coli* and *Staphylococcus aureus*. The presence of pathogenic bacterial infections often causes disease in chickens, making livestock productivity often decline. To avoid infection due to these bacteria, antibiotics are generally given (Khusnan et al., 2008). Continuous use of antibiotics can cause residues in meat. Therefore the use of natural additive feeds (phytobiotics) in poultry feed can reduce the negative impact of antibiotics. Noni is one of the medicinal plants that can be used as phytobiotics. Noni plants have bioactive compounds such as anti-bacterial, anti-fungal and anti-oxidant (Singh, 2012). Noni fruit is able to activate the lymphosid follicles that are in the Fabricius exchange which function to produce lymphocyteswhich will differentiate into b-cells and plasma cells as anti-body producers (Razak et al., 2012). Poultry given noni fruit extract can boost productivity because residues can be avoided by utilizing Noni as a natural feed aditive (Hidayati, 2006). Noni fruit has secondary compounds which are very useful for the performance of poultry, containing anthraquinone compounds, alkaloids, and glycosides. This compound is found in Noni leaves and fruit whose main function is to overcome digestive and anti-bacterial problems (Solomon, 1999). Anthraquinone in Noni fruit ranges from 5-36 g/100 g of Noni dry ingredients, the anthraquinone content in Noni fruit is 1.20% higher than the anthraquinone content in aloe vera leaves (Bintang et al., 2008). These compounds are useful for

inhibiting the growth of Gram-positive and negative bacteria that can eradicate pathogenic bacteria in the digestive tract and also make the pH of the digestive tract become acidic which allows the protein-breaking enzyme to work optimally. In the study of Rahayu (2013) the use of 3 ml of Noni juice on poultry provided the best performance of edible weights. Subsequent research on the use of 2 ml of Noni juice in drinking water has a significant effect and low feed conversion of poultry (Sujana et al., 2009). Noni fruit as immunostimulant will improve body health through increasing the body's resistance which can be measured from hematological conditions including measuring erythrocyte levels, leukocytes, hemoglobin, hematocrit and blood glucose. Noni acts as an anti-oxidant includes scopoline, nitric oxide, vitamin C and vitamin A. Vitamin C in Noni plays a role in avoiding stress by inhibiting the increase of corticosteroid hormones from the adrenal gland, and can counteract free radicals, by protecting erythrocytes from free radicals causing an increase the percentage of erythrocytes in transporting hemoglobin which binds to oxygen so that the health of chickens increases (Barcley et al., 2000). In addition to having a positive nature, if Noni fruit juice is used continuously at high doses it can cause negative effects, because Noni juice contains polyphenol compounds that cause a feeling of tighten (Nurhayati et al., 2006). Chemical compounds contained in medicinal plants if given at doses that exceed the tolerance limit in the body of an animal will have negative impact on the performance of chicken organs (Vermurugan and Citarasu, 2010). The results of Fenita (2012) study that the use of Noni juice in drinking water at a dose of 3 ml/litre gave a real effect and low feed conversion of poultry. Based on the explanation, the purpose of research is to determine the effect of the use of Noni juice fruit in the drinking water on the performance and hematologic indicator on Sentul Chicken.

MATERIAL AND METHODS

The study used 120 DOC Sentul chickens with the average of body weight was 27.92 gram (coefficient of variation 8.0%). The Sentul chicken kept in cage until the age of 12 weeks. 24 cages were used and were measured as 90 cm x 90 cm x 60 cm (length x width x height), each cage consisted of 5 chickens. The Noni fruit variety used is *Morinda citrofolia* variety, contains a water content of about 89.10%. Noni fruit is washed and each peeled, then cut into small pieces and then blended without added water, filtered to separate fiber and liquid (Fenita, 2008). The liquid produced is then mixed into drinking water according to the treatment. The feed ingredients of ration comprised of yellow corn meal, soy-bean meal, rice bran, fish meal, $CaCO_3$ and bone meal. Rations were prepared based on protein and metabolic energy requirement for Sentul chicken growth phase, ie. 17% protein and metabolic energy 2850 kcal/kg (Widjastuti, 1996). The treatments were P0: control ration without Noni juice fruit/litre drinking water, P1: Ration + 1 ml of noni juice/1 litre of drinking water, P2: Ration + 2 ml of Noni juice/1 litre of drinking water , P3 : Ration + 3 ml of Noni juice/1 litre of drinking water , P4 : Ration + 4 ml of Noni juice/1 litre of drinking water and P5: Ration+ 5 ml of Noni juice/1 litre of drinking. The composition, nutrient and metabolizable energy contents are showed in Tables 1 and 2.

Table 1. The composition basal ration

The feed ingredients	%
Yellow Corn Meal	56.00
Soy-Bean Meal	12.00
Rice Bran	21.50
Fish Meal	9.25
Caco3	0.50
Bone Meal	0.75

Note: Ration from Academic Leadership Grant (ALG) 2015

Table 2. The nutrient and metabolism energy content in Basal Ration

Nutrients	
Crude Protein (%)	17.04
Crude Fat (%)	5.92
Crude Fiber (%)	4.51
Calcium (%)	1.16
Phosphorus (%)	0.36
Lysine (%)	1.21
Methionine (%)	0.40
Metabolizable Energy (kcal/kg)	2781

Experiments were conducted experimentally using Completely Randomized Design, consisting of 6 treatments and 4 replications. Data were analyzed using Variance Analysis and differences between treatments using Duncan Multiple Test. Variables observed were water consumption, feed consumption, body weight, feed conversion, and hematologic indicator of Sentul chicken

RESULTS AND DISCUSSIONS

Performance Sentul Chicken

The effect of Noni Juice Fruit on water consumption, feed consumption, body weight, and feed conversion Sentul chickens shown in Table 3.

From Table 3, it can be seen that the average consumption of drinking water given the treatment of the use of Noni fruit juice has decreased compared to the control treatment without Noni juice (P0). The results of the statistical analysis showed that the treatment using noni juice in drinking water had no significant effect ($P>0.05$) on drinking water consumption. These results indicate that the consumption of drinking water per treatment is in the same range. This condition indicates that Sentul chicken is tolerant of the taste and smell of Noni juice added to drinking water to a dose of 5 ml/litre. In Noni fruit has an active compound from the aromatic group which gives taste to drinking water. According to Wang (2004) in Noni fruit there is a content of tannin, capric acid and caprylic acid which have aromatic properties. This shows that Noni juice given to a dose of 5 ml/litre does not have a negative effect on Sentul chicken. In line with the research of Nurhayati et al., (2006) the use of Noni juice to a level of 10% in drinking water is safe to use for poultry.

Based on Table 3, feed consumption given the treatment of use of Noni juice tends to experience decline compared to the control treatment. The results of analysis of variance showed that the use of Noni juice in drinking water had no significant effect on the feed consumption. This means that the use of noni juice to a dose of 5 ml does not have a negative effect on feed consumption. Noni fruit has polyphenol compounds, tannins and saponine which can cause a feeling of tighten and rancidity, but because of the provision of Noni juice through drinking water so that it does not affect the ration palatability, consequently it does not affect the consumption of rations. Addition of Noni fruit juice did not significantly reduce palatability, because the noni fruit used a ripe fruit so that the taste of tighten has diminished. In accordance with the opinion of Nurhayati et al. (2006) stated that the level of polyphenols will decrease with the maturation of Noni fruit which is characterized by reduced taste.

From Table 3, it can be seen that the final body weight of Sentul chickens at 12^{th} week ranged from 670.80 - 874.88 grams. From the results of the variance analysis showed that drinking water added with Noni juice had a significant effect ($P<0.5$) on the final body weight Sentul chicken. The final body weight of the treatment P1, P2 and P3 had a higher average real body weight compared to treatments P0, P4 and P5. Noni juice contains antioxidants, anti-bacterial and additives that can improve the performance of the digestive tract of the poultry, so that it can produce higher body weight. Anti-bacteria found in herbal plants can reduce the growth of pathogenic bacteria in the intestine. Noni fruit

contains active ingredients anthraquinone, acubin and alizarin, these substances are useful for optimizing the performance of digestive enzymes in the poultry. (Bangun and Sarwono, 2002). Noni fruit also contains the proxeronase enzyme which will form an active substance in the digestive organ called xeronine. Xeronine will bully the enzymes to function more perfectly so as to optimize the absorption of nutrients so that it will produce a higher body weight. The decrease in body weight in treatments P4 and P5 was caused by the crude fiber content consumed too high so that the performance of the proxeronine enzyme was less optimal which caused body weight to decrease. This is in line with Nurhayati (2006) research in male chickens that the use of Noni fruit juice at the level of 5% through drinking water has decreased body weight,

Table 3 shows that the average feed conversion of Sentul chicken during the study ranged from 4.07 - 5.02. The results of the variance analysis show that silver has a significant effect on ration conversion. Giving Noni fruit juice at a dose of 1-3 ml/litre of drinking water has a significant effect on the value of lower ration conversion. The amount of ration consumption is not significantly different, but produces a different body weight, thus affecting the ration conversion value. This means that more efficient consumption of rations is used to produce higher body weight. The anthraquinone content in noni fruit can be consumed properly so that it will affect the ration conversion value. In addition, the content of l-arginine in Noni fruit helps to optimize ration conversion by increasing the relaxation of blood vessels so that the absorption of nutrients is optimal (Rahayu et al, 2013).

Effect of Noni Juice Fruit on Hematologic Value of Sentul Chicken Blood

Mean hematologic values (number of erythrocytes, leucocytes and haematocrit) of Sentul chicken can be seen in Table 4.

Table 3. The average of were water consumption, feed consumption, body weight, feed efficiency Sentul chickens

Variable	P0	P1	P2	P3	P4	P5
water consumption (ml)	7348.47 a	7234.85 a	7065.70 a	6777.20 a	6260.45 a	62350.45a
Feed Consumption (ml)	3796.47 a	3665.47 a	3490.10 a	3440.87 a	3345.40 a	3340.80 a
Body weight (g)	702.13 b	830.27 a	874.88 a	811.92a	680.86 b	670.80 b
feed conversion	5.02 b	4.04 a	4.02 a	4.06 a	4.83 b	4.88 b

Description: P0= 0% Noni Juice, P1=1 ml Noni Juice, P2= 2 ml Noni Juice, P3=3 ml Noni Juice, P4= 4 ml Noni Juice, P5= 5 ml Noni Juice. Mean values within a row having different superscripts are significantly different by least significant difference test.

Table 4. Effect of Noni juice fruit in the drinking water on number of erythrocytes, haemoglobin, leucocytes and haematocrit of Sentul chicken blood

Treatments	Number of Erythrocytes (items x 10^6)	Haemoglobin (g/dL)	Number of Leucocytes (item x 10^3)	The Haematocrit value (%)
P0	2.01 b	10.15 b	20.05 b	26.41 b
P1	2.67 a	12.57 a	23.65 a	29.53 a
P2	2.50 a	12.27 a	22.30 a	28.53 a
P3	2.67 a	12.37 a	23.32 a	29.82 a
P4	2.71 a	11.25 a	22.35 a	30.07 a
P5	2.59 a	12.47 a	22.42 a	31.42 a

Description: P0= 0% Noni Juice, P1=1 ml Noni Juice, P2= 2 ml Noni Juice, P3=3 ml Noni Juice, P4= 4 ml Noni Juice, P5= 5 ml Noni Juice. Mean values within a row having different superscripts are significantly different by least significant difference test.

Table 4 shows that erythrocytes from each treatment giving a Noni juice (P1, P2,P3, P4 and P5) a higher tendency of treatment without Noni juice (P0), erythrocyte ranges from 2.01 x 10^6-2.67 x $10^6/mm^3$, the amount of hemoglobin ranges between 10.25 - 13.85 g/dL, the number of Sentul chicken leucocytes ranges from 21.30- 23.45 x $10^3/mm^3$. From Table 4, it can be seenthat giving Noni juice through drinking water until the level of 5 ml/litre of drinking water (P1–P4) significantly increases the number of erythrocytes compared to P0 (control). This means that the giving of Noni juice through drinking water can be acts

antioxidant so that it can protect erythrocytes from free radicals so that erythrocytes can carry out its function of transporting hemoglobin which binds to oxygen. Noni as an anti-oxidant can capture free radical compounds by giving electrons from the -OH group so as to produce stable compounds, this leads to physiologically increased chickens (Barcley et al., 2000). Anti-oxidants are capable to protect membrane of erythrocytes from oxidation reactions, erythrocytes have the function of channeling nutrients to the body's tissues and carrying oxygen from the lungs to the tissues and carbon dioxide from the tissues to the lungs. The number of normal erythrocytes in chickens according to Bell, (2002) is about 3.0 x 10^6/mm^3. Blood leucocyte results of the study ranged from 21.30 - 23.45 x 10^3/mm^3 still in the normal range. The results of variance showed that the treatment had a significant effect on the number of leukocytes. This means that the addition of Noni juice in drinking water as an immunostimulant can increase the body's resistance through increasing leukocytes as the body's defense against infection. The function of leucocyte is to help the body fight various infectious diseases as part of the immune system. In line with the opinion of Smith (1988) that chicken blood leucocyte ranges from 16 - 40 x 10^3 /mm^3. Increasing the number of leukocytes in the treatment of Noni fruit juice indicates the body is able to fight infection. Therefore the administration of Noni juice can increase immunity so that it can act as a natural or herbal immunostimulant, which can increase the body's resistance against infection so that the physiology of chicken's health can be maintained.

Hemoglobin shows that the treatment has a significant effect ($P<0.05$) on the amount of hemoglobin. This means that the use of noni juice can increase metabolism so that the need for oxygen bound by hemoglobin increases. Blood hemoglobin levels describe their ability to raise oxygen for oxidation in the body's metabolism. According to Kusumasari et al (2012), the number of erythrocytes in normal conditions is positively correlated with hemoglobin levels which are when the amount of erythrocytes in the blood increases, the hemoglobin level also increases. The role of Noni juice as an antioxidant is able to protect erythrocytes from free radicals so that there is no damage to the erythrocyte membrane, this causes hemoglobin that is bound to erythrocyte can perform its role well. Chicken blood hemoglobin levels in the study were in the normal range, according to Jain (1993) normal hemoglobin in chickens was in the range of 7.0-13.0 g/dL, whereas according to Sturkie (2000) normal hemoglobin levels in chicken broiler blood were 9.8%.

The measurement results of Sentul chicken hematocrit values ranged from 27.53 to 32.41%. The amount of hematocrit treatment of noni juice through real drinking water ($P<0.05$) is higher than the control treatment. Haema-tocrit is the percentage of red blood cells in all blood volumes, where red blood cells are responsible for carrying oxygen from the lungs throughout the body . The components of Noni fruit are natural antioxidants derived from plants and are known to have an anti-oxidant effect in cells without side effects (Inano et al, 2000). Giving Noni fruit in drinking water can act as an anti-oxidant in counteracting free radicals, causing an increase in the percentage of erythrocytes which also shows the per-centage of haematocrit. Normal haematocrit values in chickens are 29.0-40% (Sturkie, 2000). The results of this study indicate that hematocrit levels are still within normal limits. Normal animals have a hematocrit value comparable to erythrocytes and hemoglobin levels.

CONCLUSIONS

1. The best performance of heat chickens (water consumption, feed consumption, body weight and feed conversation) were given Noni fruit juice 3 ml/litre of drinking water.
2. Noni fruit juice can be used until 5 ml/litre in drinking water without affecting chicken health (erythrocytes= 2.01 x 106 - 2.67 x 10^6/mm^3, leukocytes = 21.30 - 23.45 x 10^3/mm^3, hemoglobin 10.25 - 13.85 g/dL, and hematocrit = 27.53-32.41%.) and Noni juice can be natural antibiotics from herbal for Sentul chicken.

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RESEARCH ON THE INFLUENCE OF SOME PROBIOTICS ON THE PRODUCTION PERFORMANCE OF BROILER CHICKENS

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Abstract

The objectives of this research consist describe the principles mechanisms of action, selection and synthesizing criteria of probiotics and their application in poultry industry. Biotechnology plays a vital role in the poultry feed industry. In broiler chickens feeding, there have been tested probiotics species belonging to Enterococcus faecium NCIMB 11181 and Enterococcus faecium NCIMB 10415 to reduce broiler chickens mortality and increase body weight and started with products that need to be tested and verified under these conditions. The experiment was initially performed on two successive series of broilers. The birds were weighed at the beginning of the experiment. Day-old Ross 308 broiler chickens (n=484) unsexed were distributed in three halls, in separate compartments. The data were analysed using SPSS 20.0 software (SPSS Inc., Chicago, IL). Statistical differences among means of the treatments were compared using the Tukey's multiple test. Comparisons were considered statistically significant at P≤ 0.05.

Key words*: broiler, probiotics, parameters, testing.*

INTRODUCTION

The origin of probiotics dates back to 1930, when Metchinikoff's studies described the beneficial effects of the use of lactobacilli from yoghurt by human beings. The term of "probiotic" was first used by Lilly and Stillwell in 1965 and then by Parker in 1974 in order to define: "the organisms or the substances which contribute to the intestinal microbial balance" (Vaubelle et al., 1990). Fuller (1989) also defined probiotics as being feed additives based on live microorganisms (bacteria, yeasts, moulds) which have a beneficial effect on the intestinal microbial balance of the animal organism.

Microorganisms used in animal feed as probiotic products may contain one or more bacterial strains. (Dumitru et al., 2018). In the European Union (EU) microorganisms added as feed supplementation are bacterial strains, often Gram-positive belonging to the following genus: *Bacillus (B. cereus* var. *toyoi, B. licheniformis, B. subtilis), Enterococcus (E. faecium), Lactobacillus (L. acidophilus, L. casei, L. farciminis, L. plantarum, L. rhamnosus), Pediococcus (P. acidilactici), Streptococcus (S. infantarius)*. Probiotic bacteria used in animal nutrition prevent digestive disorders or increase the zootechnical performance (Agawave et al., 2004). Also, probiotic bacteria stimulate the endogenous microorganisms which are able to modify the intestinal microbiota in order to increase the health status and improve feed efficiency (Alkhalf et al., 2010). Probiotic used in animal nutrition improved parameters such as: feed conversion by efficiency of improving intestinal microflora, the growth of non-pathogenic bacteria suppression of growth of intestinal pathogens and accessory for digestion and nutrients utilization (Pană, 2000). (Drăgan et al., 2016). Recently, it was shown that adding of probiotic containing *Enterococcus faecium* microorganism to broiler diets increased the jejunal villus height and ileal villus height. Moreover, increased intestinal villi height was reported after addition of *Bacillus subtilis* in association with prebiotics. **(**Wageha Awad et al., 2008).

It is assumed that an increased villus height is paralleled by an increased digestive and absorptive It is assumed that an increased villus height is paralleled by an increased digestive and absorptive function of the intestine due to increased absorptive surface area, expression of brush border enzymes and nutrient transport

systems. It is well known that many substances can affect the intestinal villi development.
This study was to test two variants of probiotics to reduce broiler chickens mortality and increase body weight.

MATERIALS AND METHODS

The experiments were carried out in Medgidia, the number 1 farm Avicola Medgidia, which has a number of 13 halls with a capacity of 18,000 chickens per hall, the population density being of 18 chickens per metre for all the halls (Figure 1).
The experiment was initially performed on two successive series of broilers. Broilers come from the European Union, Gradus Bulgaria and also weighed on entering of the experiment. Day-old Ross 308 broiler chickens (n=484) unsexed were distributed in three halls, in separate compartments. The climatic conditions and lighting program were computer-operated and followed the commercial recommendations. The halls in which it was run experimentally were chosen to ensure fast access and to closely monitor the experiment, at the same time the chicks that entered the experiment were selected and weighed
Research has begun on the need to eliminate early-life mortality and increase body weight, in broiler chickens and started with products that need to be tested and verified under these conditions. The products to be tested to colonize the intestine, stimulate the growth of a beneficial microflora, and inhibit the growth of pathogenic germs (*Escherichia coli*, *Pseudomonas*, *Salmonella*, *Clostridium*).
The *Enterococcus. faecium* NCIMB11181 preparation used in this study was a commercial product purchased from Romvac România, which contained a total bacteria count 100 000 UFC/mL. The commercial probiotic used in this study was based on two *Enterococcus* strains: *Enterococcus faecium* NCIMB 11181 (Bioenterom-Romvac) and *Enterococcus faecium* NCIMB 10415 (Commercial product-Romvac). The probiotic was administrated in the drinking water of broiler using an automated dispensing system for medication dosing.
The experiments were assigned to 3 groups: one control pool and two testing pools with *Enterococcus faecium* NCIMB 11181 (Bioenterom-Romvac) and *Enterococcus faecium* NCIMB 10415 (Commercial product-Romvac).
The data were analysed using SPSS 20.0 software (SPSS Inc., Chicago, IL). Statistical differences among means of the treatments were compared using the Tukey's multiple test. Comparisons were considered statistically significant at $P \leq 0.05$.
During the first 3 days of life, chickens receive in the drinking water antibiotics as Enrofloxacina 10% (1 ml/L) and Colicrid (0.5 ml/L). *Enterococcus faecium* was not administered to the first group.
For the following 3 days, the probiotic is administered in a dose of 0.6‰ of second group and the third chicks group.
The chicks were feed with the starter diets from days 1 to 14 and grower feed from day 15 to 31 and finishing feed from day 32 to 35 days, also chicks were weight individually at the beginning of the experiment as well as at the end of feeling period at day 35. Once were weighed and the dead chickens.
The chickens were monitored daily, removed the dead where appropriate, weighed 7 days, 14 days, 21 days, 28 days and 35 days.

Figure 1. Hall presentation

RESULTS AND DISCUSSIONS

Probiotics have been proven to be beneficial for broiler breeding *Enterococcus faecium* is a *Lactobacillus* genus that shows many positive effects on broiler growth.
Drawing a comparison between the results obtained from differences resulted in relation to the body weight evolution and the mortality percentage.
In Tables 1 and 2, Figures 2 and 3 are presented the parameters during the experimental period

Table 1. Average weight of chickens at set time intervals (g) - series 1

Strain	0 days	7 days	14 days	21 days	28 days	35 days
Witness lot	42± 0.047	189± 0.047	489± 0.05	932± 0.049	1461± 0.047	1885± 0.049
NCIMB 10415	42± 0.043	194± 0.044	510± 0.04	952± 0.04	1490± 0.038	1969± 0.039
NCIMB 11181	42± 0.04	206± 0.035	520± 0.035	961± 0.037	1499± 0.037	1985± 0.038

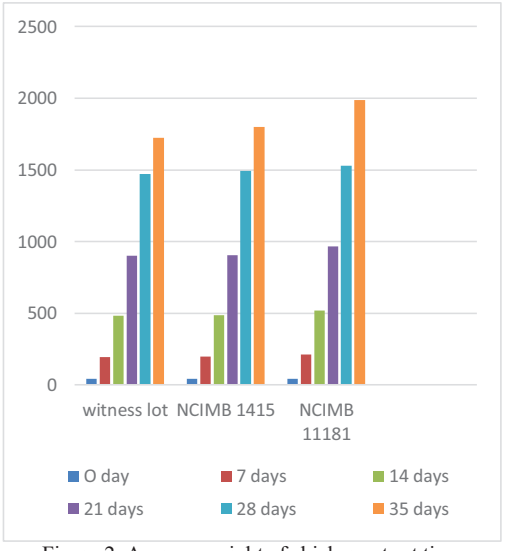

Figure 2. Average weight of chickens at set time intervals (g) seria 1

Table 2. Average weight of chickens at set time intervals (g) - series 2

Strain	0 days	7 days	14 days	21 days	28 days	35 days
Witness lot	42± 0.047	187± 0.0014	500± 0.007	927± 0.031	1469± 0.139	1849± 0.31
NCIMB 10415	42± 0.043	194± 0.0006	510± 0.005	962± 0.029	1520± 0.155	1920± 0.119
NCIMB 11181	42± 0.04	194± 0.0013	520± 0.005	983± 0.027	1569± 0.06	1986± 0.174

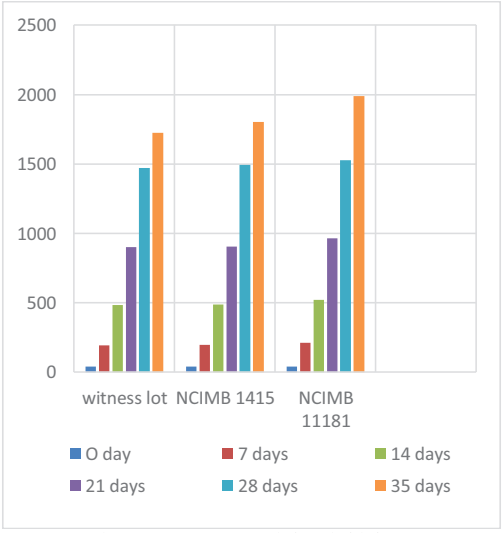

Figure 3. Average weight of chickens at set time intervals (g) series 2

Table 3. Evolution of mortalities (%) series 1

Strain	7 days	14 days	21 days	28 days	35 days
Witness lot	0.81± 0.013	1.25± 0.08	1.69± 0.015	2.12± 0.016	2.38± 0.09
NCIMB 10415	0.72± 0.017	1.01± 0.036	1.42± 0.037	2± 0.06	2.23± 0.012
NCIMB 11181	0.51± 0.013	0.74± 0.015	1.15± 0.014	1.45± 0.05	1.91± 0.06

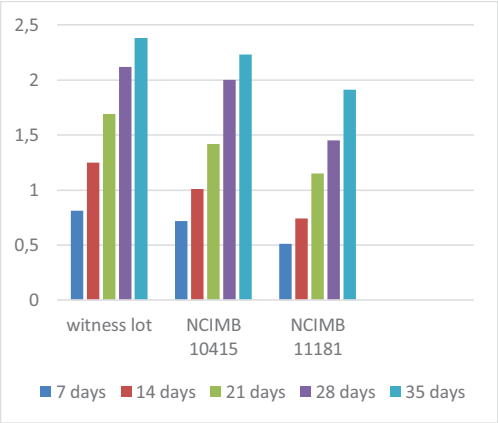

Figure 4. Evolution of mortalities (%) series 1

Table 4. Evolution of mortalities (%) series 2

Strain	7 days	14 days	21 days	28 days	35 days
Witness lot	0.92± 0.021	1.25± 0.09	1.69± 0.015	2.12± 0.07	2.58± 0.1
NCIMB 10415	0.66± 0.0174	0.91± 0.03	1.38± 0.037	1.97± 0.04	2.12± 0.01
NCIMB 11181	0.41± 0.01	0.74± 0.011	1.15± 0.015	1.45± 0.045	1.88± 0.056

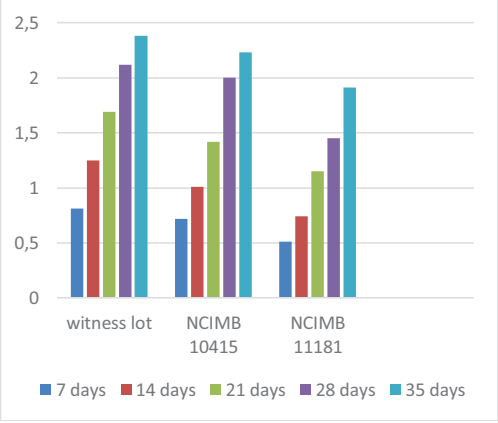

Figure 5. Evolution of mortalities (%) series 2

It`s significant differences between experimental groups was registered in term of BW ($P \leq 0.05$).

Probiotics utilisation with strains of *Enterococcus faecium* NCIMB 11181 ensures the fast colonisation of the gastrointestinal tract (GIT) with a beneficial flora having a 18-minute/generation multiplication rate, blocking the cell receptors in the intestine for pathogenic bacteria.

Stabilizes digestion, increases feed conversion and absorption of nutrients, increases weight gain, stimulates intestinal barrier.

Also, these probiotics assure an efficient fight against diarrhoea, eliminating toxins, *Escherichia coli* and *Salmonella* from the gastrointestinal tract, offer protection of the intestinal mucosa by forming a biofilm at this level and a and involve an increased antibiotic resistance.

On the other hand, our current results showed that dietary inclusion of probiotics exerted a similar effect in improving growth performance of starter broilers compared with the antibiotic treatment. Enhanced growth performance of broilers receiving dietary antibiotics depends largely on consequent reduction of the microbial population of the alimentary tract that competes with the host for nutrients.

In addition, the previous studies suggested that antibiotics work as growth promoter probably by inhibiting the production and excretion of catabolic mediators by intestinal inflammatory cells, and the subsequent reduction in intestinal microflora.

In contrast, probiotic supplementation modulated the gut environment and enhanced gut barrier function via the fortification of the beneficial members of intestinal microflora, the competitive exclusion of pathogens, and the stimulation of the immune system. Therefore the mode of action for the probiotics differs from that of antibiotics in birds, although both of them could improve the performance of starter broilers.

Figure 6. Farm presentation

Enterococcus faecium produces organic acid which entails the decrease of the pH level in intestines and creates an unfavourable environment for the multiplication of some pathogenic bacteria.
Enterococcus faecium stimulates the defence mechanisms of the animal organism by increasing the antibody level and enhancing the activity of macrophages. Administration of probiotics as growth stimulators is performed for long periods of time as they have a relatively short action time.

CONCLUSIONS

The use of the probiotic product based on *Enterococcus faecium* NCIMB 11181 strain had the following advantages: increased weight rates, decreased number of mortality losses, lower price as compared to that of similar products. The *Enterococcus faecium* NCIMB 11181 strain was efficient by increasing the growth performance, in production and digestibility.
It may be concluded that including probiotics (*Enterococcus faecium* NCIMB 11181) in the feeding of broiler chickens is effective for the immune status.

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NUTRITION CONSULTANT BASED ON MACHINE LEARNING FOR PREECLAMPSIA COMPLICATIONS

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Abstract

The current study aims to advise the alimentation of pregnant women who suffer from preeclampsia, because we live in a society which is characterized by speed and an impact needs to be done over the nutritional behaviour of the women expecting babies. Pregnant women should take care of their alimentation and acknowledge the health consequences which might appear. Fast food and soft drinks have to be avoided for a healthy life style, as well as during pregnancy. The statistical data regarding the nutrition and medicine for the prevention and treatment of preeclampsia is retrieved from the World Health Organization. In the paper have been analyzed several trials done on a number of persons which varied according to the comparison criteria. Machine learning is used for the pregnant women to assess their dietary habits based on their daily meals and taken medicine descriptions which are written in natural language. As a conclusion, salt restriction, antioxidants, calcium, vitamin D supplementation, antiplatelets have been some of the most frequent recommendations.

Keywords*: preeclampsia, nutrition, consultant, natural language processing, machine learning.*

INTRODUCTION

We live in a society where everything develops fast and people should be aware of their nutritional behaviour in order to be healthy. Pregnant women need to change their alimentation and be conscious regarding the health evolutions which can appear.

Fast food and soft drinks have to be excluded from the alimentation, mostly during pregnancy. A study done on 200 pregnant women from Downey, California, United States, reported that 96% of them did eat fast foods, namely burgers, French fries, chicken (Santiago et al., 2013). As a soft drink, Cola was consumed by 60.2% of the studied persons. The prenatal diet which contains mostly fast food endangers the fetus, because the level of fat and salt increase. In this case, the offspring is most likely to develop obesity in life and have altered genes that promote the eating behaviour (Tamashiro et al., 2009).

The content of caffeine from Cola can provoke miscarriage (American Pregnancy Association, 2019). Based on a study done at the Aarhus University Hospital from Denmark, high levels of caffeine promoted the occurrence of stillbirth after the second trimester (Wisborg et al., 2003). Based on studies done on first-time mothers, the consumption of vegetables and fruits is low (Wen et al., 2010). A poor nutrition leads to cardiovascular diseases, diabetes, cancer and weight gain (World Health Organization, 2019).

A 10 years study performed in Norway based on the New Nordic Diet which consisted on eating the main meals, as well as consuming fruits, root vegetables, cabbage, potatoes, whole grain bread, oatmeal, fish, milk and drinking water six times more than sugary drinks (Hillesund et al., 2014). The outcome of respecting such a diet was a lower risk of preeclampsia (Hillesund et al., 2014).

52% of the pregnant women prove to have iron deficiency (Abu-Ouf et al., 2015). Iron influences the haemoglobin value. Based on a study done in India at the Navodaya Medical College, a high maternal haemoglobin value is associated with a greater risk of developing preeclampsia (Manjunatha et al., 2015).

A high intake of fat, sodium and a low consumption of fruits, fibers, vitamin A, C and olive oil influence the occurrence of preeclampsia (Yusuf et al., 2019). Magnesium

taken from dairy products, bread, cereals, vegetables and meat, as well as supplements which contain it, reduce the risk of fetal growth restriction and preeclampsia (Zarean et al., 2017).

In the next section is presented a study done on the nutritional ingredients, along with the paper's algorithm which suggests the best diet. The later mentioned feature is done using the nutritional ingredients, their quantities and the list of medicines which are taken by the pregnant women. Section 3 describes the obtained results. The last section outlines the conclusions and the future work.

MATERIALS AND METHODS

In 2018, the World Health Organization performed a study which comprised 11 trials, having in total 5162 pregnant women (World Health Organization, 2018). The study demonstrated that diet and exercises prevent hypertension in pregnancy. A healthy diet comprises an intake of energy, vitamins, proteins, minerals in adequate quantities.

Several nutritional ingredients are analyzed in Table 1 according to the maximum advised quantity, along with their advantages and overdose disadvantages.

Table 1. Prevention and treatment of preeclampsia

Nutritional ingredient	Quantity	Advantages	Overdose problems
Alpha-linolenic acid (Pan et al., 2012; Phang et al., 2019)	< 1.10g/day	Lower risk of cardiovascular disease	Preeclampsia, higher infant birth weight
Salt (Scaife et al., 2017)	5g/day	Helps the thyroid, maintains the body hydrated	Hypertension, plasma volume expansion
Caffeine (American Pregnancy Association, 2019; Wisborg et al., 2003; Morgan et al., 2013)	300 mg/day	Treats headache, is efficient for weight loss, stimulates of the central nervous system	Miscarriage, stillbirth
Organic vegetables (Torjusen et al., 2014)	Up to 95% of the consumed food per day	Low risk of atopic diseases, reduced risk of preeclampsia	Higher hemorrhagic stroke risk
Fruits (Brantsaeter et al., 2009)	> 255 g/day	Reduced risk of preeclampsia	High level of fructose, can lead to diarrhoea
Fibres (Qiu et al., 2008)	>= 21.2 g/day	Reduced risk of preeclampsia, attenuate dyslipidemia	Bloating, gas, constipation
Fish (Imperial College London, 2019)	2 portions/week	Low high blood pressure	Depending on the fish origin, can lead to mercury poisoning, vomiting, diarrhoea, cramps, headaches, fatigue, fainting
Dairy products (Miller et al., 2019)			
Calcium	500 mg/day	Bone calcium balance, low risk of preeclampsia	Creates kidney stones, weaken the bones
Iron (Fisher et al. 2017)	1040 mg/day	Creation of extra blood, moves oxygen from lungs to the entire body	Vomiting, diarrhoea, pain, dehydration
Water (Parent, 2012)	2 L/day	Physical and mental health stability, normalized cholesterol and blood pressure	Nausea, vomiting, headache, low level of sodium, weakness, seizures
Olive oil (Assaf-Balut et al., 2019)	40 mL/day	Less urinary tract infections, low risk of premature birth	Weight gain, vitamin E overdose
Vitamins			
Folic acid (Liu et al., 2018; Slomski, 2018)	800 µg/day	Reduced risk of preeclampsia	Does not prevent preeclampsia
Vitamin A (Maia et al., 2019)	800 µg/day	Growth of embryonic cells	Drowsiness, irritability, vomiting, blurry vision
Vitamin C (Fu et al., 2019)	1 g/day	Reduced risk of prelabour membrane rupture	Diarrhoea, vomiting, heartburn, cramps, headache, insomnia

Through antioxidant, anti-inflammatory or vasoactive proprieties, micronutrients are good candidates for preeclampsia prevention.
Micronutrients are represented by essential elements which are needed by the body in small quantities, namely vitamins and minerals.
Based on a study done on 65,220 pregnancies in Denmark, the alpha-linolenic acid which is an omega 3 fatty acid, proved to be present in the alimentation of the women who suffered from severe preeclampsia (Arvizu et al., 2019).
A study done in Netherlands proved that caffeine determines the increase of the systolic blood pressure during the first and third trimester, but it does not determine the presence of elevated diastolic blood pressure values (Bakker et al., 2011).

According to the Danish National Birth Cohort study which consisted on 55,138 pregnant women who were analyzed, it was observed that a diet of vegetables and fish decreased by 21% the risk of preeclampsia development (Imperial College London, 2019). Adequate intake of calcium taken from dairy products proved that the risk of preeclampsia occurrence reduced by 62% (Miller et al., 2019). The calcium intake (> 1 g/day) may reduce the risk of preeclampsia in women with low-calcium diet (Cormick et al., 2019).
Multivitamins proved to decrease the risk of preeclampsia, the confidence interval reaching 95% for evaluating the connection between the supplementation with folic acid and the risk of developing preeclampsia (Liu et al., 2018).
The folic acid can also be given to pregnant women, being the created form of folate which is a B vitamin. The intake of folic acid before and at the beginning of the pregnancy proved that it might lead to giving birth to infants with neural tube defects. Due to this cause, the dose has been lowered to 400, up to 800 µg per day (Dolin et al., 2018). Folic acid deficity and anemia influence the occurrence of stillbirths (Yakoob et al., 2009).
The iron quantity needed during pregnancy is cause by the requirements of the fetus (270 mg/day), the placenta (90 mg/day), the loss of the iron in the maternal body (230 mg/day), the expansion of the maternal red blood cell (450 mg/day) (Fisher et al. 2017).

SmartCarb is a mobile system which assists the pregnant women who suffer from gestational diabetes to monitor their diet (Hu et al., 2018). The application uses deep learning neural networks for recognizing food images which were taken using the mobile phone's camera. After the image is analyzed, the value of carbohydrates is displayed and in this way, the amount of food which should be consumed is suggested.
Another web and mobile application solution comprises a corpus for machine learning that diagnoses and offers health tips based on surveys that are completed by pregnant women (Saranya et al., 2017). The corpus comprises the previous responses taken from doctors and dietitians. The solution uses decision trees and cluster analytics which are part of supervised machine learning.
The solution of the current paper consists in an application which advices the diet of the pregnant women who suffer from preeclampsia. The algorithm takes as input from the pregnant women the list of food ingredients, along with their quantities and the list of medicines which are taken by them.
The outcomes determine the classification of the persons based on their alimentation into healthy diet, being grouped into two subsequent batches corresponding to true positive and false negative diagnosis. There is also the case when the users were detected as not having a healthy diet, being grouped into two batches corresponding to true negative and false positive diagnosis.
The Viterbi machine learning algorithm was used to assess if the diet of the pregnant women who suffer from preeclampsia is healthy or not. The algorithm computes the most possible path through the use of the hidden Markov model series (Brown et al., 2010). The input dataset comprises the nutritional ingredients, their quantities and the list of medicines which are taken by the pregnant women. The states of the hidden Markov model contain the probabilities for emissions and transitions.
Initially, the sequence contains the states when the person who is ill because of preeclampsia. The Viterbi algorithm creates new states that close to the ones of the provided input. The most similar sequence of states is calculated up to the point when the final state is reached. This

is done by utilizing the transitions from the state which was before and the most probable one that results, as well as the probability of the noticed state which is related to a hidden state.
Every transition state determines the computation of a triple that consists of the probability of the entire paths from the start state until the reached one, the Viterbi sequence and the state's probability.
The probability of the following state is computed by adding the probabilities of the entire paths that go to itself. Each source state has the total probability calculated for the entire paths which lead to itself. The triggered value is multiplied by the probability of the emission if the present state changed and the transition probability beginning from the source state that goes to the following one.
Preeclampsia and nutrition knowledge influence the process of training when the source states are determined. According to the tuples which have information about the consumed nutritional ingredients, their quantities, and the list of medicines which are taken by the pregnant women, the algorithms determines a sequence of observations that have been created by the most probable states. The following formulas are used:

$$M_{1,healthy} = P(y_1|healthy) * InitP_{healthy}$$
$$M_{t,healthy} = max\ (P(y_t|healthy) * T_{x,healthy} * M_{t-1,x}) \quad (1)$$

where $M_{1,healthy}$ is the probability of the most probable initial state which depends on the probability of being healthy, $InitP_{healthy}$. $M_{t,healthy}$ belongs to the probability of the most probable state sequence $S(x_1, ..., x_t, y_1, ..., y_t)$ where the states are x and the observations are y for the first t observations which have healthy as its final state, namely the right diet. $T_{x,healthy}$ is the transition matrix that contains the transition probabilities from a state x towards the final state, healthy diet. The triggered sequence of states is the one which contains the greatest values for $M_{t,healthy}$, where t takes values from 1 to healthy.
The pregnant women who suffer from preeclampsia have an elevated blood pressure, respectively over 140/90 mm Hg. An increased sensitivity is useful for the early detection of an healthy diet. According to this, the doctor can decide upon the alimentation of the pregnant woman.
The proposed algorithm for detecting a healthy diet for the pregnant women who suffer from preeclampsia has two stages. At the first step are determined the optimal number of hidden Markov model states. The next step contains the addition of the new hidden Markov model states by applying the Viterbi algorithm and the event log which contains the tuple of ingredients, their quantities and the list of medicines which are taken by the pregnant women. Every event can be assigned to multiple states. A hidden subprocess is represented by a hidden Markov model state. An event is a consideration of the subprocess. A group of hidden subprocesses realizes a complex system which is a deep neural network that represents the full target system utilized for the early detection of healthy diet for the pregnant women.

RESULTS AND DISCUSSIONS

The current paper utilizes approximate values of the hidden Markov model and the novel determined metrics are included to evaluate the transition values.
For demonstration purposes, the input sets were split into groups, like the data which belongs to the patient who visit a clinic from Bucharest, Romania. The input sets contain information about the nutritional ingredients, their quantities and the list of medicines which are taken by the pregnant women. According to this, the healthy diet is determined. The control batch contained 10 sets and the experimental batch had 100 sets.
It was considered that a part of the pregnant women participated actively. On the other side, 6 of them, had incomplete presence and they have been excluded. For the rest of the pregnant women who participated actively, namely 104 persons, there are two cases when the detection of the healthy diet has been determined as being positive, as well as negative. The positive detected pregnant women have been divided into two groups: true positive (TP) for 38 persons and false negative (FN) diet identification for 2 persons. It has been noticed that the women who had a diet characterized by salt restriction, antioxidants, calcium, vitamin D supplementation, and the use of antiplatelets, the healthy diet was most

likely to be identified. Similarly, for the negative detected pregnant women are other two cases: true negative (TN) for 45 persons and false positive (FP) diet identification for 19 persons.

For the group of 104 pregnant women, the overall accuracy was of 79%, with a sensitivity of 95% and a specificity of 70%. The obtained values are better compared to the ones triggered by the detection of seizures for pediatric cardiac arrest (Du Pont-Thibodeau et al., 2017), having the sensitivity equal to 77% and the specificity equal to 65%. The results demonstrate that the described model is good for determining a healthy diet for the pregnant women who already suffer from preeclampsia.

CONCLUSIONS

In this paper has been described the Viterbi algorithm for determining a healthy diet for the pregnant women who have preeclampsia. This was done using the list of food ingredients, along with their quantities and the list of medicines which are taken by them.

The presented solution allows the user to analyze the evolution of their diet. The proposed healthcare solution for the observation of the diet changes enhances the user's quality of life and the probable unwanted outcomes can be avoided successfully. The results are encouraging for the analyzed control and experimental batches.

The next step of the presented work is the enhancement of the early automatic detection of gestational diabetes. This unsupervised detection will be good in the future for the staff from the healthcare domain.

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ENRICHING THE DIET IN POLYUNSATURATED FATTY ACIDS FOR LAYING HENS USING FLAXSEED MEAL AND RICE BRAN

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Abstract

Vegetable raw materials rich in polyunsaturated fatty acids, especially in α linolenic (C18:3n3) are represented by flaxseed meal (51.67 ± 0.062% α-linolenic acid) and rice bran (1.79 ± 0.023% α-linolenic acid). A 6-wk study was conducted on 80, Tetra SL layers (38 weeks) assigned to two groups (C and E), to evaluate the effect of using raw materials rich in PUFA in layer diets, on layer performance. Hens were housed in an experimental hall under controlled environmental conditions, in 3-tier batteries (4 layers/cage), and 16h/24 h light regimen. The commercial (C) diet had 2.800 kcal ME, 17.8% CP and 0.885 gα - linolenic acid/ 100 g total FAME. Unlike for diet C, the experimental (E) diet formulations used 2.5% flaxseed meal and 10% rice bran which increased the concentration of α-linolenic acid (4.575 g/100 g total FAME) in this diet. The average daily feed intake, feed conversion ratio, laying percentage and egg weight were monitored throughout the experimental period. Eighteen eggs per group were collected during the final experimental day, and physical measurements were performed on the eggs. Daily feed intake (121.088 g/day/hen) and feed conversion ratio (1.965 kg feed/kg egg) were not influenced by using flaxseed meal (2.5%) and rice bran (10%) in laying hens diet compared with the C group. Also, the laying percentage was higher (P>0.05%) at the E group (95.23%) compared to the C group (94.4%).

Key words*: fatty acids, flaxseed meal, rice bran, laying hens, zootehnical performances.*

INTRODUCTION

The current increase of severity and incidence of degenerative diseases in humans, associated with stress, is largely attributed to the exhaustion of omega-3 fatty acids and antioxidants from the modern diet. Hens eggs are considered a functional food, since they are a source of high-quality proteins, vitamins, minerals and lipids, such as phospholipids and polyunsaturated fatty acids (PUFA) (Nau et al., 2010; Zdrojewicz et al, 2016; Heflin et al., 2018; Marin et al., 2011). The use in feed of laying hens, of plants rich in polyunsaturated fatty acids is a natural way to obtain a qualitative enrichment from the nutritional point of view, by modifying the nutritional components of the egg (Narahari et al., 2005; Benakmoum et al., 2013). In general, oilseeds and their meals are incorporated into the bird ratios both as a source of omega 3 PUFA and for their energy-protein content (Aziza et al., 2010). Of these, flax *(Linumus itatissimum L.)* represents the most widely used raw material included in the diet of laying hens to obtain eggs enriched in omega 3 PUFA (Al-Nasser et al., 2011; Yassein et al., 2015). Generally, flaxseeds are a valuable source of fat contains about 34% oil and has a high content of ALA (>50%), particularly PUFAs (Cherian and Quezada, 2016). The use of flax, in different forms, in animal feed, has led to an increase in the level of PUFA ω-3 acids in food of animal origin (Criste et al., 2009; Panaite et al., 2016). Another source of feed, with a high fat content but with antioxidant properties, is rice bran (Rohrer and Siebenmorgen, 2004; Sumantha et al., 2006). Being rich in lipids, it degrades very easily, which is why enzymatic inactivation of lipase by short-term heat treatments is necessary (Paucar-Menacho et al., 2007; Simone et al., 2012).Unfortunately, feed and foods enriched in PUFAs are exposed to rapid deterioration of nutritional and organoleptic qualities due to the oxidation of double carbon bonds, specific to the molecular structure of PUFAs. Lipid oxidation products have harmful biological effects (Schroepfer, 2002) and

therefore, it is important not only to improve the nutritional value of the feeds but also to minimize the lipid oxidation (rancidity) to provide healthy foods, pleasant to smell and taste (Hayat et al., 2010; King et al., 2012). As an undesirable effect, the enrichment of eggs in PUFA ω 3 leads to the propagation of lipid oxidation processes in egg yolk (Promila et al., 2017; Saracila et al., 2017). This is why PUFA-rich diets have to be supplemented with antioxidants (Galobart et al., 2001). Dietary supplementation with antioxidants is one of the most effective ways to minimize lipid peroxidation in egg yolk, because these compounds are transferred to egg yolk (Sahin et al., 2010; Nour et al., 2018; Panaite et al., 2019).

The purpose of this study was to evaluate the effects of using in laying hens feed, a diet enriched in PUFAs, by using flaxseed meal and rice bran, on the production performance and nutritional quality of the eggs obtained.

MATERIALS AND METHODS

Birds and Housing

The feeding trial was conducted in the experimental halls of The National Research-Development Institute of Animal Biology and Nutrition (IBNA-Balotesti, Romania) according to a protocol approved by the Commission of Ethics of the institute. A 6-wk study was conducted on 80, Tetra SL layers (38 weeks). The hens were weighed individually and assigned to two groups (40 hens/group) depending on their body weight. The layers were housed in an experimental hall under controlled environmental conditions, in 3-tier cages (4 layers/cage) which allow the daily recording of the ingesta and leftovers. The environmental conditions in the hall was according to Tetra SL layers breeder guide (temperature: 23.12±2.03°C and humidity: 44.83±6.20%). The light regimen (16 h daily) was according to the prescriptions for the particular category of poultry. The layers had free access to the feed and water.

Experimental diets

The laying hens received diet formulations according to the particular species, hybrid and feeding requirements. The basal diet formulation (Table 1) was similar for both groups (2800 kcal metabolisable energy and 17.8% crude protein). Unlike for diet C, the experimental (E) diet formulations used 2.5% flaxseed meal and 10% rice bran. The diet formulation for group C was characterized by a content of 0.885 g % total fatty acids. Diet E differed from diet C by increasing the concentration of α-linolenic acid (4.575 g % total fatty acids) in compound feed.

Table 1. Diet formulation and estimated chemical composition of experimental diet

Specification	Experimental diets	
	Control	Flaxseed meal+rice bran
Corn, %	53.74	48.49
Soybean meal, %	23.85	25.48
Flaxseed meal, %	-	2.5
Rapeseed meal, %	-	-
Rice bran, %	-	10
Antioxidant, %	-	0.015
Sunflower oil, %	2.7	1.84
Monocalcium phosphate, %	1.35	1.25
Calcium carbonate, %	8.79	8.81
Salt, %	0.40	0.41
Methionine, %	0.12	0.17
Choline, %	0.05	0.05
Premix*, %	1	1
Total, %	100	100
Analysed		
Dry matter, %	87.24	87.68
Metabolisable energy, kcal/kg	2800	2800
Crude protein, %	17.8	17.8
Calcium, %	3.9	3.9
Phosphorus, %	0.63	0.61
Lysine, %	0.89	0.89
Methionine, %	0.42	0.44
Met+cist, %	0.73	0.73
Threonine, %	0.68	0.65
Tryptophan, %	0.2	0.19

*1 kg premix contained: 1350000 IU/kg vitamin A; 300000 IU/kg vitamin D3; 2700 IU/kg vitamin E; 200 mg/kg vitamin K; 200 mg/kg vitamin B1; 480 mg/kg vitamin B2; 1485 mg/kg pantothenic acid; 2700 mg/kg nicotinic acid; 300 mg/kg vitamin B6; 4 mg/kg vitamin B7; 100 mg/kg vitamin B9; 1.8 mg/kg vitamin B12; 2500 mg/kg vitamin C; 7190 mg/kg manganese; 6000 mg/kg iron; 600 mg/kg copper; 6000 mg/kg zinc; 50 mg/kg cobalt; 114 mg/kg iodine; 18 mg/kg selenium

Measurement of laying performance and egg quality analysis

Throughout the trial we monitored layer performance: average daily feed intake (g/layer/dayfeed), conversion ratio (feed intake/egg mass; g/g), laying percentage (%) and egg weight (g) and egg quality parameters. Samples of flaxseed meal and rice bran, compound feeds and eggs were collected and analysed chemically. The basic chemical composition determinations were done according to Regulation (CE) 152/ 2009 (sampling and analytical methods for the official inspection of feeds).The fatty acids content was determined by gas chromatography as described by Panaite et al. (2016). Feed

samples were collected initially and then 14 and 28 days afterward and analyzed for fat acidity index, peroxide value and Kreistest. The fat acidity index (expressed as mg KOH/g fat) was determined in fat extracted with chloroform–methanol by titrating with 0.1 N KOH, and using phenolphthalein as indicator. The peroxide value was determined by iodometric titration according to the method described by the American Oil Chemists' Society (AOCS, 2009) and it was expressed as milli equivalents of peroxide per kilogram of sample. Kreis test was performed on the extracted fat and it was based on the production of red colour as a consequence of the reaction between phloroglucinol and epihydrin aldehyde, a compound present in rancid fats. The absence of a pink colour however indicates no incipient rancidity.

In the end of the trial we collected 18 eggs/group. Measurements were performed on egg weight and its components (albumen, yolk, shell) using an analytical scale (Kerm scales, precision 0.001), eggshell breaking strength, using an Egg Force Reader (Sanovo engineering A/S, Denmark); the pH measurements (albumen and yolk), using a portable pH meter Five Go F2-Food kit with LE 427IP67, Sensor MetlerTolledo. Albumen height, Haugh unit (HU) and yolk colour were considered as the parameters of internal egg quality. Albumen height (mm) was measured by using stage micrometer manually. And based on albumen height, HU was calculated using the equation proposed by Haugh (Stadelman, 1995). Yolk colour (the colour scale from 15, dark orange, to 1, light pale) was determined by comparing yolk colour with the Roche yolk colour fan (Hoffman-La Roche Ltd., Basel, Switzerland), according to the CIE standard colorimetric system. After measurements of the internal and external egg quality, six yolk samples (3 eggs/sample) were formed from the collected eggs (18/group) and assayed for the fatty acids content.

Statistical Analysis

The analytical data were compared using variance analysis (ANOVA) with STATVIEW for Windows (SAS, version 6.0). The experimental results were expressed as mean values with standard errors of means (SEM). The differences are considered statistically significant for $P < 0.05$.

RESULTS AND DISCUSSIONS

Table 2 shows the basic chemical composition of the flaxseed meal, rice bran and of the experimental diets. The flaxseed meal is a feed ingredient rich in crude protein (34.57%). Also, is a good source of fats, 68.40 % of which being polyunsaturated fatty acids (PUFA). Of the PUFA from the flaxseed meal fat, 51.67% are omega-3 fatty acids, and 16.63% are omega-6 fatty acids (Table 2). The chemical composition of the flaxseed meal differs according to the genotype, environmental conditions, oil extraction methods and technologies, etc. (Shim et al., 2014). The literature studies show that the flaxseed meal displays the largest variations of the chemical composition, which depend on the oil extraction process. The crude protein content found was in the range of 29.97-43.30%, ether extractives 1.13-15.69%, crude fibres 8.33-12.94% and 3.87-6.40% ash (Mueller et al., 2010; Olteanu et al., 2017; Vlaicu et al., 2018).

Rice bran is a by-product from milling process of paddy rice to produce polished rice. In general, it contains 12-20 % of total kernel and the rice bran was high in protein (13.77%) and lipid (12.56%). These results, especially the chemical compositions of rice bran, were confirmed by the results obtained by Moongngarm et al. (2012). At the same time, he confirms that the rice bran indicated the strongest antioxidant activity which can conduct at slowing down the peroxidation of linoleic acid. Hence, the ferric thiocyanate formation will be slow (Suja et al., 2005).

On the other hand, the diets (Table 2) were isocaloric and isonitrogenous containing 17.89% CP (diet C) and 17.94% CP (diet E), respectively 2800 kcal ME/kg diet for both diets (Table 1). The α-linolenic acid concentration of the compound feeds given to the experimental group was 4.575%, higher than in C (Table 2). The addition of flaxseedmeal and rice bran to the experimental groups made the omega-3 PUFA concentration to increase about 3.76 times compared to the control formulation (Table 2), therefore also improving omega-6/omega-3 ratio.

Tabelul 2. Chemical composition of the raw materialsand the experimental diets

Specification		Raw materials		Experimental diets	
		Flaxseed meal	Rice bran	Control	Flaxseed meal+rice bran
• *Basic chemical composition**					
Dry matter, %		90.24	89.31	90.30	89.93
Organic matter, %		84.95	82.14	76.64	75.19
Crude protein, %		34.57	13.77	17.89	17.94
Crude fat, %		9.79	12.56	4.09	4.80
Crude fiber, %		8.56	8.86	4.46	4.10
Ash, %		5.29	7.17	13.66	14.74
Nitrogen-free extractives, %		32.02	46.95	50.20	48.35
• *Linolenic acid content and polyunsaturated fatty acids (PUFA) profile*					
Linolenic α C18:3n3, g acid/ 100 g total FAME		51.67	1.79	0.885	4.575
ΣPUFA, g/100g total fatty acids, of which:		68.40	43.34	46.13	50.87
ω-3		51.67	2.27	1.11	4.18
ω-6		16.63	41.07	45.02	46.69
ω-6/ω-3		0.32	18.07	40.41	11.16
• *Lipid peroxidation*					
Peroxide value (ml thyosulphate 0.1 N/g fat)	initial	0.32	0.31	0.468	0.47
	after 14 days	-	-	0.574	0.589
	after 28 days	-	-	0.817	0.917
Fat acidity (mg KOH/g fat)	initial	12.99	12.66	13.86	12.4
	after 14 days	-	-	16.01	16.25
	after 28 days	-	-	17.86	18.47
Kreiss test	initial	negative	negative	negative	negative
	after 14 days	-	-	negative	negative
	after 28 days	-	-	negative	negative

Where: PUFA – polyunsaturated fatty acids; PUFA ω-3-omega 3 polyunsaturated fatty acids; PUFA ω-6- omega 6 polyunsaturated fatty acids; *on dry matter basis. **Analyses performed by the Laboratory of Chemistry and Nutrition Physiology – IBNA Balotești.

The high concentration of unsaturated fatty acids in experimental diet tends toward to lipid peroxidation. Although peroxide value and fat acidity were slightly higher in the diets supplemented with flaxseed meal and rice bran compared to the control diet, their values were below the maximum limits set for compound feeds according the Romanian Standard STAS 12266-84, after both storage periods (14 and 28 days). Kreis test was negative in all samples throughout the period of study.

These results are probably due to the antioxidant capacity coming from rice bran. Numerous earlier studies reported that animal performance is affected by feeding peroxidized lipids (Hung et al. 2017).

Layer performance data (Table 3) show that the flaxseeds meal and rice bran had a positive influence on the production parameters: average daily feed intake (g CF/layer/day), feed conversion ratio (kg CF/kg egg), laying percentage (%), and in the average egg weight, but the results were not statistically supported.

The physical quality parameters of the eggs collected in the end of the trial (Table 3) didn't show any significant differences in egg weight or in the weight of its components.

On the other hand, the albumen pH was significantly ($P \leq 0.05$) higher at the control group than the experimental group, fed with flaxseed meal and rice bran.In the experimental group, the yolk colour intensity increased compared to group C, but not significantly.

After 6 experimental weeks, the colour intensity in egg yolk from E group increased by 1.04% compared to C group.

The results obtained in this study regarding the influence of the dietary flaxseeds meal and rice bran on layer performance and egg quality are in agreement with those obtained by Ianni et al. (2019).

Table 3. Influence of the dietary flaxseeds meal and rice bran on layer performance and egg quality (average values/group)

Specification	Control	Flaxseed meal+rice bran	SEM	P-value
Average daily feed intake (g CF/layer/day)	122.38	121.09	0.442	0.1430
Feed conversion ratio (kg CF/kg egg)	2.004	1.965	0.013	0.1371
Laying intensity (%)	94.405	95.237	0.413	0.3169
Egg weight (g), of which:	65.26	66.65	0.429	0.1142
- albumen	39.75	41.11	0.390	0.0878
- yolk	16.83	16.71	0.165	0.7168
- shell	8.67	8.86	0.100	0.3587
Albumen pH	8.72[a]	8.52[b]	0.026	<0.0001
Yolk pH	6.23	6.12	0.043	0.1837
Yolk colour intensity	5.42	5.61	0.099	0.3311
Eggshell breaking strength (kgF)	4.11	4.08	0.085	0.8578
Haugh units	88.98	87.93	0.154	0.2768

Where: [a-b] Mean values within a row having different superscripts are significantly different by least significant difference test ($P \leq 0.05$). SEM: standard error of the mean. *Analyses performed by the Laboratory of Chemistry and Nutrition Physiology – IBNA Balotești.

Regarding the chemical composition of the egg yolk, from the data presented in Table 4 it can be observed that by using a diet rich in polyunsaturated fatty acids, respectively of a diet that included flaxseed meal and rice bran, the fat content of the egg yolk was not affected.

Table 4. Influence of the dietary flaxseeds meal and rice bran on chemical composition of egg yolkand polyunsaturated fatty acids (PUFA) profile

Specification	Control	Flaxseed meal+rice bran	SEM	P-value
• *Basic chemical composition of egg yolk (%)**				
Dry matter content	52.58	53.45	0.415	0.6151
Crude protein	16.79	16.98	0.545	0.6878
Crude fat	30.71	30.30	0.543	0.7226
Ash	1.92	2.03	0.049	0.2855
• *Linolenic acid content and polyunsaturated fatty acids (PUFA) profile (g acid/ 100 g total FAME)*				
Linolenic α C18:3n3	0.24[a]	0.85[b]	0.100	<0.0001
PUFA of which:	27.89[a]	29.63[b]	0.259	0.0278
ω-3	1.39[a]	4.99[b]	0.282	<0.0001
ω-6	26.49[a]	24.63[b]	0.429	0.0207
ω-6/ ω-3	19.12[a]	4.94[b]	2.141	<0.0001

Where:PUFA–polyunsaturated fatty acids; PUFA ω-3 - omega 3 polyunsaturated fatty acids; PUFA ω-6 - omega 6 polyunsaturated fatty acids; [a-b] Mean values within a row having different superscripts are significantly different by least significant difference test ($P<0.05$). SEM-standard error of the mean;*on dry matter basis; **Analyses performed by the Laboratory of Chemistry and Nutrition Physiology – IBNA Balotești.

A significant increase ($P \leq 0.05$) was recorded both for the amount of α-linolenic acid, representing a significant increase by 3.54 times higher in group E compared to group C, as well as for the content of the PUFA content of the yolks from laying, significantly increase ($P \leq 0.05$) for all omega-3 polyunsaturated fatty acids determined for group E compared to group C. All of the data presented in Table 4 shows that the ratio of omega-6/omega-3 decreased significantly in the experimental group (hens fed with flaxseed meal and rice bran during the experimental period) compared to the hens from group C. Aziza et al. (2013) obtained similar results by using 10% flaxseed meal which had no changes in yolk weight compared with eggs from hens fed the control diet. Also, no significant difference in α-linolenic acid or other n-3 fatty acids in the eggs from hens fed 10% flax meal were reported (Aziza et al., 2013), but feeding flaxseed meal resulted in higher egg production ($P<0.05$) compared with the control. The egg weights and Haugh units increased, and eggshell thickness decreased significantly ($P<0.05$) in diet with 10% flaxseed meal. The egg yolk content of α-linolenic acid and total omega-3 PUFA was higher in flax meal group than in C ($P<0.05$). Regarding the average daily feed intake Vlaicu et al., 2017 found that was lower in groups fed flax meal and rosehip as antioxidant compared with C.

CONCLUSIONS

The results of this study clearly demonstrate that the dietary inclusion of 2.5% of flaxseed meal and 10% rice bran significantly increased the content of PUFA in laying hens' egg yolks; it also notably decreased their the reportomega-6/omega-3. The simultaneous enrichment of hens' feed with flaxseed meal and rice bran like natural antioxidants, did not affect the production performance and the internal and external physical parameters of the eggs, but led to the improvement of their nutritional quality by increasing the content in PUFA fatty acids, especially by increasing the content of alpha-linolenic acid (omega-3).

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COMPARATIVE STUDY ON HEMATOLOGICAL AND BIOCHEMICAL CHARACTERIZATION BLOOD PROFILE IN BIRDS GROWN IN FARMS OF ROMANIA

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Abstract

Current research is a part of a research program aimed to study the relationship between morphology and physiological status of turkey pineal gland in relation to the degree of somatic development. Research is carried out at S.C. Galli Gallo Codlea, which is the only unit in Romania for breeding and slaughter of turkeys. The measurements were performed on an important livestock specie for modern husbandry industry, namely turkey, the expansion of this area being useful for providing knowledge on the relationship between technology growth for modulation of microclimate parameters and growth performance. Were followed hematological and biochemical blood parameters,with the purpose of providing a parallel between turkey (Meleagris gallopavo-gallopavo) and chicken (Gallus gallus).

Key words*: hematology, biochemistry, turkey, chicken.*

INTRODUCTION

Hematological and biochemical determinations are essential in veterinary diagnosis. Clinical examinations together with laboratory determinations lead to a prompt and early diagnosis. The introduction of rapid hematological and biochemical tests facilitates the achievement of a metabolic profile in birds that leads to the improvement and restoration of growth and nutritional conditions.

Research regarding the metabolic profile in poultry is related to the fact that they are characterized by an intense metabolism and any nutritional imbalances are have immediate repercussions in the general metabolism of birds with strong effects on health, that are reflected in the production (Vatn et al., 2000; Simeanu, 2009).

Hematological investigations aim at identifying the origins of different haematological disturbances in order to obtain appropriate productions.

Hematological values and biochemical determinations in chickens and turkeys have been made to reflect the health and body homeostasis. Establishing haematological status enables maintenance by interpreting the influence of variations in poultry practice (food, microclimate parameters, light regime) (Simeanu, 2004; Simeanu, 2018).

MATERIALS AND METHODS

As biological material was used:

- turkey BUT Big 6 hybrid: by sex, grown on permanent litter in "blind" halls
- chicken Lohmann Brown hybrid: by sex, grown on permanent litter

Measurements of metabolic parameters were made by performing haematological profile (WBC, Lym., Neu., Mon., Eo., RBC, MCV, HCT, MCH, MCHC, RDW, Hb) by automated analyser, the principle of fluorescence using flow cytometry conductor laser and hydrodynamic focusing and biochemical profile (total protein, cholesterol, triglycerides, uric acid, calcium, magnesium, ALT, amylase, ALP, albumin) by photo spectrometric method (Kheiri et al., 2006).

Blood sampling was conducted from brachial vein in both hybrids at the age of 120 days at turkeys an 56 days at chickens.

Morphostructural pineal gland was observed by histological examination.

Level microclimate factors were provided in

accordance with the provisions of specific technological guide of these birds' categories.
Throughout the experiment, feeding was done *ad libidum* (Law et al., 1990; Foye et al., 2006).

RESULTS AND DISCUSSIONS

The need for haematological profile in birds, chickens, respectively turkeys lies in the fact that they are characterized by an intense metabolism and possible nutritional imbalances are reflected promptly in their metabolism.
Usually, at birds, the white series is less used, however it can provide essential information on the health of individuals.
The hemogram is a basic screening test, being one of the most commonly required laboratory tests, often the first step in establishing a hematological status and the diagnosis of various hematological and non-hematological conditions. Quantification of hematological parameters is sometimes associated with blood smear examination that brings precious information, further focusing the research on other specific tests.
Gender, age, and certain conditions such as: shock, massive i.v. administration of fluids, etc., which can lead to the dehydration, respectively hyperhydration of the individual, as well as certain treatments should be communicated to the laboratory. It is preferable to avoid as much stress as possible at the time of harvesting.
In the case of regular (daily or every other day) monitoring of certain parameters, the blood sample for the hemogram must be obtained at the same time of the day (due to the circadian physiological fluctuations of some parameters) (Fischbach, 2009).
The number of erythrocytes is the basic test for erythropoiesis. Erythrocytes are further investigated by measuring the hemoglobin and hematocrit, and based on them; the analyser calculates the erythrocyte counts: VEM, HEM, CHEM and RDW, which qualitatively characterize the erythrocyte population.
The number of erythrocytes as a single parameter is of low diagnostic value; a correct assessment of the body's erythrocyte mass can only be obtained in correlation with the hematocrit. The number of erythrocytes is influenced by changes in plasma volume, such as in pregnancy or in hydro-electrolyte balance disorders (Means, 2004).
The RBC count, HCT, HGB, MCV, MCHC, and RDW are also used to determine the presence and severity of anaemia (Tvedten, 2010).
In our research we obtained distinct significant differences in expression of neutrophils between this two studied populations and significant differences in expression of monocytes (Table 1).
The number of erythrocytes varies by age and sex and the amount of hemoglobin is influenced by the composition of feed rations. Interpreting the obtained results on the total number of erythrocytes and the amount of haemoglobin were obtained significant differences. Also, significant distinct differences were seen in haemoglobin expression.
Determination of biochemical profile indicators were conducted to obtain information on possible differences tooth two populations.
Total protein showed a mean value of 25.20 g/L at turkeys and 38.29 g/L in chickens. Following the statistical interpretation of results were obtained very significant differences, which can be correlated with higher feed intake and thus combined with a high intake of protein.
Protein intake in poultry influences protein, albumin and uremia levels, and so a feed that is poor in protein given over a 14-day period leads to a lower egg production.
In current practice it is important for birds to measure the enzymatic activity. In laying hens, it was noticeable that after the laying period hepatic and bone metabolism had undergone through some changes (Table 1, Figure 1).
AST (TGO) - aspartate aminotransferase is an enzyme that is part of the transaminase class and catalyses the transfer of the amino group from aspartate to the ketone ketoglutarate group with oxaloacetic acid formation. Unlike ALT that is mainly found in the liver, AST is found in several tissues: myocardium, liver, skeletal muscle, kidney, pancreas, brain tissue, spleen, thus being a less specific indicator for the liver function. At the hepatic cell level, AST isoenzymes are found in both cytosol and mitochondria.
In the interpretation of the obtained results there were very significant differences

regarding the expression of enzymes, increased values being observed in hens, AST had an average value of 16.56 U/I versus 7.1 U/I in the turkey. ALT also had a mean value well above the normal limit of 1227 U/I versus 287.9 U/I recorded in turkeys.

Table 1. Hematological values at chicken and turkey

Parameter	Turkey			Chicken			ANOVA test
	$\bar{X} \pm s_{\bar{x}}$	s	V%	$\bar{X} \pm s_{\bar{x}}$	s	V%	Significance
WBC (m/mm^3)	22.540±3.04	7.46	33.12	11.93±3.78	9.27	154.61	$\hat{F}$ = 4,76; $F_{5\%}$ (1;10) = 4,96; $\hat{F} < F_{5\%}$ => ns
LYM (%)	35.33±2.0	5.11	14.48	48.18±5.8	14.35	239.19	$\hat{F}$ = 4,27; $F_{5\%}$ (1;10) = 4,96; $\hat{F} < F_{5\%}$ => ns
MON(%)	9.68±0.7	1.71	17.73	12.40±0.9	2.38	39.69	$\hat{F}$ = 5,14; $F_{5\%}$ (1;10) = 4,96; $F_{1\%}$ (1;10) = 10,04; $F_{5\%} < \hat{F} < F_{1\%}$ =>*
NEU (%)	54.15±2.7	6.71	12.40	33.05±4.2	10.53	175.50	$\hat{F}$ = 17,12; F5% (1;10) = 4,96; F1% (1;10) = 10,04; F0,1% (1;10) = 21,04; F1%<< F0,1% =>**
EO (%)	0.96±0.96	1.67	173.20	7.14±3.89	8.71	174.31	$\hat{F}$ = 2,54; $F_{5\%}$ (1;10) = 4,96; $\hat{F} < F_{5\%}$ => ns
BA (%)	0.35±0.16	0.39	113.92	0.41±0.15	0.37	6.27	$\hat{F}$ = 0,09; $F_{5\%}$ (1;10) = 4,96; $\hat{F} < F_{5\%}$ => ns
RBC (M/mm^3)	2.00±0.35	0.87	43.86	2.98±0.11	0.27	4.56	$\hat{F}$ = 8,12; $F_{5\%}$ (1;10) = 4,96; $F_{1\%}$ (1;10) = 10,04; $F_{5\%} < \hat{F} < F_{1\%}$ =>*
MCV (fl)	127.46±4.46	10.93	8.57	108.75±0.84	2.07	34.49	$\hat{F}$ = 16,98; $F_{5\%}$ (1;10) = 4,96; $F_{1\%}$ (1;10) = 10,04; $F_{0,1\%}$ (1;10) = 21,04; $F_{1\%} < \hat{F} < F_{0,1\%}$ =>**
HCT (%)	26.13±5.33	13.06	50.00	32.33±1.04	2.56	42.76	$\hat{F}$ = 1,30; $F_{5\%}$ (1;10) = 4,96; $\hat{F} < F_{5\%}$ => ns
MCH (pg)	80.51±14.21	34.80	43.23	39.33±0.3	0.91	15.26	$\hat{F}$ = 8,39; $F_{5\%}$ (1;10) = 4,96; $F_{1\%}$ (1;10) = 10,04; $F_{5\%} < \hat{F} < F_{1\%}$ =>*
MCHC (g/dL)	65.53±13.32	32.65	49.82	36.18±0,2	0.64	10.82	$\hat{F}$ = 4,85; $F_{5\%}$ (1;10) = 4,96; $\hat{F} < F_{5\%}$ => ns
RDW	7.93±0.1	0.42	5.32	6.51±0.21	0.52	8.71	$\hat{F}$ = 26,62; $F_{5\%}$ (1;10) = 4,96; $F_{1\%}$ (1;10) = 10,04; $F_{0,1\%}$ (1;10) = 21,04; $F_{1\%} < \hat{F} < F_{0,1\%}$ =>***
HB (g/dL)	13.63±0.3	0.78	5.75	11.71±0.3	0.92	15.36	$\hat{F}$ = 15,05; $F_{5\%}$ (1;10) = 4,96; $F_{1\%}$ (1;10) = 10,04; $F_{0,1\%}$ (1;10) = 21,04; $F_{1\%} < \hat{F} < F_{0,1\%}$ =>**

$\bar{X} \pm s_{\bar{x}}$ = mean ± standard deviation of the mean; s = standard deviation; V% = coefficient of variation

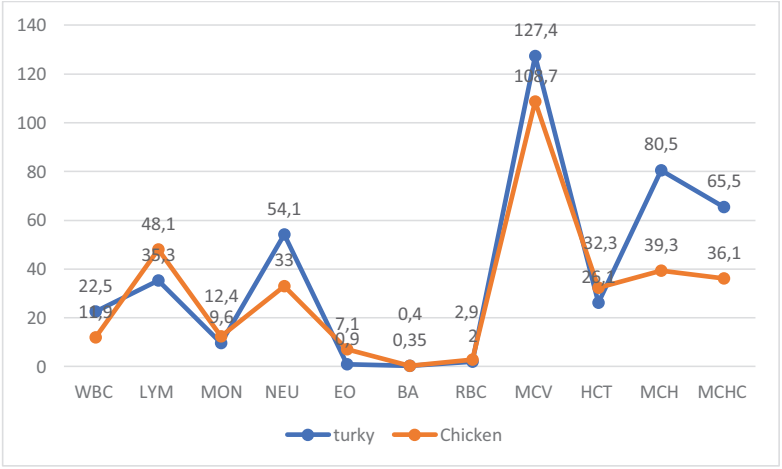

Figure 1. Variation of hematological values of turkey and chicken

Significant differences were obtained separately in AST, ALT and amylase expression enzymes. Significant differences were recorded at magnesium and uric acid values.
Triglycerides from the adipose tissue, but also other tissues, are the most important reservoir of energy inside the body. In the adipose tissue they are stored in the form of glycerol, fatty acids and monoglycerides, which are converted inside the liver into triglycerides that enter the VLDL (80%) and LDL (15%) (Tabel 2, Figure 2).

Table 2. Biochemical values index at turkey and chicken

Parameter	Turkey			Chicken			ANOVA test
	$\bar{X} \pm s_{\bar{x}}$	s	V%	$\bar{X} \pm s_{\bar{x}}$	s	V%	Significance
Total proteins (g/L)	25.20±1.84	4.52	17.96	38.29±2.08	5.09	13.31	$\hat{F} = 22,1$; $F_{5\%}(1;10) = 4,96$; $F_{1\%}(1;10) = 10,04$; $F_{0,1\%}(1;10) = 21,04$; $F_{1\%} < \hat{F} < F_{0,1\%}$ =>***
Cholesterol (mg/dL)	111.36±10.04	24.61	22.10	164.06±9.41	23.071	14.06	$\hat{F} = 3,39$; $F_{5\%}(1;10) = 4,96$; $\hat{F} < F_{5\%}$ => ns
Triglycerides (mg/dL)	177.36±49.39	120.99	68.21	150.31±6.52	15,98	10.93	$\hat{F} = 0,29$; $F_{5\%}(1;10) = 4,96$; $\hat{F} < F_{5\%}$ => ns
Calcium (mg/dL)	11.64±0.97	2.40	20.60	9.43±0.37	0.91	9.70	$\hat{F} = 4,46$; $F_{5\%}(1;10) = 4,96$; $\hat{F} < F_{5\%}$ => ns
Magnesium (mg/dL)	3.31±0.04	0.10	3.05	5.1±0.55	1.35	26.31	$\hat{F} = 10,97$; $F_{5\%}(1;10) = 4,96$; $F_{1\%}(1;10) = 10,04$; $F_{0,1\%}(1;10) = 21,04$; $F_{1\%} < \hat{F} < F_{0,1\%}$ =>**
ALT (U/I)	16.56±1.72	4.22	25.51	7.1±0.20	0.49	6.97	$\hat{F} = 29,69$; $F_{5\%}(1;10) = 4,96$; $F_{1\%}(1;10) = 10,04$; $F_{0,1\%}(1;10) = 21,04$; $F_{1\%} < \hat{F} < F_{0,1\%}$ =>***
AST (U/I)	1227.26±127.74	312.90	25.496	287.96±7.49	18.36	6.37	$\hat{F} = 53,88$; $F_{5\%}(1;10) = 4,96$; $F_{1\%}(1;10) = 10,04$; $F_{0,1\%}(1;10) = 21,04$; $F_{1\%} < \hat{F} < F_{0,1\%}$ =>***

$\bar{X} \pm s_{\bar{x}}$ = mean ± standard deviation of the mean; s = standard deviation; V% = coefficient of variation

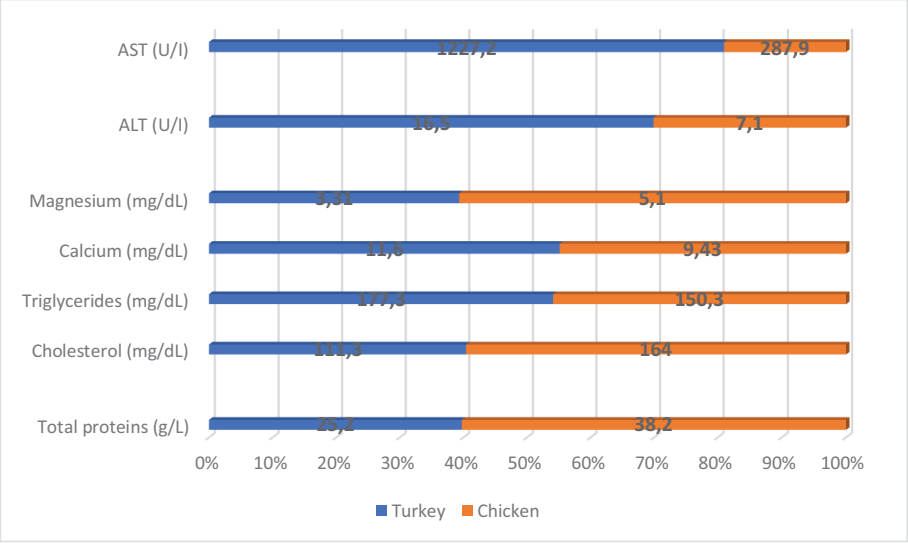

Figure 2. Variation of biochemical values of turkey and chicken

Hypertriglyceridemia together with hypercholesterolemia are independent risk factors for atherosclerotic disease. Also, thetriglyceride level is required for LDL-C calculation.

Calcium is the major mineral component of the bones. 99% of the amount of calcium in the body is found inside the bones and teeth, which represent a huge reservoir for maintaining seric calcium levels, the rest being distributed in biological fluids and soft tissues. Calcium ions play an important role in transmitting nerve impulses, muscle contraction, cardiac function, and coagulation processes.

The hormonal regulation of calcium metabolism as well as for phosphorus is complex. The reciprocal relationships between the small intestine, the skeleton, the kidney and the endocrine system, in particular the parathyroids, maintain the calcium and phosphorus homeostasis. Also, calcitonin, vitamin D, estrogen, androgen are factors that influence calcium levels.

The amount of protein in the blood affects the calcium level, because 45% of the serum calcium is protein-bound. Thus, decreasing serum albumin causes a decrease in total serum calcium. In our research there were no significant differences between the studied individuals.

Magnesium is an element that, although found to be present in small amounts inside the body (0.05% of the total body weight), is of great structural and functional importance.

70% of the total magnesium content of the human body (about 14g) is found inside the bone composition, along with Ca and P, and the rest is distributed in soft tissues (especially skeletal muscles) and various fluids. About 1% is found in plasma, 25% is protein-bound, and the rest remains in the form of ionized Mg^{2+}. Inside erythrocytes there is inappreciable amount of Mg, approx. 5.2 mEq/L. As for the cellular distribution of magnesium, most of it is found inside the mitochondria and nucleus. In addition to its plastic role in bones and soft tissues, Mg performs many functional roles, including activator of some enzymes (over 300 enzymes involved in carbohydrate metabolism, protein and nucleic acid synthesis, the most well-known Na^+/K^+ - ATP-ase). Together with Na^+, K^+ and Ca^{2+} ions, magnesium regulates neuromuscular excitability and coagulation mechanism.

The actions of calcium and magnesium are closely linked, the deficiency of one of these elements significantly affecting the metabolism of the other (magnesium is needed for both intestinal absorption and calcium metabolism). In the muscle cell, magnesium acts as a calcium antagonist. Magnesium deficiency will result in the mobilization of calcium from the bones, with the possibility of abnormal calcifications in the aorta and kidneys. It is therefore advisable to consider the calcium level when assessing the magnesium level. Also, hypomagnesiemia is associated with hypopotasiemia in 60% of cases. Significant magnitude values were recorded in the expression of magnesium, with higher values in the turkey, 5.1 mg/dL, compared with the 3.31 mg/dL value from chickens.

CONCLUSIONS

This paper aims to characterize, through the light of presented data, a control group of females and males, maintained under conditions of microclimate and technological factors. Track parameters underlying the lots referential experimental realization that change lighting system influence on endocrine function is responsible for coordinating epiphyseal metabolisms involved in growth and development.

Hematological and biochemical results fall within the reference values of this two populations and are characterize the normal physiological status of individuals.

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STUDY REGARDING SOME REPRODUCTIVE PARAMETERS IN SHAGYA ARABIAN MARES FROM RĂDĂUȚI STUD FARM

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Abstract

The paper was based on analyzing 119 Shagya Arabian mares (Dahoman, El-Sbaa, Gazal, Hadban, Koheilan, Mersuch, Shagya and Siglavy-Bagdady bloodlines), reared in Rădăuți stud farm, between 1996-2016 years regarding fecundity percent, gestation length, abortion rate and foaling-interval parameters. The main purpose was to establish if the reproduction indices registered at females taken into study respect the limits indicated by the literature which can establish if the population can be included into the breeds` standard; the results showed that the fecundity percent had lower values than the literature describes and the foaling-interval exceeded the limits given by the literature, facts that reveal some aspects related to the reproduction process need to be reconsidered.

Key words*: Shagya, fecundity, gestation, abortion, foaling-interval.*

INTRODUCTION

This study had the purpose to analyze some reproduction parameters of Shagya Arabian mares, from Rădăuți stud farm and to establish if there are significant differences between genetic lines; also there was an opportunity to see if the breed can be found in the species standard regarding these parameters.

The Shagya Arabian horse breed had the famous Shagya stallion as a genitor which was paired with the oriental mares, not necessary Arabian in Mezohegyes stud farm (Hungary) in 1834. Gozlan stallion arrived in 1874 from Lipizza and 1885 is the year when Obayan was bought from Syria. In the beginning of 20th century the stud farm achieved two exceptional horses, Siglavy-Bagdady and Mersuch and between the World Wars, Poland provided Kuheylan Zaid and Germany Sven Hedin (renamed Kemir).

Also a multitude of stallions were imported from Egypt (Dulugeac, 2005).

The Rădăuți stud farm was set in 1792 to produce valuable horses for the Austrian army, as 1400 stallions, working horses, pregnant mares and foals were sent from Vășcăuți village to the 16 sections of Rădăuți stud farm. Barberino stallion and two Pure Arabian horses, Hussein and Manachi were the founders of this stock (Rădăuți stud farm registers 2016).

After the war ended, in 1919, the stud farm was rebuilt starting with Gazal, Siglavy-Bagdady I, Dahoman XXII and Shagya XV and 31 mares from different bloodlines. The first studbook was drafted in 1924, following to be brought descendants of El-Sbaa and Beck in 1936-1941. (Schipor, 2007)

Rădăuțistud farm currently (2019) holds 273 horsesplaced in its 3 sections: Rădăuți where the training livestock can be found, Mitoc (Frătăuții Noi village) where the breeding stock is and Brodina where the male youth is located. In 2019, the stock is represented by: 39 stallions breed for public mounting (35 are Shagya Arabian breed, 3 Semigreu Românesc, 1 Lipizzaner), 8 Shagya Arabian stallions, 57 Shagya Arabian mares, 21 young horses aged 0-6 months, 22 horses aged 6-12 months, 34 horses aged 12-24 months, 26 horses aged 24-36 months, 20 training horses (all Shagya Arabian breed), 3 sport and recreation (2 Shagya Arabian and 1 Romanian Sport Horse), 7 working horses and 36 Shagya Arabian horses for sale (Rădăuți stud farm registers 2016).

Regarding the fecundation and abortion rate in *Equus caballus* species, the literature indicates

that fecundation process (the fusion of male and female gametes followed by zygote formation and quick segmentation triggering) takes 12-16 hours and that the abortion can be noninfectious (abnormalities of the fetal annexes, neuro-hormonal, nutrition, traumas, genetic factors) or infectious (*Salmonella abortusequi* and rarely diplococcus, staphylococcus, *Sigella equirulis*, colibacillus) (Dumitrescu, 1986).

Fecundity percent is about 80-90% within the stud farms and 60-70% in other types of farms, depending on some conditions. The average fecundity level for Arabian is 90% and the breeding perspective follows reaching 95% (Vancea et al., 1980).

According to a study based on 1393 Thoroughbred mares reared in 22 stud farms from Newmarket, UK, 59.9% of the inseminated mares at 15 days after ovulation had a 94.8% fecundity percent. This value went to 89.7% by the 35th day and to 87.5% by the October pregnancy test. In 1983 the percent of mares which gave birth at term was 77% while in 1998 the registered value of this parameter was 82.7% (Morris et al., 2010).

In a study published in 2012 on equine abortion, stillbirth and neonatal mortality, the abortion was defined as the miscarriage before 300th day of gestation, the stillbirth was known as the delivery of a dead foal after 300 days of gestation and the neonatal mortality was the death of foals within seven days post-foaling (Marenzoni et al., 2012).

Likewise, another paper which had the objective to review the same subject described above, within the Animal Health Trust data, based on a 10 years timeframe revealed the diagnoses of 1252 fetuses and neonatal foals. The results showed that 38.8% of them were problems associated with the umbilical cord, 35.7% were related to comprising umbilical cord tension and only 3.1% were long cord/cervical pole ischaemia disorder. Values regarding other noninfectious causes of abortion or neonatal death were 6.0% for twinning, 13.7% for *intrapartum* stillbirth and 9.8% for placentitis (associated with infection – *E. coli* and *Streptococcus zooepidemicus*). The percent of neonatal infections not related to placentitis was 3.2% and cases of EHV-1 and EHV-4 were 6.5% (Smith et al., 2010).

The gestation length (the period of time between fecundation and birth) is 337-339 days for half-breeds (Velea et al., 1980) and 310-340 days for the rest of horse breeds (Dumitrescu, 1986) this parameter can be influenced by heredity, age of mares, sex of the product, conditions during the gestation etc.

The foaling-interval length (the period between two consecutive births) mentioned in the literature is about 337-338 days (Dumitrescu, 1986).

The results obtained in this study, regarding these reproductive parameters, showed that in Rădăuți stud farm this process is well mastered, but needs some improvement to increase several values related to some aspects. In stud farms, there's a constant need of developing the reproduction process, because their purpose is to provide high quality biological material while optimizing the process of obtaining it.

Shagya Arabian horse breed has abilities for riding, endurance, jumping over obstacles, but also hippo-therapy and recreation. Among the objectives of breed improvement, there are included: increasing body size, energetic capacity in gallop races, reproductive indices and constitutional resistance.

A study conducted between 1992-2003 on Dahoman, El-Sbaa, Gazal, Hadban, Koheilan, Mersuch, Shagya, Siglavy-Bagdady and Nedjari genetic bloodlines, composed of 8 batches of stallions and 9 batches of mares, had the purpose to analyze the objectives of amelioration; the paper indicated that at the time progress was already recorded on some indices and performances identified in gallop races. There was an increase in population average withers height on Gazal, El-Sbaa, Hadban and Nedjari genetic bloodlines and the thorax and cannon girth, were bigger at Gazal and Hadban genetic lines, compared to the average of the population. Regarding the performance obtained on the gallop race of 2400 m, males and females from Koheilan and Gazal bloodlines have achieved the best results (Manole et al., 2004).

MATERIALS AND METHODS

We studied 119 mares reared in Rădăuțistud farm, from Mersuch, El-Sbaa, Koheilan, Shagya, Siglavy-Bagdady, Dahoman, Hadban

and Gazal bloodlines, which started breeding in 1996.

In this paper, data retrieved from Rădăuți stud farm, by calculating the main estimators–descriptors (average, variance, standard deviation, standard error of average, coefficient of variation) for the genetic line level and by making interline comparisons, was made using the unifactorial ANOVA algorithm.

RESULTS AND DISCUSSIONS

From the Table 1 it can be observed that the minimum absolute fecundity percent for the studied mares was 0 for 50% of the 8 analyzed bloodlines. Regarding the average values, the fecundity percent was 55.96±6.93% (El-Sbaa bloodlines) 78.41±4.42% (Siglavy-Bagdady bloodline). Also, the females from Gazal genetic line were close to the average minimum percent (61.63±12.22%) but the Mersuch bloodline was identified as closest to the maximum average percent (75.76±8.32%).

Given the fact that the fecundity percent should be 80-90% (Velea et al., 1980)in stud farms and for Arabian breed 90% (according to the literature) it can be claim that the studied mares registered lower values than this limit, though the average percent of 78.41±4.42% identified at Siglavy-Bagdady bloodline showed that the standard threshold can be almost reached. The data obtained mark the existence of a series of problems regarding reproduction management or bad maintenance of mares that can cause the occurrence of these low values and catch the attention to an urgent rehabilitation.

Table 1. Fecundity analysis for the 8 bloodlines of Shagya Arabian breed (%)

Bloodline	$\bar{X}$	± St. Dev.	$\pm s_{\bar{x}}$	V%
Mersuch	75.76	31.14	8.32	41.101
El-Sbaa	55.96	28.94	6.92	51.727
Koheilan	69.40	26.69	6.31	38.466
Shagya	69.28	26.50	6.29	38.251
Siglavy-Bagdady	78.41	14.65	4.42	18.693
Dahoman	71.79	26.18	6.76	36.475
Hadban	72.24	21.41	5.72	29.644
Gazal	61.63	34.56	12.22	56.085

The Figure 1 shows that the average fecundity percent had the minimum value in case of Gazal bloodline (62.50%) and the maximum for Mersuch bloodline (79.03%), the second value being also in accordance with the literature (Morris et al., 2010).

For all analyzed genetic lines, the calculated coefficient of variation oscillated between 18.69-56.08%, indicating thus a high influence of individuality within the lines, on the fecundity level.

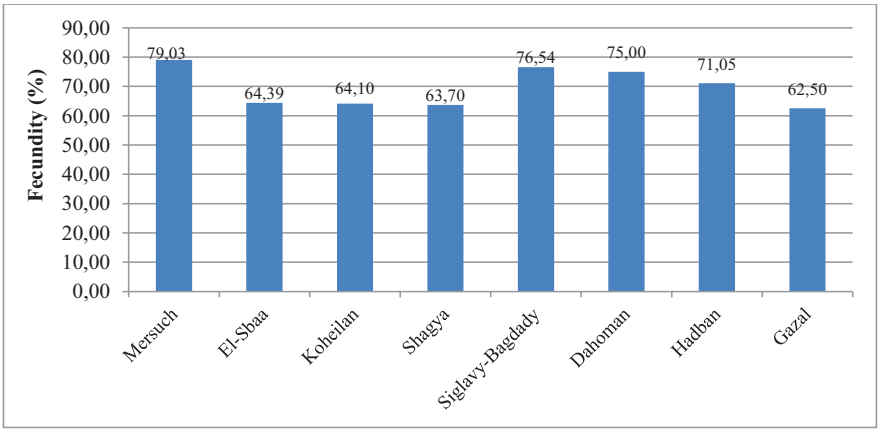

Figure 1. Average fecundity percent in studied mares (%)

The Table 2 reveals that the minimum absolute gestation length and the maximum absolute gestation length, were both found at Dahoman bloodline (300 and 361 days).
Regarding the average gestation length, this parameter was found between 336.2±2.55 days (Hadban mares) and 341.9±2.55 days (Gazalmares). Also, the females from other genetic lines were close to the average minimum length (Dahoman: 336.5±3.23 days and Mersuch: 336.6±2.88 days) or to the maximum limit (Shagya: 341.8±2.35 days).

Table 2. Gestation length of analyzed mares from the 8 bloodlines (days)

Bloodline	n	$\bar{X}$	± St. Dev.	$\pm s_{\bar{x}}$	V%	Min.	Max.
Mersuch	14	336.6	9.681	2.587	2.88	319	354
El-Sbaa	18	338.6	13.23	2.643	3.82	304	358
Koheilan	21	338.9	10.32	1.339	3.05	306	357
Shagya	18	341.8	8.029	1.892	2.35	322	355
Siglavy-Bagdady	11	337.7	9.52	2.870	2.82	319	354
Dahoman	15	336.5	10.88	2.808	3.23	300	361
Hadban	14	336.2	8.587	2.294	2.55	318	355
Gazal	8	341.9	8.717	3.082	2.55	325	360

The Figure 2 indicates that the average gestation length had the minimum value at Hadban bloodline and the maximum at Gazal, which was influenced probably by the reproduction management or by the sex of the product.
For all analyzed genetic lines, the calculated coefficient of variation oscillated between 2.35-3.82% and indicated a reduced influence of individuality within the lines regarding gestation length, revealing a homogeneous character.
However, using the variance analysis algorithm, the unifactorial variant, significant statistical differences were observed ($p<0.05$) between the average gestation length, found at Shagya and Dahoman bloodlines.

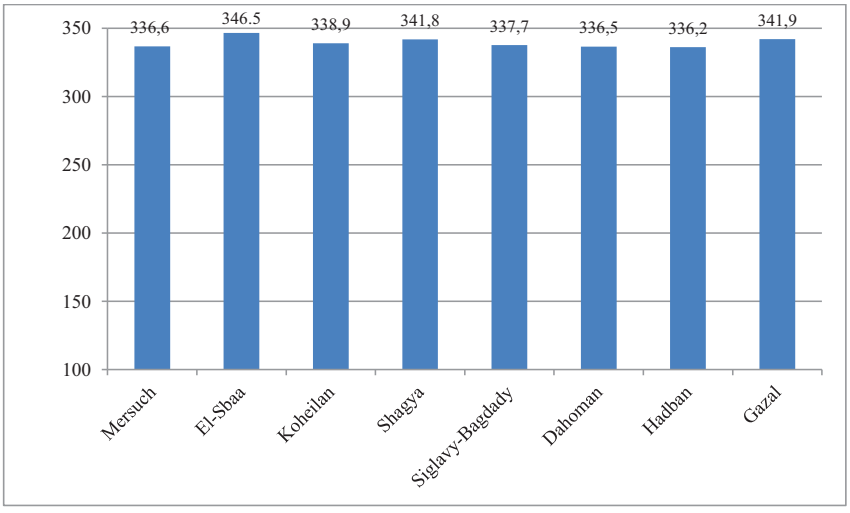

Figure 2. Average gestation length in studied mares (days)

The Table 3 indicates the abortion rate for all females analyzed from all the bloodlines of Shagya Arabian breed. The highest percent of abortion was registered at Hadban bloodline (11.12%) and the lowest at Gazal bloodline (0.00%). The maximum number of gestations was found at El-Sbaa bloodline (85) from which 92.94% ended up with a healthy foal (79 births) and the minimum number of gestations was registered at Gazal bloodline (25) but from which all of them ended with giving birth to a foal (100%).

Table 3. Abortion rate for all 8 bloodlines (%)

Bloodline	N	Number of gestations	Number of births	Abortion (%)
Mersuch	14	49	47	4.09
El-Sbaa	18	85	79	7.06
Koheilan	21	75	72	4.00
Shagya	18	79	74	6.33
Siglavy-Bagdady	11	62	61	1.62
Dahoman	15	75	71	5.34
Hadban	14	54	48	11.12
Gazal	8	25	25	0.00

The Table 4 shows that the individual value for foaling-interval registered a minimum at Siglavy-Bagdady bloodline (230 days) and a maximum at El-Sbaa (1376 days).
The average values oscillated between 409.0±59.13 days (Gazal bloodline) and 539.4±57.47days (Hadban bloodline).

There were not noticed high individual oscillations between bloodlines (the coefficients of variation registered close limits: 54.80-59.13%) indicating the analyzed characters were homogenous.

Table 4. Foaling interval values (days)

Bloodlines	n	$\bar{X}$	± St. Dev.	$\pm s_{\bar{x}}$	V%	Min.	Max.
Mersuch	14	412.1	225.87	60.36	54.80	340	721
El-Sbaa	18	468.1	275.90	65.03	58.94	257	1376
Koheilan	21	481.9	264.31	57.67	54.84	322	908
Shagya	18	526.1	298.79	70.42	56.79	344	1066
Siglavy-Bagdady	11	459.7	257.53	77.64	56.02	230	803
Dahoman	15	485.5	283.34	73.15	58.36	278	1092
Hadban	14	539.4	310.03	82.85	57.47	347	1154
Gazal	8	409.0	241.84	85.50	59.13	343	1051

The Figure 3 indicates that the minimum and the maximum value of foaling-interval parameter (409 and 539.4 days) exceeded the limits found in the literature (337-339 days).

This can occur because of the management of reproduction; however, it is preferable that this parameter be within the limits given by the literature to ensure obtaining one foal a year from each mare.

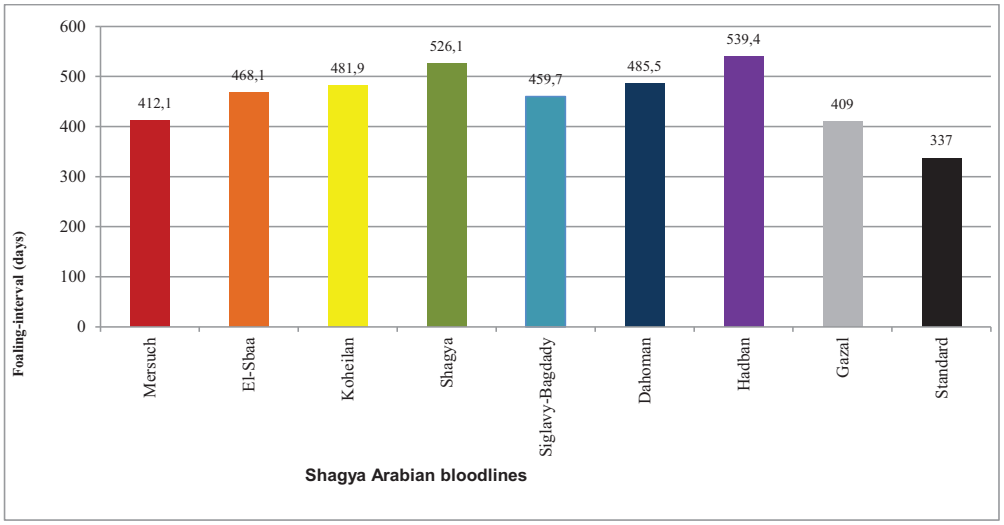

Figure 3. Average foaling-interval values in the studied mares (days)

CONCLUSIONS

The results revealed by this study showed that all the genetic bloodlines were at a lower level than the limits described by the literature for the fecundity percent; however, Siglavy-Bagdady, Mersuch, Hadban and Dahoman bloodlines registered average values close to 80% minimum percent found for this parameter. The coefficient of variation calculated for fecundity percent indicated a high influence of individuality within the genetic lines (18.69-56.08%) and, therefore, the need for selection to increase the values and to homogenize this trait.

Regarding the gestation length, the limits at half-blood breeds found in literature are between 337-339 days (Dumitrescu, 1986), so it can be claim that only El-Sbaa, Koheilan and Siglavy-Bagdady bloodlines had average values within this interval (338.6±3.82 days, 338.9±3.05 days and 337.7±2.82 days). The coefficient of variation calculated for this parameter indicated a homogeneous character (2.35-3.82%).

The abortion rate indicated the highest level of 11.12% at Hadban bloodline and surprisingly the lowest level of 0% in Gazal bloodline case; the other calculated values were placed between 1.62-7.06%, which are acceptable for this parameter.

Regarding the foaling-interval parameter, there were noticed very wide limits within bloodlines (230-1376 days); the average values calculated were placed between 409.0±59.13 days (Gazal bloodline) and 539.4±57.47days (Hadban bloodline) which indicated that they have exceeded the limits given by the literature (337-338 days) (Dumitrescu, 1986). The coefficients of variation registered values between 54.80-59.13%, which proves that the analyzed characters were heterogeneous, hence the need to control all factor involved in order to render homogeneity to this trait.

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PRESERVATION OF RAMS' SPERM AT +2-+4°C REGION OF MOLDOVA

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Abstract

Quantitative and qualitative indicators of sperm obtained from rams Moldavian Karakul were studied. The average volume of the ejaculate was 0.84 ml, 91% mobility and 2.5 mlrd / ml concentration. The action of the BD-1 preparation synthesized at the Institute of Microbiology and Biotechnology of the Academy of Sciences of the Republic of Moldova was experimented as an additional component introduced into the basic environment regarding the quality of the ram seminal material, preserved at 2-4°C. It has been found that the introduction in the composition of the basic medium for 6% dilution of seminal material of BD-1 has a positive influence on the preservation of the semen. After 144 hours of incubation at 2-4°C the sperm mobility was 68% compared to the control group where this index was 45%. Artificial insemination of the sheep with refrigerated sperm after 144 hours allowed 71.9% sheep fecundity. The proposed technology for conserving the semen of ram at 2-4°C which composition proposes the dilution medium for semen, containing: glucose, sodium citrate, egg yolk, BD-1, allows the results of artificial insemination of sheep to be made more efficient.

Key words: *ram, breed, sperm, dilution medium, preservation, fecundity.*

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INTRODUCTION

Theoretical studies in the biology of animal sperm have opened up great prospects for opportunities not only in the accelerated development and widespread introduction of the method of artificial insemination of farm animals, but also in the preservation of genetically most valuable and endangered species and species of animals.

Methods of storing sperm in a non-organism are based on a decrease in the metabolic processes of spermatozoa, which allows them to increase the time of their survival and preserve their fertilizing ability (Milovanov, 1962). Currently, the most widely used in sheep farming is the short-term storage of diluted sperm at a temperature of 2-4°C (Shaydulin, 2007). To store sperm at a temperature of 2-4°C, it is diluted with a special medium (GTsZH) prepared according to the following recipes, per 100 ml of bidistilled water: glucose-0.8 g, sodium citrate-2.8 g, yolk-20 ml. The shelf life of sperm at 2-4°C is very small and as a rule they are used during the day. Even if the spermatozoa have a progressive movement after 3 days or more, their fertilizing ability is sharply reduced. Such a short period of storage of sperm causes difficulties in the work of items of artificial insemination of sheep (Voevodin, 2012). This requires the improvement of this method in order to increase the shelf life of sperm without reducing the fertilizing ability of spermatozoa. In this regard, further research is needed in the field of improving synthetic media for storing sperm, both in freezing conditions and in the frozen state. It is possible to increase the effectiveness of artificial insemination of sheep by using various biological active compounds in environments (Nauk, 1972, 1973).

MATERIALS AND METHODS

The object of research was Moldavian type rams of Karakul breed. In the experiments used clinically healthy rams - producers. Sperm was taken into an artificial vagina, the quality of freshly obtained sperm was determined by standard methods for volume, concentration, and motility was determined using the computer program "CEROS". For the experiments, sperm with a mobility of at least 80% and a concentration of at least 2.5 billion / ml was used.

All the original components intended for the preparation of synthetic media for dilution of sperm had a purity of "HCH" or "analytical grade" and were tested for harmlessness to sperm in accordance with approved quality

control methods. Environments were prepared according to the standard technique; their quality was checked by the method of biocontrol.

As an additional component introduced into the composition of the basic medium (GTsZH), the drug BD-1 was developed, which was developed at the Institute of Microbiology and Biotechnology of the Academy of Sciences of the Republic of Moldova in order to increase the shelf life of semen of rams.

The drug BD-1 was tested with its introduction into the composition of the main medium in different concentrations from 1 to 10%.

All comparative experiments in studying the action of the drug BD-1 were studied on separate ejaculates.

The quality of the stored sperm at 2-4°C in each prepared medium after dilution of the sperm was tested for motility every 24 hours, using the CEROS program.

RESULTS AND DISCUSSIONS

Further improvement of the method of storing sperm involves the selection of rams, the sperm of which is suitable for use and does not reduce the loss of spermatozoa during storage, improve the safety of biological usefulness and, accordingly, the effectiveness of artificial insemination. Values of the level of semen products of rams, allows you to send in the right direction their use, which is very important in the effectiveness of their use.

During the experiments, special attention was paid to the study of the quantity and quality of sperm obtained from sheep of the Moldavian type of Karakul breed, as well as improving the synthetic environment for diluting the ram sperm and its protective properties. At first, the assessment of rams producers of the Moldavian type of Karakul breed on the quality of sperm production (Table 1) was reviewed and verified.

Table 1. Average data of indicators of freshly received rams semen

	units	*Statistical Parameters*				
		n	M±м	V %	V min	V max
amount	ml	15	0.84±0.06	28.53	0.4	1.2
mobility	%	15	91±1.01	4.38	90	100
concentration	milliard/ml	15	2.78±0.05	24.48	2.06	2.92
The total number of spermatozoa in the ejaculate	milliard	15	2.59±0.05	13.38	2.42	2.86

It has been established that rams of the Moldavian type of Karakul breed is characterized by variability of sperm values. The data presented in the table show that the average volume of ejaculate in ram producers was 0.84 ± 0.06 ml, with fluctuations between rams from 0.4 ml to 1.2 ml.

The mobility of freshly obtained sperm was 91.0 ± 1.01%. The concentration of sperm in 1 ml of sperm was 2.78 billion, and the total number of sperm in the ejaculate averaged 2.59 billion / ml.

The experimental data obtained show that the sperm production of ram-makers of the Moldavian type of Karakul breed is lower compared to the standard indicators of the Karakul breed.

Biocontrol quality of diluents allows to determine the effect of the developed media on the mobility and survival of spermatozoa outside the body.

Another series of experiments was carried out to improve the technology of preserving sperm during cooling. After collecting the semen, the ejaculates were subjected to microscopic and macroscopic analysis using the CEROS program. Ejaculates approved for treatment were diluted with GTG medium, which included BioR with membranotropic and antioxidant properties, synthesized in concentrations from 1 to 10%, developed by the Institute of Microbiology and Biotechnology of the Academy of Sciences of Moldova.

Biocontrol quality of diluents allows to determine the effect of the developed media on the mobility and survival of spermatozoa outside the body. The mobility and survival of sperm at 2-4^{o}C are presented in Table 2.

Table 2. Mobility and experience of spermatozoa at + 2 - +4°C

Time between researchings, h	indices, %	control GTJ	*BD-1 concentration*									
			1%	2%	3%	4%	5%	6%	7%	8%	9%	10%
After dilution	*motility*	*81.3 ±4.6*	*82.0 ±4.7*	*72.0 ±14.5*	*84.0 ±6.3*	*86.8 ±1.5*	*84.8 ±4.8*	*84.3 ±2.2*	*88.3 ±1.5*	*84.5 ±3.7*	*85.3 ±4.8*	*79.3 ±4.9*
	progressive	*39.3 ±2.3*	*40.8 ±2.7*	*47.0 ±6.9*	*38.3 ±3.3*	*4.3 ±3.0*	*42.3 ±1.6*	*38.5 ±3.2*	*40.8 ±3.4*	*41.0 ±5.0*	*45.5 ±4.3*	*38.5 ±4.3*
24	*motility*	*81.3 ±3.2*	*797 ±6.4*	*80.3 ±5.0*	*82.3 ±3.5*	*88.3 ±3.2*	*83.3 ±3.7*	*81.0 ±3.6*	*82.3 ±3.8*	*80.3 ±2.3*	*83.7 ±0.9*	*82.7 ±4.4*
	progressive	*33.0 ±1.0*	*32.2 ±3.7*	*32.0 ±2.1*	*31.0 ±2.1*	*34.0 ±3.1*	*38.3 ±4.4*	*37.7 ±5.9*	*35.3 ±3.7*	*31.7 ±2.2*	*35.0 ±3.5*	*35.3 ±7.5*
48	*motility*	*81.7 ±3.7*	*71.0 ±5.1*	*82.0 ±6.8*	*73.0 ±2.9*	*76.0 ±2.3*	*78.0 3±5.2*	*77.0 ±5.1*	*84.0 ±5.5*	*84.3 ±6.1*	*81.7 ±3.2*	*80.0 ±3.2*
	progressive	*38.7 ±3.4*	*28.0 ±4.0*	*33.3 ±6.4*	*31.0 ±3.5*	*34.0 ±4.3*	*37.0 ±4.6*	*35.3 ±4.3*	*41.0 ±7.8*	*33.0 ±3.6*	*36.0 ±5.0*	*28.7 ±4.7*
72	*mobility*	*71.3 ±1.8*	*65.7 ±5.9*	*77.7 ±3.4*	*70.3 ±0.9*	*76.3 ±4.5*	*73.3 ±6.2*	*74.7 ±2.7*	*78.0 ±4.0*	*77.7 ±1.8*	*73.0 ±3.0*	*75.3 ±5.5*
	progressive	*28.3 ±1.2*	*18.7 ±1.7*	*25.0 ±2.5*	*31.3 ±3.5*	*29.7 ±4.1*	*31.3 ±5.8*	*30.7 ±2.3*	*32.7 ±5.6*	*27.0 ±1.5*	*32.0 ±3.1*	*27.3 ±4.8*
96	*mobility*	*72.3 ±5.5*	*60.3 ±12.4*	*72.0 ±5.8*	*73.3 ±2.9*	*73.7 ±6.4*	*73.7 ±8.4*	*73.7 ±4.8*	*77.7 ±5.0*	*78.3 ±3.8*	*81.0 ±3.8*	*81.3 ±5.6*
	progressive	*28.3 ±6.1*	*24.0 ±5.5*	*23.0 ±4.7*	*24.3 ±3.3*	*28.7 ±4.3*	*29.0 ±3.5*	*26.7 ±0.9*	*29.7 ±4.8*	*28.3 ±1.8*	*28.3 ±2.4*	*29.7 ±9.6*
120	*mobility*	*52.0 ±6.0*	*52.5 ±12.9*	*53.5 ±5.2*	*64.3 ±5.0*	*65.3 ±3.7*	*68.5 ±6.2*	*67.3 ±8.8*	*74.0 ±4.9*	*69.3 ±9.4*	*68.8 ±4.2*	*65.5 ±4.8*
	progressive	*12.5 ±3.0*	*12.5 ±4.6*	*10.8 ±3.3*	*21.3 ±3.0*	*18.3 ±3.4*	*27.3 ±2.8*	*21.0 ±5.1*	*18.5 ±1.8*	*23.5 ±8.4*	*23.5 ±4.6*	*22.3 ±6.2*
144	*mobility*	*45.7 ±8.2*	*45.7 ±8.2*	*31.7 ±16.6*	*36.3 ±7.4*	*37.0 ±4.7*	*60.3 ±7.6*	*69.0 ±2.5*	*68.0 ±5.0*	*56.3 ±3.3*	*61.0 ±3.6*	*61.7 ±0.3*
	progressive	*8.7 ±5.2*	*3.0 ±2.5*	*4.3 ±1.9*	*8.7 ±2.0*	*6.3 ±2.4*	*13.0 ±6.6*	*16.0 ±6.0*	*14.7 ±0.3*	*14.0 ±1.5*	*15.0 ±3.5*	*13.3 ±4.5*
168	*mobility*	*25.8 ±10.0*	*33.5 ±13.9*	*21.5 ±11.0*	*45.3 ±8.4*	*43.0 ±18.4*	*47.5 ±8.9*	*48.8 ±6.9*	*57.8 ±2.5*	*56.5 ±7.5*	*45.8 ±6.1*	*48.8 ±12.8*
	progressive	*6.0 ±5.4*	*3.8 ±1.9*	*2.5 ±2.2*	*6.8 ±4.9*	*13.3 ±7.5*	*12.0 ±5.6*	*5.5 ±1.7*	*11.8 ±5.7*	*7.5 ±2.0*	*4.0 ±1.3*	*6.3 ±2.8*
192	*mobility*	*15.3 ±8.3*	*12.7 ±7.2*	*21.0 ±3.8*	*31.0 ±6.9*	*37.0 ±14.0*	*44.3 ±5.8*	*50.3 ±6.1*	*53.0 ±3.5*	*45.3 ±11.8*	*29.0 ±5.7*	*44.3 ±9.8*
	progressivee ssive	*1.0 ±0.6*	*1.0 ±0.6*	*1.3 ±0.3*	*1.7 ±0.7*	*5.7 ±3.7*	*6.0 ±2.0*	*7.0 ±4.5*	*8.0 ±3.5*	*8.7 ±5.4*	*2.0 ±0.6*	*3.7 ±1.5*

The experimental data obtained show that the tested drug BD-1 is not toxic to spermatozoa in the tested concentration ranges. After dilution of the sperm with various test media, the motility of the sperm was within 80%, and the number of spermatozoa with straight-line movement was within 40%.

With an increase in the storage time of diluted sperm at 2–4°C, these figures sharply decreased. After 144 hours of sperm storage, sperm motility in the experimental group, where the concentration of the drug BD-1 was in the range of 6-7% was 68-69%, whereas in the control group this indicator decreased and amounted to only 45.7 ± 8.1%.

Similar changes have occurred with the number of sperm with a straight-line movement. In the experimental group, where the concentration of BD-1 was 6-7% after 144 hours of sperm storage, this indicator ranged from 16 to 15%, whereas in the control group, this indicator was 4.7%.

Investigation of the effect of seed dilution on the safety of spermatozoa by cooling was carried out using spermatozoa from designated rams. Ejaculates were diluted 1: 3 and 1: 4. Samples were kept at four degrees Celsius, sperm motility was determined regularly at established intervals (24 hours) until it reached zero. The breeding medium was prepared in the laboratory of the Scientific and Practical Institute of Biotechnology in Animal Husbandry and Veterinary Medicine.

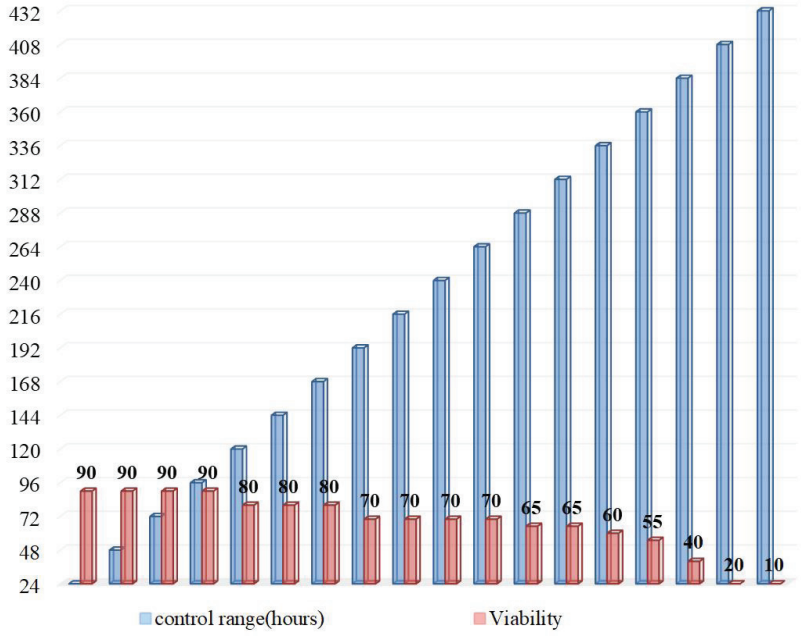

Figure 1. Viability of diluted sperm 1:3

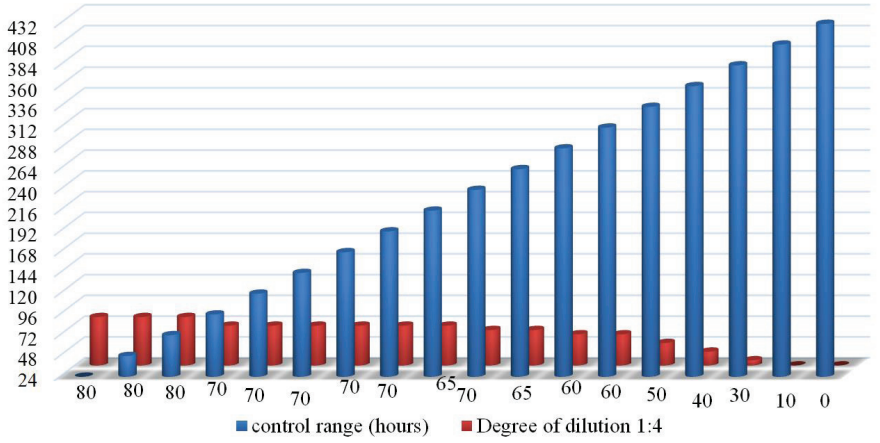

Figure 2. Viability of diluted sperm 1:4

From the data in diagram 1 and 2 it can be seen that, regardless of the degree of dilution, the ability to maintain sperm motility has longer periods. The best results are achieved if the dilution is 1: 3. In this case, after 6 days, the mobility of chilled sperm is still 80%, decreasing to 55% on the 15th day and finally 10% (18th day)

As a result of the research, an improved synthetic medium of the following composition was proposed: for 100 ml of bidistilled water, glucose 0.8 g, sodium citrate - 2.8, chicken egg yolk - 20 ml, BD-1 - 6% and antibiotics.

The proposed improved environment was tested under production conditions by artificial insemination of sheep. After production, the sperm was diluted 1:2 and 1:3 and stored in a refrigerator at 2-4℃. The heat period at sheep's was detected by the special ram-detector. Insemination is twofold. Data on the results of artificial insemination of sheep are presented in Table 3.

Table 3. Results of artificial insemination of sheep

Inseminated sheep, heads	camed back in the period of estrus	
	head	%
74	30	40.5

The data presented show that 30 days after the last insemination 30 heads came to the hunt again, which is 40.5% of the initially inseminated.

CONCLUSIONS

Based on the results of the research it was found:

1. Our improved environment contributes to better preservation and functional usefulness of sperm.
2. Our improved environment contributes to better survival of sperm after thawing (this is confirmed by the results of sperm motility after thawing).

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PROTON TRANSVERSE RELAXATION TIMES OF FREE AND BOUND WATER IN RAT LIVER AND RED BLOOD CELLS FED ON CHOLESTEROL REACH DIET. THE EFFECT OF TREATMENT WITH EUTROPHIC MEDICATION

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Abstract

Our study done on experimental hypertensive old rats model using reach cholesterol diet associated or not with rejuvenating drugs (Aslavital/procaine was designed to investigate biophysical parameters such as the proton transverse relaxing times of intracellular free and bound water in liver tissue and of membrane permeability to water by 1H NMR method..Biological material: 32 male white Wistar rats aged 24 months old were divided into 4 groups of 8 rats each. Group A (control) group (B) received only reach cholesterol diet for 6 weeks without associated eutrophic substances. Group (C) which received high cholesterol diet associated with Aslavital treatment for 6 weeks 4mg/kg body weight intraperitoneal injections(ip). Group (D) has received associated treatment with Procain 4mg/kg body weight in order to test the antiatherosclerotic properties of this rejuvenation drug. In liver tissue, bound water , characerised by structural parameter T_{21} *presents a slight decrease în treated groups, comparatively with Control group, while free water characterised by structural parameter* T_{22}*, decreases following cholesterol administration and increases under Aslavital and Procaine treatment, in the case of Procaine this effect is more powerfull above the control values. In the case of intraerythrocyte water there are very subtile changes , but which remind of those which take place in the liver. As a mirrror reflexion of the behaviour previosly described , the proton trannsverse relaxing time of plasma water increases slightly in cholesterol treated rats and decreases under the actions of administrated drugs ,with a more pronounced effect under Procaine treatment. Conclusion: Liver hypertophy is associated with a decrease in proton transverse relaxing times as well as in the proportion of free water(m_2%), associated with the decrease in proton transverse relaxing times as a consequence of the increase in protein content of liver tissue.The global decrease of proton transverse relaxation time (T_2) in liver is mainly due to changes in the free and bound water ratio and of a decrease in proton transverse relaxing times of free water(T22) and bound water(T21). The effects of Procaine are much more increased. In general, Aslavital is behaving like a buffered Procaine with a more adequate action. There were cases in which both drugs had the same effects:for example a decrease in proton transverse relaxing time* T_{22}*, characteristic of bound water structural parameter, or did not manifest any influence upon proton transverse relaxation time* T_{21} *charateristic for free water structural parameter from liver.*

Key words*: cholesterol, rejuvenating drugs, liver, blood cells.*

INTRODUCTION

The recent data upon atherosclerosis origin have initiated a strong debate regarding the preponderant role of hypercholesterolemia in the onset of this disease in counterpart with the idea that atherosclerosis could have its origin in an inadequate immune response due to presence of vascular alterations. Despite these data an impressive amount of experimental research have shown that atherogenesis is initiated under the reciprocal influence between cholesterol, cytokine cellular secretion (IL-6 especially), appolipoprotein E and the arterial wall (Balta, 2009). Recent data have shown that cells posses two types of sensors for cholesterol: Ck receptors sensitive for extracellular cholesterol which initiate the signalling pathway responsible for gene regulations implicated in the cell cycle, cell death and homeostasis of cell cholesterol level and cytokine including IL-6 and LxR alfa receptors sensitive to intracellular oxysterols and control genes implicated in cell death, cellular cholesterol homeostasis and cytokine IL-8 (Balta, 2009).

The understanding of membrane permeability mechanisms to water and of changes in intracellular water structure will might improve the actual view about various diseases in which

water transport is directly involved or the medication influences the cellular water state (Balta, 2009). Such aspects are well revealed by the most modern nuclear magnetic resonance (NMR) techniques (Gatina et al., 1998; Petcu et al., 1995).

Water crosses cell membranes by two routes: by diffusion through the lipid bilayer and through water channels (aquaporins) (Benga, 2012).

They were termed initially as major intrinsec proteins (MIPs) but now are also known as water channels, glycerol facilitators and aquaglyceroporins, yet recent studies suggest that they facilitate the movement of other low molecular –weight metabolites as well (Zhang et al., 2007). AQP-1is found in erythrocyte membranes, in epitelia and its expression was recently confirmed in the arterial wall and in capillary endothelia in the smooth muscle vascular cells and in the atherosclerotic plaques (Shanahan et al., 2000).

Taking into account this distribution it might be supposed that vascular cells and erythrocyte membrane permeability too water is well correlated; they being modulated by the same AQP-1, controlled by the same circulating factors. The role of arginine vasopressin and atrial natriuretic peptide in aquaporine regulation of water channel activity (Schrier et al., 2001).

These aspects facilitate evaluation of cardiovascular status by NMR relaxometric measurements of blood erythrocytes. This is a most suitable technique for studies of erythrocyte membrane permeability in physiological and pathological states such as arterial hypertension experimentally induced by feeding rats on reach cholesterol diet. Erythrocyte membrane has the capacity to renew during its life span (120 days) and imagistic is a useful tool to evidence modifications in water permeability and the results may contribute to a better understanding of aging process as well as pathological mechanisms of arterial hypertension (Stoian et al., 2012; Marin et al., 2018).

OBJECTIVE

Our study done on experimental hypertensive old rats model using reach cholesterol diet associated or not with rejuvenating drugs (Aslavital/Procaine was designed to investigate biophysical parameters such as the proton transverse relaxing times of intracellular free and bound water in liver tissue and of proton transverse relaxation times of intraerythrocyte and plasma water by 1H Nuclear Magnetic Resonance (1H NMR) method.

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MATERIALS AND METHODS

Biological material

Our study has been done on: 32male White Wistar rats aged 24 months old divided into 4 groups of 8 rats each: group A control, group B fed for 6 weeks on reach cholesterol diet only, group (C) and (D) received along with reach cholesterol diet also rejuvenation drugs treatment as follows: group (C) treated with Aslavital injections (4 mg/kg body weight intraperitoneal (IP); Aslavital contains in its composition; Procaine chlorhydrate, glutamic acid (as activator factor) and benzoic acid (as an antiaterogenic factor). This drug has a regenerative eutrophic, antiatherogenic (lipotrop) action and regulates fat metabolism and cholesterol levels which is used for prophylactic and curative treatment of cerebral and cardiovascular aging (Aslan, 1962; MacFarlane, 1975) restoration of the deformability of 'irreversibly' Sickled Cells by Procaine Hydrochloride.Group (D) –which received along with reach cholesterol diet for 6 weeks tretament with Procaine injections Procaine solution 4 mg/kg body weight (IP). Procaine is an ester composed of PABA (para-amino benzoic acid) and DEAE (diethyl amino ethanol). Both of these are water soluble B-vitamins. PABA stimulates the production of folic acid and vitamins K and B1. It has its greatest beneficial effects in the hair, glands and intestines (Aslan, 1962; MacFarlane, 1975). DEAE is a precursor to choline and acetylcholine. These factors are well known for their importance in nerve function. 9 Esters typically are joined by weak covalent bands. In the case of Procaine, the looseness of the bonds allows the PABA and DEAE to enter the body easily. Once inside, they separate and pursue their singular missions. PABA has an electrical charge, which makes it difficult to absorb. When joined together in the procaine molecule, however, PABA and DEAE become ionized.

Since they no longer have a charge, the body readily attracts and absorbs them (Aslan, 1962; MacFarlane, 1975). It has been obtained restoration of the deformability of 'irreversibly' Sickled Cells by Procaine Hydrochloride treatment (Baker et al., 1975).
Procaine treatment has been done in order to establish if this drug formula is efficient in preventing atherosclerotic effect of high reach cholesterol diet.
After 6 weeks treatment, rats have been antestethised with Na penthobarbital and peripherial blood was harvested on heparin and an adequate volume of $MnCl_2$ in such a way to obtain in extracellular compartment a concentration of 20 mM $MnCl_2$.
Then the animals were sacrificed by cervical dislocation, 24 hours after the last dose, then thoracic cage was opened and fragments of liver of $1cm^3$ were collected for assessment of T2 by1H NMR.
Samples were stored on ice until NMR measurements had been done (within maximum 1h) in order to prevent biochemical damage of tissue. Before the assay, the samples were brought to room temperature (24^oC).

Determinations of 1H Nuclear Magnetic resonance (1H NMR)

^1H NMR method has been used for evaluating Proton transverse relaxing times of free and bound water in liver, as well and Proton transverse relaxation time changes in free water against the total water ratio from liver under the effect of Cholesterol treatment associated or not with Aslavital or Procaine modifying intra erythrocyte water and also of extra erythrocyte water, as well as for evaluation of times for exchange of water and calculus for water permeability.
The method`s principle: Consists of characterising of a system composed of two compartments - A and B –of the two relaxing times - T2a and T2b – of the same type of nuclei originating from the same compartment (Revnic et al., 2007).
^1H NMR determinations have been done at room temperature on a ^1H NMR AREMI`78 Spectrometer (0.6T; proton resonance in impulses at a frequency of 25 MHz. The estimation of T2 was done by CARR-PURCELL-MEIBOOM-GILL pulse sequence, with 32 spin chhoes ranging from 8 to 256 ms after the 90 degree pulse,each point being the average of 16 measurements with an interval of 1 ms between impulses (Revnic et al., 2007).

Proton transverse relaxation times and data processing

The data were fitted to a bi-exponential as well as to a monoexponential curve. X^2 values were analyzed using the Student *t* test, and we arrived to the conclusion that a model with two relaxation times is adequate and therefore, we attributed the two relaxation times obtained from our data to bound water which are engaged in supporting motion of protein chain substrate and free water in which a large number of solutes are dissolved.
The two values obtained for T2 by means of a computerized program were T21for the bound water and T22 for the free water. Literature data (Gillis et al., 1991) pointed out that at high frequencies(between 10-200 MHz, bound water may be used in its usual meaning ,that is the hydration shell bound to the protein by electrostatic interactions.
The obtained relaxation times ascribed to the bound water and free water are the apparent times of the considered compartments.
It have been measured transverse proton relaxing times in intracellular compartment in the presence of water exchange between intracellular and extracellular compartment fed with Mn^{2+} obtaining in such a way the apparent relaxing time $T2^1$.

RESULTS AND DISCUSSIONS

Figure 1 represents the value of transverse proton relaxing times (T2) of water in liver of control and treated animals. There is a decrease in this parameter in cholesterol treated animals, which is more obvious in groups which received Aslavital treatment; increase values than in controls were observed also in Procain treated . In other words, Aslavital and Procain have different effects upon water from animals` liver .Globaly, water becames more bound in case of Aslavital treatment than in case of Procaine.
Analiysing in detail proton transverse relaxing times of bound water and free water from liver of control and cholesterol and Aslavital and of

cholesterol and Procaine treated rats (Figure 2), we can observe that bound water , characerised by T_{21} structural, presents a slight decrease în ltreated groups, comparatively with Control group, while free water characterised by structural parameter T_{22}, decreases following cholesterol administration and increases under Aslavital and Procaine treatment, in the case of Procaine this effect is more powerfull above the control values.
Modification of negative sense of transverse proton relaxing times , therefore gradualy reduction of the degree in mobility of proton from the structure of bound and free water, in the liver of treated animals are due to modifications in free water to bound water ratio – bound water in the detriment of quantity of free water, which decreases at half versus Controls (Figure 3). It must be mentioned that the treatment with above mentioned drugs, even modifies the structure of free and bound water does not act upon proportion of free water quantity.

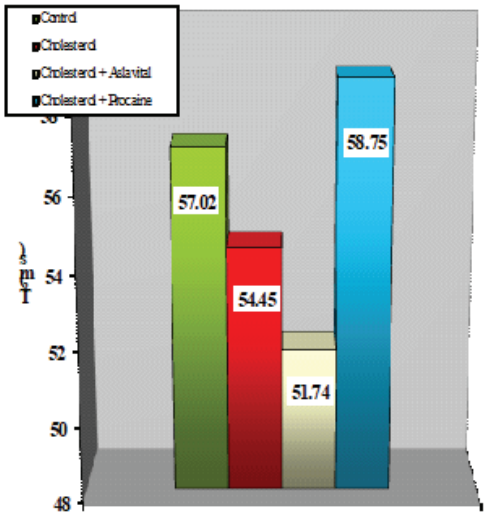

Figure 1. Proton transverse relaxation times of water (T_2) from control rat liver treated with cholesterol, Aslavital and Procaine in different combinations

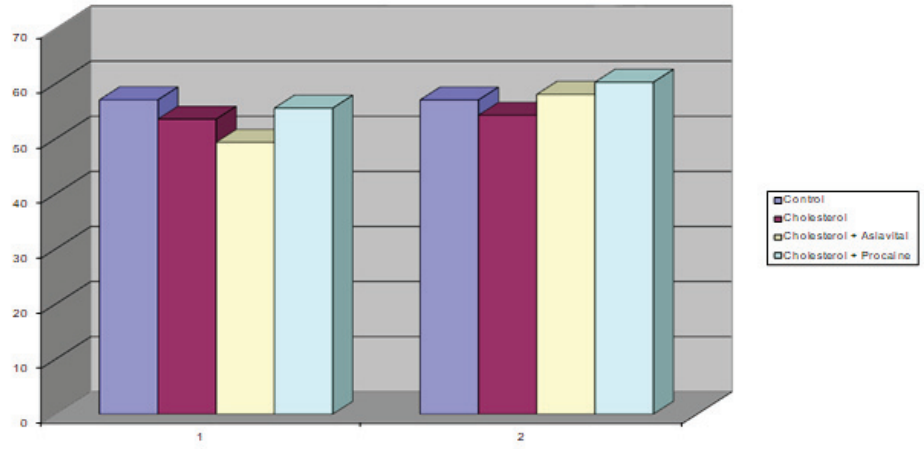

Figure 2. Proton transverse relaxation times of free (T21) and bound (T22) water from control rat liver or from rats treated with cholesterol, Aslavital and Procaine in different combinations

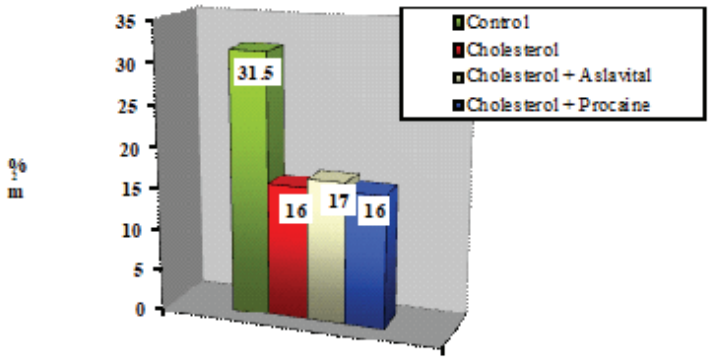

Figure 3. Free water mass ratio against total quantity of water mass from control rat liver and from trated rats with cholesterol, Aslavital and Procaine

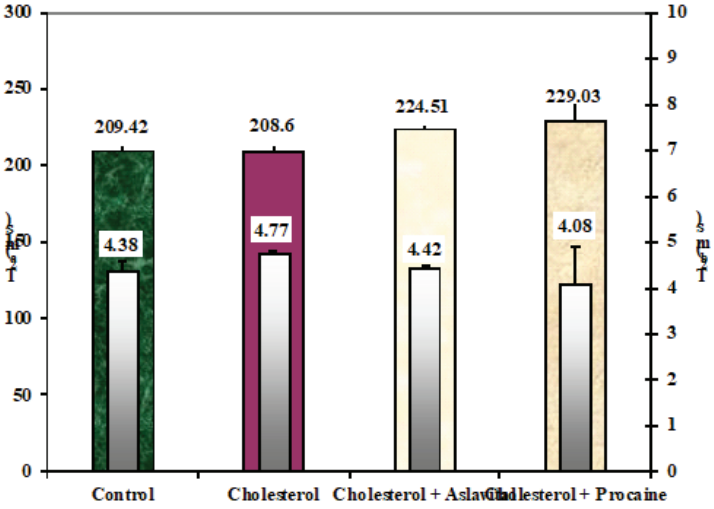

Figure 4. Proton transverse relaxing times of plasma water (T2b) and intraerythrocyte water (T2a) in control rats and in treated rats with cholesterol, Aslavital and Procaine

In the case of intraerythrocyte water there are very subtile changes , but which remind of those which take place in the liver.

Therefore, the transverse proton relaxing time of intraerythrocyte water ($\mathbf{T_{2a}}$) decreases slightly in cholesterol treated group,but thne under the effect of Aslavital and Procaine to slightly increase. (Figure 4*)*.

As a mirrror reflexion of the behaviour previosly described, în plasma the proton trannsverse relaxing time ($\mathbf{T_{2b}}$) of plasma water increases slightly in cholesterol treated rats and decreases under the actions of administrated drugs, with a more pronounced effect under Procaine treatment.

CONCLUSIONS

There is a global decrease of proton transverse relaxation times in rat liver which is mainly due to changes in the free($\mathbf{T_2}$) and bound water ($\mathbf{T_1}$) ratio.

Liver hypertophy is associated with the decrease in these proton transverse relaxing times as well as in the proportion of free water (m_2%), associated with the decrease in proton transverse relaxing times as a consequence of the increase in protein content of liver tissue.

There are cases in which the two drugs Aslavital and Procaine have the same effects ie: either lead to a decrease in liver of proton

transverse relaxing time of bound water ($\mathbf{T_1}$) or do not act upon a certain parameter of free water ($\mathbf{T_2}$) mass ratio of liver.

In the case of intraerythrocyte water there are very subtile changes, in proton transverse relaxing times but, which remind of those which take place in the liver.i.e.

The proton transverse relaxing time of intraerythrocyte water ($\mathbf{T_{2a}}$) decreases slightly in cholesterol treated group, but under the effect of Aslavital and Procaine they manifest a slight increase.

In plasma the proton trannsverse relaxing time of plasma water ($\mathbf{T_{2b}}$) increases slightly in cholesterol treated rats and decreases under the actions of administrated drugs, with a more pronounced effect under Procaine treatment.

Membrane permeability to water (MPW) may be accounted for as an index of recovery of cardiovascular system, important in maintaining a dynamic equilibrium with phenomenon of vascular destruction due to the high blood pressure.

Aslavital /Procaine treatment exhibits upon the studied parameters effects which emerge in the same direction, the Procaine effect is more pronounced. In general, Aslavital is behaving as a buffered Procaine with a more adequate action.

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THE RELATIONSHIP BETWEEN ABUNDANCE, DIVERSITY WITH COWS SKIN DEFECTS ACCORDING TO DIFFERENT ALTITUDE, HUMIDITY AND TEMPERATURE IN THE REGION OF SOUTHERN MINAHASA

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Abstract

This study aims to study the relationship between diversity and abundance of flies with cow skin defects according to differences in temperature and humidity in North Sulawesi. This research was carried out in two different regions: in the highland region, and in the lowland region. In the highland data was collected in cattle farms in Minahasa District such as in Kawangkoan and in Tompaso. The activities in lowland realized in South Minahasa Regency such as in Tengah, Poigar. Identification of fly species carried out according to differences the temperature and humidity. The results showed that insect abundance in cows skin defects was significantly affected by humidity (P = 0.023), but was not influenced by altitude (P = 0.341) or temperature (P=0.145). Cow skin defect was influenced by 66.8% by height, humidity, temperature, abundance, and diversity, and the remaining 43.2% was influenced by other factors. The diversity has a significant influence by altitude (P=0.034) and temperature (P=0.048) but was not the same to the humidity (P=0.138). It was obtained R2 value of 0.085 or 8.5% which meant that the diversity was influenced 8.5% by altitude, humidity, and temperature, and about 91.5% was influenced by other factors.

***Keywords**: abundance, diversity, path analysis, skin defect.*

INTRODUCTION

Flies as ectoparasites in cattle in Indonesia have become a priority for mitigation, but still lack on information such as a list of species that are existing cow skin defects in this area, including their biological and ecological geographical distribution. Koningsberger (1903), revealed the infestation of flies to livestock in Indonesia and reported findings in the form of *Tabanus ruficantris* flies, *Chrysops dispar, Stomoxys calcitrans* and *Haematobis exigua* sp. Partoutomo et al (1981), reported that the types of flies found in cattle located in North Sulawesi were, *Haematobia irritans, H. exigua, Sarcophagi* sp., *Musca conducens.*

The flies faund in cattle farm such as *Muscidae, Calliporidae, Tabanidae* and *Hippoboscidae*, and *Stomoxys calcitrans* fly cages (Muscidae) both male and female are blood eaters which are carried out twice a day. Haematobia irrtans and *Haematobia exigua* (Muscidae) known as cattle flies that found in Indonesia (Toar et al., 2018), India, Malaysia, China, Philippines and America (Soulsby, 1982). Both female and male blood-sucking flies, the main landlady are buffalo, cattle and horses (Rumokoy et al., 2017). The high or low diversity and abundance of flies depends on the season, rainfall, humidity, this condition depends a lot on the height of the cattle center area.

According to Kette (1977), it is estimated that a cow can tolerate flies of up to 100-300 birds, without adverse effects, but in fact 500 - 1000 tails even 5000 flies are found in this animal and are at very risk of causing negative influences, such as a decrease Animal weight can also cause skin quality problems, with injuries to livestock. According to Herms and James (1961), it is estimated that 100 - 300 flies on an animal can still be tolerated without adverse effects, but 500 flies will have a significant effect on livestock health. The diversity and abundance of flies in cattle according to geographical area (altitude) of livestock centers can be identified after capture.

Some fly species are known to play a very large role in cow skin defects. Flies live foraging on wounds to potentially increase the damage to cow skin. Mites, ticks and mites are ectoparasites whose entire life depends on the body of the host (cattle), with the mouth part used to pierce and suck the blood of livestock.
Flies from various species that cause a skin defects can be found in almost all parts of the animal's body, many contributing to increasing damage to the tails of livestock. Furthermore, fly density is a condition for a species to enlarge the skin defect, so it is important to know the diversity, abundance, breeding of flies in an area and type of defect and the number of defects and defect positions according to the overall topography of the animal's body.
The distribution of diversity and abundance of flies that cause skin defects is important to be studied according to differences in temperature and humidity with regard to the extent of defect, type of defect, number of defects and position of defects in North Sulawesi.

MATERIALS AND METHODS

This research was realized in two different regions: in the highland region, and in the lowland region. The data from highland was collected in cattle farms located in Minahasa District such as in Kawangkoan and in Tompaso. The activities in lowland realized in South Minahasa Regency such as in Tengah, Poigar. Identification of fly species carried out according to differences of temperature and humidity in high and low land. Data were analysed by using Ordinary Least Square (OLS) analysis according to Faraway, (2002) and described a path effect of each variable.

RESULTS AND DISCUSSIONS

The effect of height, humidity, and temperature on abundance is illustrated in the Table 1.
The R2 value 0.059 showed that abundance was low affected by height, humidity, and hemperature, and the remaining 94.1% is influenced by other factors. The first equation obtained is:
Abundance = 0.114 Z Altitude - 0.369Z Humidity - 0.211 ZSuhu + e^1

Table1. OLS on Abundance (Y_0)

Independent variable	Beta coefficient	T_{caunt}	Sig
Height	0.114	0.958	0.341
Humidity	-0.369	-2.324	0.023
Temperature	-0.211	-.469	0.145
$R^2 = 0.059$, $t_{tabel} = 1.987$ Dependent variable → abundance			

Table 1 represent a path coefficient obtained from the OLS beta coefficient between the altitude variable to Abundance is 0.114, with a t_{count} of 0.958 and Sig of 0.341. Because the absolute value of $t_{count} < t_{tabel}$ (0.958 <1.987) and Sig> 0.05 (0.341> 0.05), it can be concluded that there is no influence of Altitude on Abundance. This means that regardless of the height value, it will not affect the high or low abundance.
The beta coefficient of humidity variable on abundance was negative 0.369, with a t_{count} of negative 2.324 and sigmificance value of 0.023. Because the absolute value of $t_{count} > t_{table}$ (2.324> 1.987) and Sig <0.05 (0.023 <0.05), then there was an effect of humidity on bundance. Because the path coefficient is negative (-0.369) indicated a negative relationship. This means that the higher the Humidity then the less abundance will be resulted.
Path coefficient was obtained from the beta coefficient of OLS results of temperature variables to Abundance was negative 0.211, with t_{count} value -1.469 and Sig of 0.145 which the absolute value of $t_{count} < t_{table}$ (1.469 <1.987) and Sig> 0.05 (0.145> 0.05) showed that there was no influence of temperature on abundance. This means that regardless of the temperature value, it will not affect the high and low abundance.
The effect of altitude, humidity and temperature on diversity decribed in Table 2.

Table 2. OLS on Diversity (Y_1)

Independent variable	**Beta coefficient**	**T_{caunt}**	**P**
Height	0.253	2.149	0.034
Humidity	-0.234	-1.497	0.138
Temperature	-0.285	-2.005	0.048
$R^2 = 0.085$, $t_{tabel} = 1.989$ dependent variable→diversity			

R^2 value of 0.085 indicated that diversity was 8.5% influenced by height, humidity, and temperature, and 91.5% influenced by other factors. The second equation obtained is: Diversity = 0.253 Altitude - 0.324 Humidity - 0.285 Temperature + e^2

Table 2 shows that the path coefficientobtained from the OLS beta coefficient between the altitude variable ondiversity was 0.253, with a t_{count} 2.149 and Sig 0.034.

Because the absolute value of tcount> ttable (2.149> 1.987) and Sig <0.05 (0.034 <0.05), it can be concluded that there is an effect of Altitude on Diversity. Because the coefficient of path is positive, it showed a positive relationship. This means that the higher the altitude, the higher diversity will result.

The magnitude of the path coefficient (obtained from the beta coefficient of OLS results) between the variables of Humidity to Diversity is -0.234, with the value of t count of -1.497 and Sig of 0.138. Because the absolute value of tcount <t table (1.497 <1.987) and Sig> 0.05 (0.138> 0.05) It can be concluded that there is no effect of Humidity on Diversity. This means that high and low humidity, will not result in a change in a lot of diversity.

The magnitude of the path coefficient obtained from the OLS beta coefficient between the Temperature variable to Diversity is -0.285, with a t-count of -2.005 and Sig of 0.048. Because the absolute value of t count> t table (2005> 1,987) and Sig <0.05 (0.048 <0.05), it can be concluded that there has an effect of temperature on diversity. Because the path coefficient has a negative sign indicating a negative relationship. This means that the higher the temperature, the lower the diversity will be.

The following is an analysis of the path effects of altitude, humidity, temperature, abundance, and diversity on cattle defects.

Table 3. OLS on Skin Defect (Y_2)

Variabel Independen	Beta	t_{caunt}	P
Height	0.214	2.885	0.005
Humidity	0.124	1.246	0.216
Temperature	0.129	1.441	0.153
Abundantly	0.711	10.937	0.000
Diversity	0.265	4.022	0.000
R^2 = 0.668, t_{tabel} = 1.989 dependent variable→Skin defect of cattle			

When R^2 value is 0.668, it means that cattle defect influenced by 66.8% by height, humidity, temperature, abundance, and diversity, and the remaining 43.2% is influenced by other factors. The third equation obtained is:

Defect Skin = 0.214Z Altitude + 0.124Z Humidity + 0.129 ZSuhu + 0.711 Z Abundance + 0.265 Z Diversity + e^3

Table 3 presents that the path coefficient obtained from the beta coefficient of OLS results between the height variable on skin Defect is 0.214, with a tcount of 2.885 and P of 0.005. Because the absolute value of t_{count}> t $_{table}$ (2.885> 1,989) and P<0.05 (0.005 <0.05), it can be concluded that there is an effect of Altitude on Cattle Defect. Because the coefficient coefficient is positive, it shows a positive relationship. This means that the higher the altitude, the higher the defect of the cow will be.

The magnitude of the path coefficient (obtained from the beta coefficient of OLS results) between the variables of humidity to skin defect is 0.124, with a tcount of 1,246 and P of 0.216. Because the absolute value of t_{count} <t_{table} (1.246 <1.989) and P>0.05 (0.216> 0.05) it can be concluded that there is no effect of Humidity on Cow Defects. This means that the high and low humidity, will not result in a change in the least amount of cow defects.

The magnitude of the path coefficient (obtained from the beta coefficient of OLS results) between the temperature variables on cattle skin defects is 0.129, with a t_{count} of 1.441 and P of 0.153. Because the absolute value of t_{count}<t_{table} (1.441 <1.989) and P>0.05 (0.153> 0.05) it can be concluded that there is no effect of temperature on skin defects. This means that the high and low temperatures, will not result in a change in the least amount of cow defects.

The magnitude of the path coefficient (obtained from the beta coefficient of OLS results) between the abundance variables on cattle defects is 0.711, with a tcount of 10.937 and P of 0.000. Because the absolute value is t count> t table (10.937>1.989) and P<0.05, it can be concluded that there is an effect of abundance on cattle defects. Because the path coefficient has a positive sign that shows a positive relationship. This means that the higher abundance, the higher the skin defect will be.

The magnitude of the path coefficient between the variables of diversity on cattle skin defects is 0.265, with a tcount of 4.022 and Sig of 0.000. Because the absolute value is $t_{count} > t_{table}$ (4.022>1.989) and P<0.05 (0,000<0.05), it can be concluded that there is a diversity of defects Cows. Because the path coefficient has a positive sign that shows a positive relationship. This means that the higher diversity, the higher the cattle defect will be..

The coefficient of total determination obtained represent as following equation:

$$R^2_{total} = 1- Pe_1^2 Pe_2^2 Pe_3^2$$

$$R^2_{total} = 1 – (1-R_1^2) (1-R_2^2) (1-R_3^2)$$

Where R12 = 0.059, R22 = 0.085, and R22 = 0.668, respectively, are the R square values of the first, second, and third equation models so that the total R2 value is 0.7141 or 71.41%.

From the causal relationship between variables on the Path diagram the total determination coefficient is 0.7141 or the information contained in the 71.41% data can be explained by the path model.

So that the results of the path analysis are feasible to use.

In addition to direct influence, in path analysis also known indirect effects.

Tabel 4. Path Analisis of Indirect Effect

Coeficient of Effect indirect	Coefisient of direct effect		Coef	Sig/Non
Hight→Abundance→skin defect	Hight →Abundance = 0.114 (ns)	Abundance →skin defect = 0.711 (s)	0.081	NS
Humidity→Abundance→skin defect	Humidity→Abundance = -0.369 (s)	Abundance →skin defect = 0.711 (s)	-0.262	S
Temp.→Abundance→skin defect	Temp.→Abundance = -0.211 (ns)	Abundance →skin defect = 0.711 (s)	-0.150	NS
Hight →Diversity →skin defect	Hight →Diversity = 0.253 (s)	Diversity →skin defect = 0.265 (s)	0.067	S
Abundance →Diversity →skin defect	Kelembaban →Diversity = -0.234 (ns)	Diversity →skin defect = 0.265 (s)	-0.062	NS
Temp.→Diversity →skin defect	Temp.→Diversity = -0.285 (s)	Diversity →skin defect i = 0.265 (s)	-0.076	S

Note: Temp. = temperature, S=significance, NS=non significance

Based on the table above, the indirect effect of Altitude on cattle defects through Abundance obtained an indirect coefficient of 0.081. Because the direct influence (altitude on abundance, and abundance on skin defec of cattle) is not significant, this indicates that there is no indirect influence significant between the height of the defect of cattle through abundance. This means that regardless of the value of Altitude, it will not result in a change in Cow Defect even though Abundance increases or decreases.

The indirect effect of Humidity on Cattle defects through Abundance obtained an indirect coefficient of -0.262. Because the direct influence (Humidity to Abundance, and Abundance on Cattle Defect) are both significant, this indicates that there is a significant indirect effect between Humidity to Cattle defects through Abundance. With an indirect coefficient that has a negative sign, it indicates a negative relationship. This means that the higher the Humidity value, the greater the Cattle Defect if there is less abundance.

The indirect effect of temperature on defects of cow skin through abundance obtained an indirect coefficient of -0.150. Because the direct influence (temperature to abundance, and abundance of cattle defects) was not significant, this indicates that there is no significant indirect effect between temperature against defects of cattle through abundance. This means that regardless of the value of temperature, it will not result in changes in cattle defects even though abundance increases or decreases.

Indirect effects of height on cattle defects through diversity obtained an indirect coefficient of 0.067. Because the direct effect (Altitude on Diversity, and Diversity on cattle defects) is both significant, this indicates that there is a significant indirect effect between the height of the cattle defect through diversity. The coefficient with a positive sign indicates a positive relationship. This means that the higher the altitude (terrain), the higher the Cow Defect will be, if the diversity is also higher.

The indirect effect of moisture on skin defects through diversity obtained an indirect coefficient of -0.062. Because one of the direct effects (humidity on diversity, and diversity on cow defects) is not significant, this indicates that there is no significant indirect effect between moisture on cow skin defects through diversity. This means that regardless of the value of

humidity, it will not result in changes in the defect of cow skin even though the diversity is getting bigger or smaller.

Indirect effects of temperature on cattle defects through diversity obtained an indirect coefficient of -0.076. Because of the direct effect (temperature on diversity, and diversity on cattle defects) both were significant, this indicates that there is a significant indirect effect between temperature to cattle defects through diversity. With negative signatures the coefficient shows a negative relationship. This means that the higher the temperature, the greater the cattle defect, if the diversity is getting smaller.

CONCLUSIONS

From the results above, the following conclusions are obtained:

- Abundance Insects that cause cattle defects are only significantly affected by Humidity, but are not affected by height or temperature;
- Diversity Insects that cause cattle defects are significantly affected by height and temperature, but are not affected by humidity;
- Cow defects are influenced by height, abundance, and diversity of insects that cause cow defects.

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EUROPEAN LEGAL FRAMEWORK IN MANUFACTURING AND PROCESSING OF MILK. THE MILK PACKAGE. ELIMINATION OF MILK QUOTA. CASE STUDY: GERMANY

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Abstract

The paper aims to present the effects of the abolition of the milk quota system who was in force for more than 30 years, to analyse the new milk packake introduced by the European Union (since October 3, 2012, it is in full force). This package was prepared in 2015 with a view to the long term future of the dairy sector after the quota system expired. Also, we have analyzed and produced a study case for Germany, in respect of the two systems who were in force in EU, taking in consideration the role, position and place of the German milk market at European level.

Key words*: dairy cows, evolution, milk production, Romania.*

INTRODUCTION

The abolition of the milk quota system in 2015, agreed in the 2008 Health Check of the common agricultural policy (CAP) reform, created a new context for economic operators who have faced the milk quota system since its introduction in 1984.

In order to prepare the sector for this new operating environment, the European Commission has developed a number of new instruments in the context of the 'milk package' of 2012 as regards cooperation between farmers in the dairy sector.

The so-called "milk package" was published in May 2012, came into full force on 3 October 2012 and applies until 30 June 2020. The implementing regulations were published in June and September 2012.

The milk package aims to strengthen the position of dairy farmers in the dairy supply chain and to prepare the sector for a more sustainable and market-oriented future, in particular by drawing lessons as a result of the dairy market crisis in 2009.

Member States have now the option of making binding the conclusion of written contracts between milk producers and processors (Saraz et al., 2008).

Farmers have the possibility to negotiate, through producer organizations, the contractual clauses, including the price of raw milk. Specific rules at EU level for inter-branch organizations allow actors in the dairy supply chain to engage in dialogue and conduct a range of activities.

At the same time, they allow Member States to apply, under certain conditions, rules governing the supply of PDO / PGI cheeses.

RESULTS AND DISCUSSIONS

The milk sector has been intensively discussed during the negotiations on the CAP reform and the final agreement offers better market orientation along with a safety net for farmers in the context of external uncertainties, primarily through direct payments, but and with options for risk management under rural development programs, as well as with a new and flexible market crunch reserve in case of market difficulties.

Study case – Germany (biggest milk producer in European Union)

Milk production is the most important branch of German agriculture and the German dairy industry is the largest sector within the German food industry.

More than one fifth (21.0%) of the total cow's milk collected by dairy factories in the EU-28 in 2015 was collected in Germany, making it the biggest producer in EU.

About a quarter of agricultural holdings in Germany is producing milk.
Dairy farming in many regions is not only of great importance for the rural economic and labour market. It also makes an important contribution to preservation and care of grown cultural landscapes.
The use of grassland at many locations makes the landscape open and attractive for the living and recreation area and tourism.
By the year 2015, milk production in Germany, according to the Federal Ministry for Agriculture and Nutrition (BLE) has been steadily reaching its previous high of 32.69 million tonnes
In 2015, milk production was 14.1% higher than production in 2008 (28.66 million tonnes), or 4.3% over 2013 (31.34 million tonnes).
This growth trend continued at the beginning of 2016. So the quantities produced from January to May 2016 were higher with between 1.8 and 8.1% than in the previous year. Since June 2016, however, there has been a trend reversal here.
For example, in June 2016, milk production was at -1.8% for the first time below the previous year. This development continued and even intensified. In November the milk production was 5.7% below the previous year's level, or 3% below the production volume of the year 2014.
So, in 2016, Germany provided again almost 21% of the milk produced in EU (20.9), representing one fifth of the total, as we can see in the figure below, provided by Eurostat.

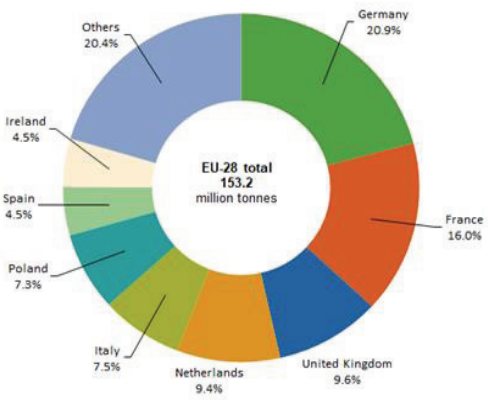

Figure 1 Milk production in EU (2016) – source Eurostat

In 2017 milk delivery again dropped slightly German. The decline amounted to 0.1 million tonnes, which is a daily decrease of 0.1%. This was the growing trend after the record 2015 interrupted for two consecutive years.
According to our analysis, with the data provided by Eurostat, starting with the reference year 2010, it is observed that the milk production in the country chosen as a case study (Germany) increased after the adoption of the milk package, with the exception of 2017 (data for 2018 not yet available in Eurostat format), when it recorded a slight decrease, as can be seen from the table below.

Table 1. Evolution of Milk Production (t/year)

Year	Production (1000 t)
2010	29.075
2011	29.764
2012	29.703
2013	30.301
2014	31.375
2015	31.879
2016	31.972
2017	31.937

The milk market remained volatile in 2018 as well. These is essentially due to a higher milk yield while global demand continued to grow more slowly than at the beginning of the year
The expansive tendencies in the milk supply in Germany and the EU is likely to continue.
Outside the EU, milk volumes are expected to continue rising, especially in North America. Oceania does not seem likely to increase if adverse weather conditions persist.
The milk market starts firmer in the year 2019 than in the previous year.
The moderate development of milk yield, which is below the previous year's level in Germany and the EU as a whole, contributes to this. Due to the feed situation, lower milk deliveries are expected in the first half of 2019 than in the previous year.
The drought also had a dampening effect on the milk due to the poorer feed qualities with higher crude fiber content.
As a result, milk volumes in Germany are unlikely to continue to grow in the 2019 or, depending on the weather and price trends, even decline.

Agricultural policy and legal framework
System change: dairy farming without quota
The liberalization of the EU dairy market has taken place in small steps over the years and

served the purpose of preparing for the abolition of the milk quota on 01.04.2015.
The abolition of the milk quota had become necessary because of the increasing integration of the EU dairy market into the world milk market through the reduction of foreign trade regimes, in particular the export refunds, which meant that the milk quota alone was not an effective measure to

Price support
The possibilities of "decoupling" the single market price level from the world market price level were no longer present.
This meant that milk producers were largely exposed to the world market in recent years, despite the existing quota system.
At the same time, they had to bear the financial burdens associated with the quota regime (especially the purchase and lease of milk quotas). The Milk Package 2012 was integrated into the CMO in 2014. It goes to the recommendations of a High Level Expert Group on Milk set up after the milk crisis of 2008/2009.
The aim of the regulations is to strengthen the position of producers within the supply chain. The following areas are affected by the regulations:
- recognition of producer organizations, their associations and industry associations;
- Member States have the option to legislate on raw milk supply contracts;
- a special antitrust exemption for recognized producer organizations and their associations, and contracts for the supply of raw milk to the affiliated farmers, to processors or collectors;
- Option for the Member States, a specific volume regulation for geo-protected cheese to adopt and renewal of a monthly reporting requirement for Member States and first purchasers of raw milk in relation to raw milk deliveries.
These rules have been included in the CMO, including special antitrust exceptions. The milk package was approved for a limited time until 30.06.2020.
The Commission has to report on the implementation and the effectiveness of the package. The last Report was presented on 24.11.2016.
In this last Report, the Commission notes that the use of the instruments anchored in the milk package are increasing.
Already 13 Member States use the possibility of mandatory contracts. Producer organizations were established and recognized in 11 Member States.
At the end of 2015, the number of producer organizations was up260, of which about 92% were recognized in Germany, France and Italy.
The Commission proposes, in its above mentioned report, the creation of producer organizations, who will continue to strengthen associations and industry associations.
In Germany, there was no deficit in bundling, even before the Milk Package came into force for the raw milk supply in producer organizations. So, the effects of the milk package were therefore limited in Germany, at least until now.
The mount of cow's milk production minus the quantities for the milk fed, for direct marketing and for natural withdrawals shall indicate the delivery volume of raw milk to dairy companies. The conventional Cow's milk produced and delivered to German dairy companies shows a significant increase in volume:
- in 1995, the delivery volume amounted to 26.9 million tonnes;
- in 2009, a quantity of 28.2 million tonnes was delivered;
- by 2016, the volume of milk delivered increased to 31.3 million tones.

In EU Terms, the amount of milk delivered throughout the EU has also increased in recent years:
- in 2009 were 134.2 million tonnes of milk delivered;
- in 2016, the quantity delivered is 153.2 million tonnes.

At European level, it is noted that the European milk package strengthens the position of milk producers within the supply chain
According to the latest European Commission report on the functioning of the so-called "milk package", European farmers are increasingly using the tools offered by the milk package, such as collective bargaining of contractual clauses through producer organizations or the use of written contracts.
The measure allowing collective bargaining is designed to strengthen the bargaining power of milk producers, while written contracts offer farmers greater transparency and better traceability.

The European Commission report shows that there are measures that can be taken at EU level to ensure a better position for dairy farmers in the supply chain.
The report also examines the additional possibilities for dairy farmers. For example, it highlights the potential of two key instruments of the Milk Package - Producer Organizations (OPs) and collective bargaining - which are not yet fully exploited by Member States, producer organizations and farmers, and outlines the different ways to increase their effectiveness, both at EU level and at Member State level.
In particular, Member States are encouraged by the milk package to take the necessary steps to stimulate the setting up of producer organizations to undertake collective actions that go beyond collective bargaining, thereby increasing the share of producers in the milk supply chain.
In addition to these recommendations, consideration should be given to extending the role of Inter-branch Organizations (OIPs).
For the full potential of the milk package to materialize, the report concludes that an extension of its application beyond 2020 should be considered.
The most important provisions of the milk package, the real key points are mandatory contracts and producer organizations.

Mandatory contracts (art. 148)
The Contracts establish the responsibilities of operators in the dairy chain, increase awareness of market signals, improve price transmission, adjust supply on demand and avoid unfair commercial practices. Following the abolition of the milk quota regime, they are a useful tool for producers and processors to plan their production volumes.
Under Article 148, Member States have the possibility to impose written contracts between farmers and processors and to force milk purchasers to offer farmers a minimum contract duration. These contracts should be drawn up prior to delivery and should contain specific elements such as price, volume, duration, payment details, collection and applicable rules in case of force majeure.
All these elements should be freely negotiated between the parties and farmers should have the right to refuse a minimum duration bid within a contract. Deliveries by a farmer who is a member of his cooperative are exempt from the obligation to draw up such contracts if the statutes or rules applicable within that cooperative provide for provisions having similar effects to the provisions described in the contract.
Seven Member States have stipulated that the contract proposed by the buyer to the farmer should have a minimum duration of 6 months, while Spain has opted for 1 year contracts and France for 5 years. Inspired by the Milk Package provisions in the United Kingdom, manufacturers and processors agreed on a voluntary code of conduct that provides for contracts to be drawn up under conditions similar to those specified in the Milk Package and covering 85% of raw milk production.
Also in Belgium a code of good practice was signed by 98% of the processors and the three most important farmers' organizations. This includes in particular quality agreements, arrangements for the notification period for farmers and purchasers, sustainability agreements and provisions on the role of producer organizations.
In Germany, for contracts negotiated through producer organizations, model contracts are used frequently, which in the future, in addition to quality, price and duration parameters will also include additional details on milk volumes.
Contracts have become particularly binding in Member States where the cooperative structure of contractual relations between producers and processors in the dairy sector has been less pronounced.
Almost 64% of all deliveries of cow's milk from the Community are carried out by farmers to processing cooperatives or to the processing cooperatives whose members are. In the case of France, no distinction could be made between deliveries to processing and collection cooperatives. As regards Germany, deliveries to private processors are mainly carried out through producer organizations or their associations.
Generally, the proportion of those types of contractual provisions is reported to be fairly stable in recent years. However, several Member States (EE, IT, LV, AT, SK) reported an increase in deliveries to private collectors,

although their share in absolute terms is rather limited.

Producer organizations (Article 152 (3))

Member States are required to formally recognize producer organizations (POs) set up by producers in the milk sector at the initiative of producers and pursuing a specific purpose which may include:

1. ensuring planning and adaptation of production on demand, particularly in terms of quality and quantity;
2. the concentration of supply and the placing on the market of the products obtained by their members;
3. optimizing production costs and stabilizing producer prices. Member States may set a minimum number of members and / or a minimum volume of marketed production that producer organizations must meet in order to be recognized.

All recognized producer organizations focus on the production of cow's milk, with the exception of one in Spain focusing on the production of sheep's milk.

A considerable number of producer organizations out of a total of 228 in the EU dairy sector, particularly in Germany and Italy, existed before the entry into force of the Milk Package.

However, the number is rising.

An association of producer organizations was recognized in Germany, resulting in a total of two. In several Member States, national recognition legislation has only recently entered into force.

The rather large variation in minimum requirements indicates the difficulty of striking a balance between the ambitious target for large producer organizations which has the potential to increase the bargaining power of producers and encouraging the establishment of POs by setting realistic thresholds.

However, it should be noted that, in the second stage, several producer organizations can reunite in an association of producer organizations which have the same possibilities of collective bargaining as a producer organization, but at a larger scale.

The possibility of extending certain rules applicable to recognized producer organizations and their associations (as well as to interbranch organizations) to non-member entities and to compulsory contributions by entities not qualifying under the reformed CAP is also currently applicable to sector organizations milk and is expected to stimulate the creation of representative organizations.

As regards the incentives offered for adherence to producer organizations, the reformed rural development policy offers in particular the following possibilities:

- in the 2014-2020 period, support for the creation of producer groups was extended to producer organizations;
- the new cooperation measures (which are potentially open to producer groups, cooperatives and interprofessional organizations) provide opportunities to support, for example, the development of new products and practices, short supply chains and local markets, as well as cooperation with small operators in organizing common working processes and sharing facilities. Combined with support under the investment measure, collective investment can benefit from higher aid rates (a possible increase of 20 percentage points);
- farmers groups can also benefit from a range of rural development measures such as investment support, participation in quality systems and information / promotion activities, agricultural, environmental and climate measures, etc.

The package also sets specific EU-wide rules for inter-branch organizations, allowing actors in the dairy supply chain to engage in dialogue and conduct certain activities, and Member States may, under certain conditions, apply rules regulating cheese offerings.

The package also involves a number of measures to increase market transparency. The measures foreseen in the milk package will be in place by mid-2020.

The Commission was mandated to report in 2014 and 2018 on the market situation and the implementation of the measures. These reports (to the European Parliament and the Council) should in particular assess the effects on milk producers and milk production in less-favored regions and include any incentives to encourage farmers to enter into joint production agreements.

CONCLUSIONS

Brexit, Common Agricultural Policy after 2020
Brexit consequences are not yet clear.
The last year of this decade begins with political uncertainties that could affect the milk market in the remainder of the year.
Uncertainty is currently mainly caused by Brexit. The vigorously growing production volumes in New Zealand and the USA also pose problems for European exports.
But even 2018 was a year of ups and downs: milk producer prices rose again in the course of the year. For a long time, milk supply has also increased. Only the drought in the summer caused less milk.
At the beginning of 2019, deliveries were around 3.3 percent down on the previous year.
Overall, however, a new record delivery of an estimated 32.4 million tonnes is expected for the past year 2018. EU milk deliveries are roughly at the high level of the previous year and show a similar course to that in Germany, but not so strongly.
The effects of the upcoming Brexit are clear. It is still unclear what market participants will expect from March 2019 onwards.
The signs point to a hard Brexit (present time, april 2019),which would lead to tariffs on the various dairy products and costly and complex customs clearances, is likely to have a negative impact on the cheese market in the EU, in particular, as the United Kingdom is a large net importer of cheese.
In the particular case of Germany, this country's dairy industry is not a friend of Brexit. To evaluate correctly the impact of Brexit on Germany we should look first at the economic informations.
In 2018 the trade volume between the United Kingdom (UK) and Germany amounted to 119 billion euros. Approx. 750,000 german jobs depend on the trade with UK.
Until present time, German companies have established investments worth over 140 billion euros. In UK are located approx. 2,500 branches of German companies that employ more than 400,000 people.
Viceversa, UK companies have 1,500 branches in Germany with around 270,000 people.
The United Kingdom is an important trading partner for dairy products. A "hard Brexit" would have unforeseen consequences for the milk market. Many countries are expected to be affected, Germany, Ireland and others in particular.
The administrative part of the process is not only threatened by high tariffs on third countries, but also accompanied by the need for customs controls with a high expenditure of time at the border.
To this end, the two ex-partners EU and UK would initially treat each other as third countries in the exchange of goods and services and also introduce veterinary controls again.
The milk markets have just stabilized after the turn of the year, while threatened by a "hard Brexit" and the resulting trade distortions would have negative consequences for the European dairy industry.
The use of Brexit's diminishing EU funds is giving rise to agricultural policyeven more in competition with the other EU policies.
Also, competition for the funding of the two pillars within the CAP will worsen.
When designing post-2020 CAP measures, the challenges faced by livestock farms will need to be given special consideration.
Business and politics must master these challenges together. Coping the recent milk crisis was a strenuous effort - including public budgets and the need for a critical situation analysis and structural changes is substantiated. There is an urgent need to make difficult provision for the future.
For the first time, the Milk Package 2012 has created the opportunity to recognize dairy industry associations. These are agricultural organizations in which, in addition to the producer, at least one further level of the value added chain must be represented (processing and / or trade).
Background of this new regulation was the realization that the dairy industry for a well-defined catalog of topics should be able to arrive at joint solutions across stages without conflicting with antitrust law. This should help the industry to meet the targeted market direction.

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RESEARCHES ABOUT INFLUENCE OF PRO-BIOTICS ON BROILER PRODUCTION PERFORMANCES

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Abstract

Same as the whole European poultry industry Romanian poultry industry is facing worldwide competition and so Romanian poultry industry had to produce a product better than in other countries. This prospect depends on several factors, among them being the use of probiotics as an alternative to antibiotic growth promoters. That is precisely why this paper is aiming to present the influence of probiotics on the production performance in broilers reared in industrial system. The study was conducted over the course of five consecutive growth series, using the three-phase feeding technology on two experimental batches - batch A that received feed containing probiotics in commercial quantity of 550 grams per ton of feed and batch B that received conventional feed without probiotic. The values obtained at 42 days are demonstrating the beneficial effect of pro-biotic products in all used feed recipes as body weight (2908.20 ± 53.30 g), mortality (1.912 ± 0.01 %) and specific consumption (1596.98 ± 38.98 g/kg) are better in batch A which is why probiotics can be used successfully to enhance production performances of industrial produced broilers.

Key words*: broilers, production performances, probiotic.*

INTRODUCTION

Poultry production in Romania during next years should be well prepared and assess its situation and establish its priorities and build up a strategy to deal with extreme harsh competition same as other world producers. Romanian European Production Indexes and Romanian European Efficiency Indexes are guaranteeing that Romanian poultry industry is going to grow appropriately both quantitatively and qualitatively to fulfill the following objectives: Romanian poultry products should cover interne populations auto-consume; Romanian poultry products competitiveness on the world market to enable our country to balance trade balance with poultry products any import quantity and value being compensated for with compensating exports of Romanian poultry products.

The objective pursued by this strategy is raising broiler's production potential in order to obtain the desired product in as short time as possible by substantially improving technological operating conditions based on the application of specific immune-prophylaxis programs.

There are different natural or synthetically feed additives which could be used as an alternative to antibiotic growth promoters to enhance technical and economical performances (Pop, 2009). Amongst these supplements there are probiotics as they are improving production parameters - body weight, specific consumption, mortality (Weis et al., 2007; Martin et al., 2012; Nawaz et al., 2016) and cellular immune responses (Huang et al., 2004; Kabir et al., 2004). It was also proven that adding pro-biotics in diet prevents the spread of pathogens (Karaoglu and Durdag, 2005; Wondwesen et al., 2017; Mohamed et al., 2013) and that pro-biotics have the potential to modulate the composition of microbial communities in the intestines (Apata, 2008; Kabir, 2009).

MATERIALS AND METHODS

Broiler production has grown spectacularly last years as remarkable productive performances

have been acquired and feeding technology has been improved and progresses have been acquired in animal health, bio-security and welfare.

Considering the ban of antibiotics as growth promoters in animal feeds probiotics are a reasonable alternative for poultry meat production with beneficial effects for production performances and pathogens inhibition and modulation of intestinal micro flora.

However, in the literature there are conflicting data which are showing that responses from performance and microbial balance were not significantly constants (Pop, 2009; Apata, 2008).

In this regard, the goal of these researches was observing and makes a contribution as strong as possible to knowledge of pro-biotic effect on production performances to improve their usage in industrial raised broilers.

Our studies and researches were performed at S.C. Avicola Buzău S.A. which is a private owned broiler production company with Ross 308 commercial hybrid.

In this aim two experimental lots were organized - Group A receiving feeds containing a commercial pro-biotic at 550 grams for ton of feeds and Group B receiving a conventional feed without pro-biotic.

Groups have day old chicks from the same hatchery and each group had 16000 heads by cycle. Chicks came from parents of same age in order to attenuate the genetic influence on results obtained.

Experimental period was five consecutive series of growth using three-phase feeding technology.

Combined feed used in experiments was prepared according to nutritional requirement of chicks according to the experimental design.

Chicks were raised in same housing conditions according to standard technology and feed and water were provided „*ad libitum*".

During the experiment for each group bird's live weight, feed intake and live ability were monitored weekly.

Statistical data processing was performed by usual means and averages and their errors and variability were revealed and significance of difference between groups was tested by multiple Student test.

RESULTS AND DISCUSSIONS

The following results were obtained after processing the data (Table 1).

Table 1. Body weight progression in hybrid Ross 308

Specification	Group				Student (t)
	A		B		
	$\bar{X}$	$S\bar{x}$	$\bar{X}$	$S\bar{x}$	
Week 1	203.08	2.6	193.50	1.64	3.1165 *
Week 2	526.20	4.8	486.30	1.86	7.7511 **
Week 3	1030.40	24.2	980.00	14.1	1.7995 NS
Week 4	1606.50	26.1	1518.90	29.3	2.2325 NS
Week 5	2368.10	32.2	2261.90	27.8	3.2017 *
Week 6	2908.20	53.3	2620.50	33.7	4.5624 *

It is found from the analysis of the dynamics of the body weight in hybrid Ross 308 that diets based on recipes with probiotics has led to achieving higher performance compared using classic recipes.

As early as the first week we may see a growth rate differentiation in favor of feed with pro-biotic.

From analysis of the data presented in Table 1 we can see the following:

- in the first week of growth, no difference between the body weight are significant: 203.08 ± 2.60 g – at group A (fed with the diet with pro-biotic) and 193.10 ± 1.64 g – at group B (fed with the diet without pro-biotic) respectively;
- in the second week of life, the values remain with performance in favor of lot A, differences in calculating Student test being distinctly significant, (t =7.7511** 526.20 ± 4.80 g at group A and 486.30± 1.86 g at group B respectively);
- the trend is the same in the following weeks: 1030.40 ± 24.20g at group A and 980.00 ± 14.10 g at group B in third week (1.7995^{NS}), 1606.50 ± 26.1g and 1518.900 ± 29.30 g in forth week (t =2.2325^{NS}), 2368.10 ± 32.20 g, 2261.90 ± 27.80 g in fifth week (t =3.2017*) respectively and 2908.20 ± 53.30 at group A and 2620.50 ± 33.70 g at group B (t = 4.5624*) at the end of last production week.

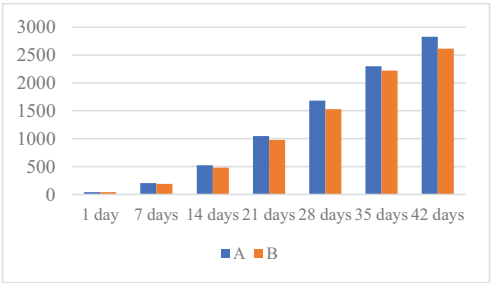

Figure 1. Body weight dynamics in hybrid Ross 308

Therefore following the results we have been able to form a picture on how rations of forage influences performance productive hens hybrid Ross 308, media weight being significantly higher when feeds were in the compound included pro-biotic

Analyzing the evolution of mortality it is observed as early as the first week of life that we have a lower mortality rate at group A. This trend is maintained throughout the period under review (Table 2).

Table 2. Evolution of the weekly mortality of hybrid Ross 308

Specification	Group				Student (t)
	A		B		
	X̄	Sx̄	X̄	Sx̄	
Week 1	0.843	0.05	1.011	0.09	1.6318 NS
Week 2	0.241	0.01	0.337	0.01	6.7884 **
Week 3	0.225	0.01	0.329	0.01	7.3541 **
Week 4	0.128	0.01	0.521	0.02	17.5760 ***
Week 5	0.244	0.04	1.091	0.06	11.7461 ***
Week 6	0.231	0.03	0.6669	0.01	13.7879 ***

In first week difference between the averages of mortalities are small and insignificant statistically (1.011 ± 0.09% at group B and 0.843 ± 0.05% at group A).

In second and third week difference between the averages of mortalities are small and insignificant statistically.

In forth week mortality at group B increases (0.521 ± 0.02%) compared to group A at which mortality decreases compared to precious weeks (0.128 ± 0.01%).

In fifth week mortality decreases at group B and difference between the two averages are very significant (1.091 ± 0.06% at group and 0.244 ± 0.04% at group A.

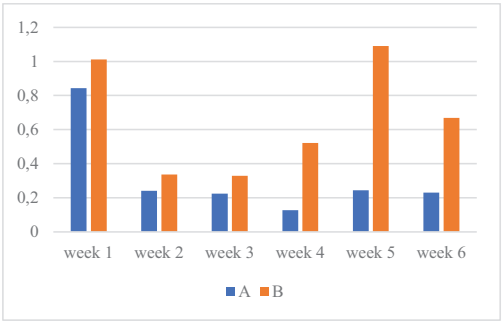

Figure 2. Weekly mortality in Ross 308 hybrid

In sixth week mortality decreases approximately by half relative to previous week at group B (0.6669 ± 0.01%) and at group A mortality still remains low (0.231 ± 0.03% t = 13.7879***)

Average weekly gain during weeks 0-6 has an evolution similar to those of body weight with a steady increase in both groups and a higher increase in forth and fifth week.

So, average weekly gain is increasing until fifth week at both groups (Table 3, Figure 3), which is the higher weekly gain registered fifth week at both groups (761.60 ± 6.75 at group A and 743.00 ± 9.81 in birds from group B).

During first week of life there are significant difference between average gains of the two groups with 165.00 ± 2.56g at group A and 155.50 ± 1.85 g at group B (t = 3.0078*).

At the end of second week difference between average weekly gains significantly in favor of group A: 322.40 ± 4.97 g compared to 292.80 ± 2.32 g (t = 5.5281*).

This trend is maintained until the age of slaughter and average gain is significantly higher in birds from group A: 504.20 ± 6.86 g compared to 493.70 ± 5.98 g in third week of life and 576.10 ± 9.72 g and 538.90 ± 8.48 g respectively in forth week and 540.10 ± 8.42 g at group A and 358.60 ± 8.41 g at group B in last week of life (t = 15.2517***).

Figure 3 is showing a fairly uniform increase of average gain until fifth week with higher performances in group A after a sudden decrease of weekly gain in last week of life

Table 3. Weekly gain evolution in hybrid Ross 308

Specification	Group				Student (t)
	A		B		
	X̄	Sx̄	X̄	Sx̄	
Week 1	165.00	2.56	155.50	1.85	3.0078 *
Week 2	322.40	4.97	292.80	2.32	5.5281 *
Week 3	504.20	6.86	493.70	5.98	1.1538 NS
Week 4	576.10	9.72	538.90	8.48	2.8839 *
Week 5	761.60	6.75	743.00	9.81	4.0814 *
Week 6	540.10	8.42	358.60	8.41	15.2517 ***

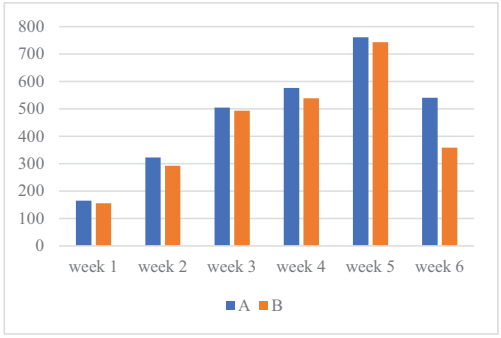

Figure 3. The average increase in weekly gain of hybrid Ross 308

In Table 4 and Figures 4 and 5 final production performances of hybrid ROSS 308 might be analyzed for the two experimental groups.

Table 4. Final production performance of hybrid ROSS 308

Specification	Group				Student (t)
	A		B		
	X̄	Sx̄	X̄	Sx̄	
Average live weight (g)	2908.20	53.30	2620.50	33.70	4.5624 *
Average daily gain (g)	68.32	1.78	61.46	1.72	2.7715 *
Cumulative Mortality (%)	1.912	0.01	3.9559	0.15	13.5962 ***
Specific consumption (g)	1596.98	38.98	1623.31	46.80	0.4326 NS
Efficiency index (points)	425.2960821		369.1510439		-

Average live weight was higher in group A (2908.20 ± 53.30 g) than in group B with an average live weight of 2620.50 ± 33.70 g.

Average daily gain was 61.46 ± 1.72 g at group B and 68.32 ± 1.78 at group A.

Results are showing that mortality is almost double at group B (3.9559 ± 0.15 %) than at group A (1.912 ± 0.01 %, t = 13.5962***).

Specific consumption recorded higher values at group B (1623.31 ± 46.80 g combined feed/1000 g gain) compared to group A (1596.98 ± 38.98 g combined feed/1000 g gain).

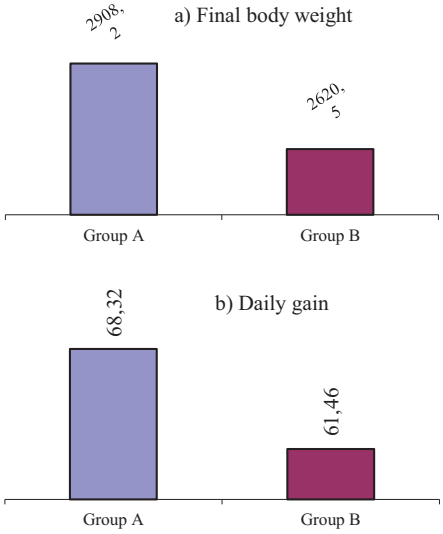

Figure 4. Final production performances in hybrids ROSS 308 (a - final body weigth, b - daily gain)

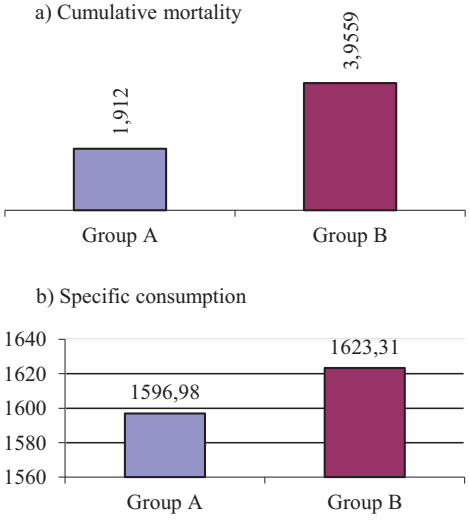

Figure 5. Final production performances in hybrids ROSS 308 (a – cumulative mortality, b – specific consumption)

CONCLUSIONS

The beneficial role of probiotic has been proven on the basis of five production parameters which allow us to draw following conclusions:

- It was demonstrated the existence of a positive correlation between providing a feed with pro-biotic and production parameters;
- weight at the end of the production cycle (42 days) was 287.7 grams higher in group receiving feed with pro-biotic (2908.20 grams) compared with group receiving classical feed (2620.50 grams);
- mortality was lower in chickens which have consumed feed with pro-biotic due to low incidence of bacterial diseases, especially infections collibacilosis due to inhibitory effect of pro-biotic on pathogen bacteria;
- average daily increase was 68.32 grams in group consuming feed with pro-biotic and 61.46 grams in the other group. This can be explained by improving the intestinal integrity, notably through the inhibition of pathogenic bacterial flora of the digestive apparatus;
- chicks consuming feeds with pro-biotic have a lower feed consumption which are decreasing production costs as feed represents a heavy part of the price cost;
- the health of offspring who ate feed with pro-biotic is clearly superior with a reduction of collibacilosis enteritis proven by the analysis bulletins performed during the experimental period as a result of the reduced incidence of digestive diseases characteristic for this category of animals.

So production parameters are superior by adding pro-biotic in broiler feed and for this reason pro-biotic might be successfully used for improving broiler production performances.

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STUDY ON THE INFLUENCE OF THE FEEDING PROGRAM ON THE PREPARATION OF HEIFERS FOR FIRST INSEMINATION

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Abstract

This study wants to demonstrate the importance of the feeding program on preparing heifers for first insemination. The study was done on a group of 35 heifers aged between 9-12 months on Agroserv Dairy Măriuța. The varieties of the experimental group belong to the Holstein breed and are inseminated at the age of 12 months and the body weight of 400 kilos. The role of the dissimilar feeding program is to help the heifersto reach the body weight 75% of the adult weight, to show the heat and to install gestation.

Key words*: inseminate, gestation, feeding program, heifers, body weigh.*

INTRODUCTION

Milk price is influenced by the big importators like China and Russia, they reduced the acquisitions from international market, which led to a surplus.

Although many regions are in full swing, demand for milk has increased significantly. In this direction, the trend is to expand over the next decade, amid the growth of the population and the change of diet in favor of dairy products. Prices will rise moderately over the next five years, after which they will stabilize around 360 euros / t, analysts said.

Only 7.5 % from mondial milk production will be traded on external markets until 2025, with the risk for imbalances for short time. In next 10 years, half from surplus produced by European Union it can be transform in skimmed-milk powder, for export and more then 30 % will be processed by the milk industry, for domestic consumption (European Commission, 2018).

This situation motivates farmers to find ways to produce more milk, choosing two ways, the first is to increase the flock and a second way is to increase the production of milk on the cow's head. The best way is to combine the two ways by establishing a performing genetics and feeding programs studied in detail.

The increase and exploitation of dairy cattle is one of the most complex types of exploitation due to the long growth period. It is precisely because of these factors that the age of breeding input is advanced compared to other species. The note on the rise and exploitation of dairy cows over time has been forced, precisely to help the farmer to have his first hen at the age of 24 months. These 24 months are permanent monitoring of females from the first day of life. Studies encourage farmers to harvest calves at the age of 12 months, the basic condition being body weight.

The study made by Penev et al. (2014) in Bulgary on 818 heifers from 17 farms shows that age of first insemination was for 16 months.

It has also been observed that where the age of breeding is lower, the number of straws per gestation is lower.

Thus, for heifers inseminated after 26 months, the number of insemination ranged from 2.7 to 3.7 straws per gestation, unlike those introduced at reproduction below 25 months, where the average number of inseminations was 2.1.

The clear conclusion is that the economic efficiency of the farm correlates positively with the early introduction to breeding.

Heifers are considerate an important source of profit. According to estimations in the

statistical records for the increase of one heifer from birth to birth, costs are between 1595 and 2935 $, which is one of the highest expenditures (Stuttgen et al., 2008). To reduce juggling costs, the U.S. Department of Agriculture (USDA) recommends reducing the age of the first calving.

Studies made by Stuttgen et al. (2008) and Lormore et al. (2005) have highlighted the fact that farmers, on those farms where the heifers have first calving between 22 and 24 months, have a higher profitability.

For each month of fertility delay over the average age of 22 months, farmers spend $ 100 extra for each heifer. These losses are caused by the quantity of less milk recovered on the market as well as by the additional costs of feeding and maintenance (Lormoere, 2005).

The age of first calving can decrease to 22-23 months, but before the first insemination is done, the female need be prepared on the basis of a strict feeding program. Before the first insemination, the heifer has to reach the criteria of height and weight, so that the calving to be normal.

MATERIALS AND METHODS

For this study data are from the zootechnical records of S.C AGROSERV MARIUTA S.R.L. Two experimental lots, each with a total of 15 heifers of the Holstein breed, aged between 9 and 12 months, were made to carry out the experiment. Experimental lots received different ratios (ration A and ration B), and the rest of the heifers received different ration, which consisted of the control group. This study was conducted between January and March 2019, temperatures being specific to temperate-continental climate in our country.

The vines were subjected to regular weighing, having a daily average gain of 900 g -1000 g from birth to the first insemination. The animals were weighed monthly at the same time, preferably before the meal, and the results obtained were used to regulate ration.

The heifers were fed at the same time, the feeding time being about 1 hour. Exit at the feeding stand is done at the same time, checking it by the responsible caretaker, thus eliminating the hierarchy of animals and their unevenness in the group.

During the experiment, the following indicators were followed: stocking density, ration type, daily average gain, calf weight, weaning, and first insemination.

Recorded data have been statistically processed and interpreted in accordance with the literature.

RESULTS AND DISCUSSIONS

Getting better heifers is done by paying special attention to the calves so that they grow harmoniously. Heifers should receive 3-4 liters of colostrum in there first 6 hours of life. Their accommodation should be in clean, dry and well-ventilated barns.

Periodically, body measurements are performed to track the individual growth plan, and based on the nutrition plan, the birth weight should double around the age of 60 days. The replacement needs to be calculated annually according to the number of animals reformed and the direction of the farm. The need may be covered by purchases of heifers or by replacement from their own herd.

The studied farm aims to maintain a number of 860 cows in milking, the consistency of this average number will be possible by producing breeding animals with a very good genetic baggage.

It should be noted that the heifers become the second or the first expense of the farmer, who in this position will have to choose a qualified staff and personified feeding program (Penev et al., 2014).

In prepuberty period, it is ideal for heifers to take weight 2.15% per day and in post-puberty period to take 1.65% per day. This increase will help the protein, the protein need to be 14-15% in prepuberty and 13-14% post-puberty. The ratio can be easily made from fodder and premix.

Energy of fodder will be calculated according with heifers weight and environment factors, generally the ratio for this category will be by 130 kcal (3)

This period from each cow life is very sensitive the nutritionist's advice is to juggle with a fiber level of 23 to 31% towards the end of the growing period.

Also they suggest to limit the administration of alfalfa hay and cereals, because this

combination can have like results meteorism. The belong ration is planning to prepare the organism for first breeding. Experimental Group 1 received ration A from the age of 5 months until confirmation of gestation and experimental Group 2 received ration A up to 9 months of age after which ration B was administered (Table 1)

Tabel 1.Diferent structures of ration for xperimental heifers group

RATION A

Fodder	**Quantity/head (kg)**
Alfalfa silage	4.5
Alfalfa hay	3
Corn meal	1.5
Wheat meal	1
Corn silage	10
Soy bean meal	0.85
Calcium	0.05
Premix	0.1
Salt	0.05

RATION B

Fodder	**Quantity/head (kg)**
Straws	2.5
Alfalfa silage	8
Soy bean meal	0.45
Corn silage	11
Salt	0.05
Premix	0.1
Calcium	0.05

The eligibility criterion for breeding is to reach a minimum of 380 kg at the age of 12 months. Since the animals in experimental group 1 received a higher energy-protein ratio throughout the test, they had a mean weight of 420 kg with variations between 400 and 460 kg.

Figure 1. Dairy Farm heifers

The heifers from group 2 was feeding with ration 2 from 9 months of age, which in terms of protein and energy is lower than ration 1. The average weight for this group at 12 months was 395 kg, varying from 365 kg to 410 kg (Table 2).

From the analysis of the two groups we can see that the calves had equal average weight at birth but at weaning they have different weights, 77.6 kg for group 1 and 92.2 for group 2.

Table 2. Weaning weight variation between experimental groups

Experimental lot 1				Experimental lot 2			
No.	Birth weight (kg)	Weaning weight (kg)	First service Weight (kg)	No.	Birth weight (kg)	Weaning weight (kg)	First service Weight (kg)
1	50	89	460	1	50	102	360
2	32	90	420	2	32	75	365
3	55	91	435	3	55	105	405
4	45	78	425	4	45	92	395
5	43	75	410	5	43	90	410
6	42	74	410	6	42	91	410
7	41	75	415	7	41	84	400
8	40	76	435	8	40	82	410
9	39	79	440	9	39	81	440
10	43	78	415	10	43	87	370
11	45	77	410	11	45	107	400
12	46	75	405	12	46	110	370
13	44	72	400	13	44	91	390
14	43	71	405	14	43	93	405
15	42	68	415	15	42	99	395
X	43.33	77.6	420	X	43.33	92.6*	395

The studies from USA show that the Holstein heifers are ready for AI when they reached 55% from the adult weight. That means the heifers must to have minimum 380 kg weight and the height 125-130 cm. A farm is considered effective when the average age of first calving is 23-24 months.

The heifers from group 1 with 420 kg average weight at first service become pregnant after 2 shots in average. The group 2 had 395 kg average weight at first service and they need 1.2 shots.

This results show us that the ration for group 2 was more efficient because the heifers were not fat and become pregnant early with higher conception rate.

From the analysis of the results of the two experimental groups we can see that the animals had equal weights at birth (43, 33 kg) and at weaning they reached 77.6 kg experimental group 1 and 92.6 kg experimental group 2.

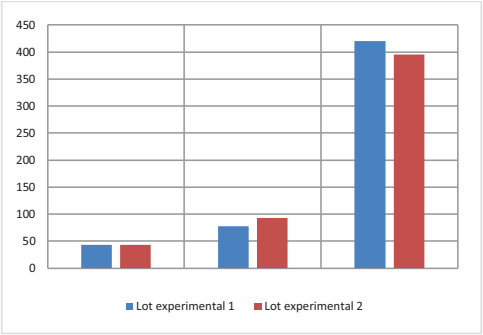

Figure 2. Results of the experiment

According to American researchers, for the Holstein breed the heifers are ready for breeding when they reach 55% of the adult size. Thus, a body weight of between 360 and 383 kg and the width of 125-130 cm is considered optimal. A farm is considered effective when all heifers are pregnant at the age of 15 months.

Table 3. Weight variation between experimental groups until first breeding

Experimental lot 1				Experimental lot 2			
No.	Birth weight (kg)	Weaning weight (kg)	First insemination Weight (kg)	No.	Birth weight (kg)	Weaning weight (kg)	First insemination Weight (kg)
1	50	89	460	1	50	102	360
2	32	90	420	2	32	75	365
3	55	91	435	3	55	105	405
4	45	78	425	4	45	92	395
5	43	75	410	5	43	90	410
6	42	74	410	6	42	91	410
7	41	75	415	7	41	84	400
8	40	76	435	8	40	82	410
9	39	79	440	9	39	81	440
10	43	78	415	10	43	87	370
11	45	77	410	11	45	107	400
12	46	75	405	12	46	110	370
13	44	72	400	13	44	91	390
14	43	71	405	14	43	93	405
15	42	68	415	15	42	99	395
X	43,33	**77,6**	**420**	X	43,33	**92.6***	**395**

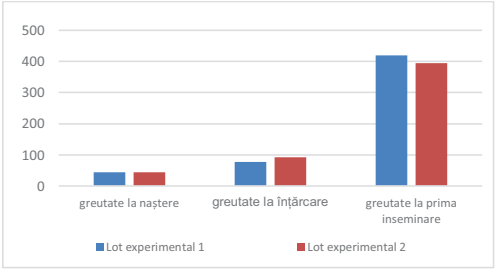

Figure 3. Weight variation from weaning to first breeding

Figure 4. Image from heifers barn

Heifers in experimental group 1 with a mean weight at first 420 kg insemination had a consumption of 2 inseminations per gestation. Experimental group 2 had a consumption of 1.2 insemination on gestation, which confirms that the optimum weight for the first insemination is 395 kilograms. Also, the fodder scheme for experimental group 2 proved effective.

CONCLUSIONS

The heifers are the most important group from farm because by the quality of them depends the future of farm.

The study made by us shows the importance of feeding plan and a good management for raising heifers such that they reached the optimal weight and height and a great body condition this meaning 380-400 kg weight and 125-130 cm height.

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RESEARCH ON THE EVOLUTION OF THE ABERDEEN ANGUS BREED IN ROMANIA

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Abstract

The purpose of this paper is to make an analysis of the evolution of the Aberdeen Angus breed in Romania. This research paper desire to be a first response to the many questions about accommodation, advantages, disadvantages, bio-acclimatization and profitability of the Aberdeen Angus cattle breed at national level, compared with the country of origin Scotland and with the countries with great success in raising this breed. The first embryo transfer was performed in 2000 and at the end of 2008 there took place the first cattle import of 120 heifers Aberdeen Angus breed from Germany. Now in 2019 we have in the Aberdeen Angus Romanian Herdbook 45.000 cattle in all area of Romania. During this research we noticed that the Aberdeen Angus breed is accommodating well in the Romanian pedoclimate conditions.

Key words*: beef cattle, evolution, Aberdeen Angus.*

INTRODUCTION

Cattle breeding is considered a main branch of livestock farming which contributes in a large percentage for providing animal originated food for the population of the globe. The investigations made over time by different research institutions specializing in human population, it is noted that the index of growth of the human population is in constant development both in the short and for the next 3-4 decades.

These factors indicate that there will be a sharp increase in demand for food resources to feed people.

Representing an important source for food with high biological value, but also appreciated as a means of labor, increasing and improving of the cattle subfamily is for mankind a surplus food, providing about 55-57% of the animal protein consumed daily. The set of technical and organizational measures in cattle exploit operation involves several processes of feeding, growth, reproduction, breeding, which helps to increase the productive potential.

The importance of cattle in economy results from the fact that the animals in the cattle family provide the following products: milk, meat, leather, manure, hair, and in countries with underdeveloped agriculture, they are used as labor force. From the historical data, the worldwide cattle existence dates back to Mesopotamia in the Middle East region, which currently belongs to the part of Iraq, Syria and Turkey.

Domestication occurred together with the development of prehistoric peoples as evidence of the Sumer people in the Mesopotamia region in the 6th millennium BC cattle have a well-developed service, which can be seen from various mosaics bequeathed to humanity. With the human population migration from rural to urban areas, in the modern era (starting with the 18th century) it was stimulated the cattle development in order to increase the production of animal origin food (David, 1976).

The massive exports of these Aberdeen Angus (Figure 1) breed began at the end of the 19th century when they competed with the local meat breeds. Compared with other meat breeds such as Shorthorn and Hereford, the migration and success of the Aberdeen-Angus breed has so far increased about 18 times since 1920.

Figure 1. Aberdeen Angus cattle in Romania

MATERIALS AND METHODS

The Aberdeen Angus breed specialized in beef production was imported for the first time in the 1960s but the results obtained were unfavorable.

After 2010, imports of animals (heifers) from Germany started. The breed has adapted very well in Romania.

The limiting factors for the increase of cattle production in Romania are mainly the following: reduced animal herds, non-assurance and quality of the forage base, maintenance and exploitation technology, reduced cow breeding index, low growth rates, low body mass at slaughter and a low slaughter yield

This study aims to review the statistical data and research carried out in this field, both at national and international level.

The working method was based on the analysis of data recorded over time in Aberdeen Angus Romania's database and on their statistical interpretation using the consecutive methods.

RESULTS AND DISCUSSIONS

Romania is a country with high agricultural potential given that the geographical configuration and climate are favorable. Our country is not part of countries that have to import substantial amounts of food because, under normal circumstances, this land can meet the food needs of a population of three and a half times higher than the current population.

At the end of 2008, the first massive import of German-born Aberdeen Angus cattle took place. It was a group of 120 heifers who found their new shelter at a newly established farm near Sibiu (Marpod Commune. This meat bovine nucleus has grown continuously through its own calves and imports, thus becoming the largest breeding and fattening farm for meat taverns in Romania.

Due to the fluctuation and lowering of the price of milk, many farmers started to opt for crossbreeding and purchases of cattle having the morpho - productive type of meat.

The formation and expansion of the Aberdeen Angus meat consumption market in Romania would lead to the education of consumer taste for good quality beef and the support of local breeders of this breed. Initially, industrial crosses with low-yielding cows from local breeds were used to obtain hybrids with good skills for meat production (Figure 2).

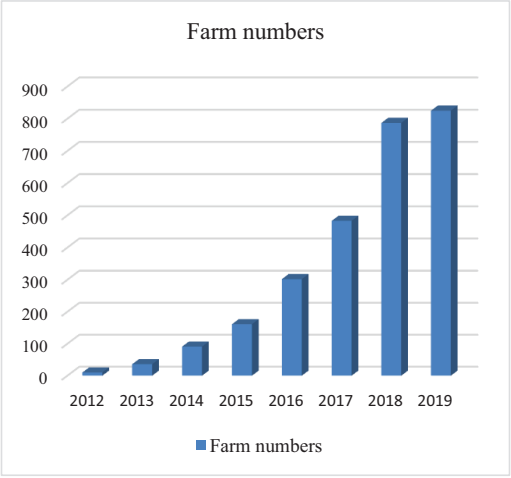

Figure 2. Evolution of Aberdeen Angus farms in Romania

The dramatic decrease in agricultural production has led us in the paradoxical situation of import, when we should have to export more, a key to the success of getting Romania's population and its economy out of the impasse. This neglect of the agricultural potential has resulted in the decline of the living standards of the population, especially in the rural area (Acatincai, 2004).

Aging of the rural population, the strong rural poverty, the poor infrastructure, the lack of production, the lack of markets, the lack of a strong and coherent agricultural policies do not support constructive few farmers left the farms to go out of state of subsistence (Marginean, 2012).

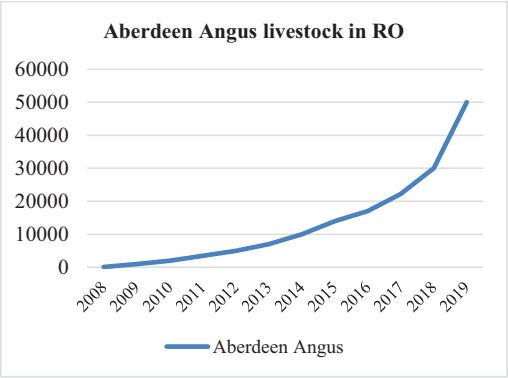

Figure 3. Evolution of Aberdeen Angus livestock cattles in Romania

By increasing the number of cattle specialized in the production of meat, it can become an important sector of agriculture in Romania (Figure 3, Table 1), with reference to meat quality requirements demanded in Europe and worldwide.

Table 1. World cattle inventory

Rank	Country	2018	% of world
1.	India	305.000.000	30,44%
2.	Brazil	232.350.000	23,19%
3.	China	96.850.000	9,67%
4.	U.S.A.	94.399.000	9,42%
5.	European Union	88.445.000	8,83%
6.	Argentina	53.765.000	5,37%
7.	Australia	25.500.000	2,55%
8.	Russia	18.380.000	1,83%
9.	Mexico	16.584.000	1,66%
10.	Turkey	14.500.000	1,45%
TOTAL		1.001.841.000 heads	

Since the worldwide population is alarmingly growing, the animal protein quality is increasingly sought after, especially in the developed countries where livestock meat is one of the sectors of agriculture considered to be an industry with huge potential for economic development and research (Ulrich, 2009).

Romania has got 237,500 km^2 area, consisting of a symmetrical relief, concentric and varied, with the main features of relief proportioned as follows: 31% mountains, 36% hills and plateaus, and 33% plains.

My conclusion is that Romania with the second surface of pasture in Europe, have a big potential to became a beef brand producer in Europe (Figure 4).

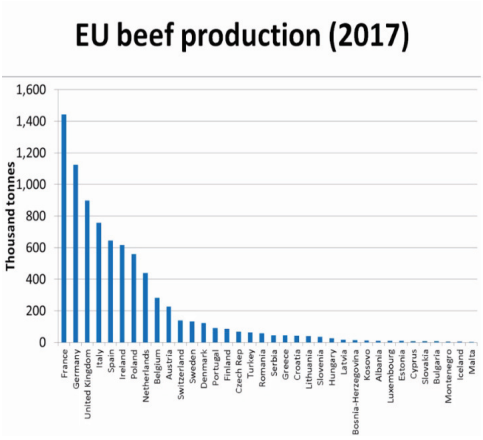

Figure 4. European beef production in 2017

Of the total Aberdeen Angus cattle population worldwide, 75% of this breed is in Australia, North America and South America. Cattle breeds directed towards meat production are in constant development, and their 70% of the population belong to the breed Angus cattle.

Worldwide, if we consider only the population growth and economic development of China where estimates predict an increase in meat consumption from 50 kg per capita / year currently being estimated in 2030 to reach consumption 70 kg per capita / year, the required annual required meat increased significantly.

The physiological and morphological-productive characters specific to Aberdeen Angus breed cattle are very suitable climatic condition of Romania (Mirita, 1982).

Romania should not be part of the countries that have to import substantial amounts of food, and the geographical configuration and the climate are favorable for the production to meet the needs of a population of two and a half times larger than the current population of Romania (Figure 5).

Producing beef on natural grasslands concomitantly with nature conservation may present a method to provide with a huge part of these demands. However, beef produced in this environment should not only show ecological value, but also have the basic quality attributes desired by consumers: shiny red color, little fat covering, tenderness, juiciness and a pleasant taste (Felicio, 1998).

Figure 5.Aberdeen Angus bull in Romania

In the most important reproduction farm for the Aberdeen Angus breed in Romania, the dynamics of body development are presented in Table 2.

Table 2 Dynamics of body development in reproduction females

Age	Average weight (kg)	Size at withers (cm)	Average daily gain (g)
Birth	35	70	-
Weaning (8months)	200	100	700
18 months	375	125	584
24 months	400	130	250
Multipurpose cows	550	135	850

A study by Personen et al. (2012) on the characteristics of the carcass and the quality meat of the Aberdeen Angus revealed the special potential of this breed (Table 3). In this experiment, the animals were fed *ad libitum* grass silage and concentrates based on barley and oats. The duration of the experiment was 345 days and the slaughter age 526 days. From the table 3 we note that the animals had a body weight of 285 kg at the time of fattening, and at the end of the experiment 705 kg, with a daily average gain of 1224 grams.

Table 3. The fattening parameters for Aberdeen Angus breed (Personen et al., 2012)

No.	Parameter	Value
1	Duration of the experiment (d)	345
2	Initial live weight (kg)	285
3	Final live weight (kg)	705
4	Live weight gain (g/day)	1224
5	Age of slaughter (d)	526
6	Carcass weight (kg)	391
7	Conformation score, EUROP	7.4
8	Fat score, EUROP	3.8

Moldovan (2012) tested the combining ability of the Aberdeen Angus breed in Romania. The results of the experiments on the ability to combine indigenous breeds (Bălţată Românească-BR, Brun de Maramureş B, Bălţată cu Negru Românească-BNR) with the Aberdeen Angus meat breed have led to different results (Table 4).

Table 4. Growth parameters of half breed obtained between Aberdeen Angus breed and local breeds in Romania (Moldovan, 2012)

Age	Birthweight (kg)	Final live weight (kg) at 14 months	Average daily gain (g)	Specific consume UNC/kg
BRXAA	30,11	633,03	1436	6,32
BXAA	30,53	644,11	1460	6,21
BNRXAA	28,58	597,89	1424	6,38

It is noted that half breed between the Aberdeen Angus breed and Brown of Maramures have achieved the best growth parameters. So, the final live weight at 14 months age was 644.11 kg, with 11 kg more than at Bălțată românescă X Aberdeen Angus (Figure 6).

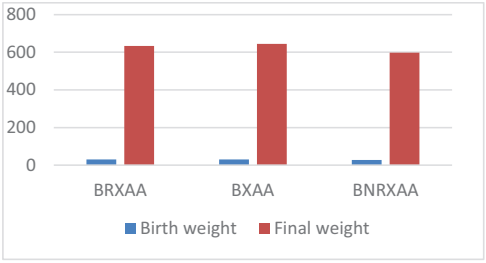

Figure 6.Growth parameters of half breed (local Romanian breeds and AA breeed)

These results lead to the conclusion that local cattle breeds in Romania show very good skills for meat in combination with the Aberdeen Angus breed.

The quality of carcasses obtained from the metisses can be improved by using the Aberdeen Angus breed using an industrial hybrid production scheme

CONCLUSIONS

Aberdeen Angus calves have low weights at birth (35 kg), but have a very good compensatory growth rate, which results in high weights at slaughter (700 kg). Average daily growth averages are on average 1200-1300 grams. It is a disease resistant breed and adapts very easily to different environmental

conditions. The carcasses weigh more than 390 kg, the slaughter yield is around 55%.
Due to the fat deposits between and within the muscle fibers, the meat is savory, and the look is marooning and preservation.
Romania is not one of the cattle meat consuming countries, with a meat consumption of 11 kg per person in 2010, but with such a natural potential, we can become producers and exporters of top countries in Europe and of the overpopulated countries from the other continents.

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MAKING AND CHARACTERIZATION OF PEGAGAN DRY EXTRACT (*Centella asiatica*) AS FEED ADDITIVES FOR ANIMAL FEEDING

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Abstract

The research on the manufacturing process and characterization of Centella asiatica dried extract by adding lactose was carried out. The ratio of "pegagan" extract to lactose is 2: 1; 1: 1; and 2: 3. Products in the form of dry extract can be used as feed additives in poultry feed formulas. The results showed that the ratio of "pegagan" extract to lactose in the ratio of 2: 1, obtained the best dry extract "pegagan"flour character. The results of the characterization of dried "pegagan" extract are: shrinkage levels of 1.52%, total ash content of 1.17%, acid soluble ash content of 0.95%, water soluble compound content of 83.32% and organic solvent soluble compound content of 13.01%. Based on the results of the characterization test, "pegagan" dry extract flour can be classified as a feed additive in poultry feed formulas.

Key words*: Extraction, pegagan, lactose, additive feed, characterization.*

INTRODUCTION

Rations are generally in the formulation of feed ingredients produced by agricultural waste and agro-industry, because agricultural products are prioritized for the food sector. Nearly 60% of feed ingredients come from agricultural waste and agro-industry. Ingredients that are formulated, in composition contain various chemical compounds which in the process of digestion will have character as an anti-nutrient, resulting in decreased productivity of livestock. In order to overcome or compensate for these conditions the feed additives need to be added.

Additive Feed Material is a feed ingredient that is added to the ration with a small amount, because if it is given in high quantities, it can produce residues in the body of livestock. The form of additive feed ingredients commonly given to livestock consists of liquid simplified added to drinking water. The form of dry simplified (dry flour) at a dose of 0.5% body weight, has not given a real effect on increasing livestock productivity (Lee et al., 2005). Herbal medicinal products be affected by quality of raw material (BPOM, 2004) This is since it has not been processed or purified as an active substance and is not exactly the dose and the low affinity of the active substance contained in the additive.

Anti-nutrient substances in feed ingredients make up rations, causing disruption of the function of livestock organs, especially the liver. In order to find out whether liver damage is occurring, a series of checks of compounds in the body are usually carried out, such as SGOT (Serum Glutamic Oxaloacetic Transaminase), SGPT (Serum Glutamic Pyruvic Transaminase), AST (Aspartate Transaminase), and ALT (Aspartate Transaminase), or immunochemical examination. AST and SGPT or ALT and SGOT are intracellular enzymes that are normally in cells (Lee et al., 2005). These enzymes function as catalysts in chemical reactions that occur in cells. When liver damage occurs due to toxic compounds, contamination of microorganisms results in changes in permeability in the cell membrane. This condition results in the transfer of enzymes in cells into the blood, this event is called serum transaminase. To overcome this condition, hepatoprotection compounds are needed which can reduce the content of SGPT and SGOT enzymes in the blood.

The oxidation reaction is the cause of damage to the liver, it can be seen from several drugs referred to as *hepatoprotective* which is an antioxidant compound. One example is silymarin compounds contained in plants (*Sylybum marianum*). Feeding in extracts ethyl acetate 17,5 mg/kg of doses *body weight* has been applied for in vivo test using *mice.* Induced by $CCL_{4;}$ They demonstrated hepato-protective effects (Lee et al., 2005). Ethyl Acetate extracts were able to reduce levels of the enzyme *alanine aminotransferase* (ALT) and *aspartate aminotransferase* (AST) by 56% and 44%. Local plants in Indonesia which contain silymarin are "pegagan" plants (*Centella asiatica*), which is a weed that grows in rice fields and irrigation areas. Silymarin is a flavonolignan that contains silybin (the main compound), isosilibin, silidianin, and silicristine (Lee et al., 2005; Tedesco et al., 2004). According to Heyne (1987) and Iswari (2002), "pegagan" plant contains almost the same as *Sylibum marianum* plants.

Based on these assumptions, the process of making feed additives is carried out in the form of dried "pegagan" extract, which functions as a hepatoprotective in livestock. *Centella asiatica* flour extract is expected to be used as an herbal medicine that has characterization, and follows the guidelines required by BPOM (Departemen Kesehatan Republik Indonesia, 2013) including: shrinkage levels, total ash content, acid soluble ash content, water soluble extract content, and levels extract dissolves organic solvents. Products from characterization can be developed as additive feed ingredients for livestock.

MATERIALS AND METHODS

Research sites. The research has been carried out in the Poultry, Non-Ruminants Nutrition Laboratory and Livestock Food Industry, Faculty of Animal Husbandry, Padjadjaran University, Sumedang, West Java, Indonesia.

Tools. Analytical scales, dark bottles (maceration processes), rotary evaporators, vaporizers, ovens, desiccators, water baths, furnaces, blenders, and laboratory glassware.

Research Materials. Pegagan plant, lactose powder, ethanol 96%, 0.2 N sulfuric acid, glacial, chloroform, and hexane acetic acid.

Preparation of "Pegagan" Extract. Pegagan plant samples (*Cantella asiatica*), washed and dried air in laminar flow, in temperature 30^0C, for 10 days, until the water content is not more than 10%, then mixed using a blender.

Extract Making. As much as 100 grams of "pegagan" dry flour, put in a maceration bottle, added 1 liter of ethanol 96%, soaked for 6 hours while stirring, then let stand for 24 hours. Macerates are separated, and the process is repeated twice with the same type and amount of solvents. All macerates were collected and evaporated with vacuum vaporizers until thick extracts were obtained. The yields obtained were weighed and recorded (BPOM, 2004).

Making "Pegagan" Dry Extract. The thick extract produced from maceration products, dried by adding lactose, by comparison (2:1); (1:1); and (2:3), then homogenized. Then added 300 ml hexane solvent for every 100 grams of dried extract mixed with lactose, stirring several times for 2 hours, let it settle, do the same for the rest, and separate excess hexane. Repeating washing with hexane, the remainder is dried in an oven at 70^0C, after drying, milling, and weighing (into powder). Furthermore, characterization of dried extract flour was obtained (Martin et al., 1961).

Characterization of "Pegagan" Dry Extract

Loss of drying rate. Extract flour was weighed in a shallow weighing bottle of 2 grams, and flattened by shaking the weighing bottle, so that the extract flour resembled a layer of approximately 10 mm thick, then put into the drying chamber (laminar), dried at 105^0C. Every drying, the bottle is left in the exicator so that it cools to room temperature. Then it was dried again at a determination temperature to a fixed weight and stated the value of drying losses in % weight per weight (Departemen Kesehatan Republik Indonesia, 2013).

Total ash content. Weigh 3 grams of extract flour, put it into a porcelain saucer that has been spawned and tasted, evenly spread, slowly gently until the charcoal runs out, cool and weigh; calculate the ash content of the material that has been dried in the air; calculate in % (weight / weight).

Acid insoluble ash content. Ash obtained from the determination of total ash content, simmer with dilute 0.2 N sulfuric acid for 5 minutes, collect the insoluble part in the acid, filter it

with crucible glass or ash-free filter paper, wash it with hot water, until constant; calculate the acid insoluble ash content of material that has been dried in the air.

Levels of water-soluble compounds. 5.0 gram of extract was macerated for 24 hours with 100 ml of chloroform LP water using a clogged flask while being shaken many times, for the first 6 hours and left for 18 hours. Strain, apply 20 ml of the filtrate to dry in a shallow dish on a flat (Petri dish) layer, heat the residue in the oven at 105^0C until the weight is fixed; calculate the levels of water-soluble compounds against the initial weight of the extract; state in % weight per volume.

Levels of compounds dissolved in organic solvents. 5.0 gram of extract was macerated for 24 hours with 100 ml of 96% ethanol, in a clogged flask, while being shaken repeatedly for 6 hours and left for 18 hours. Strain quickly to avoid evaporation of ethanol, then apply 20 ml of filtrate to dry in a flat-based cup (Petri dish) that has been tasted, heat the residue in the oven at 105^0C to a fixed weight; calculate the levels of soluble compounds of organic solvents against the initial weights, expressing in % (weight / volume).

RESULTS AND DISCUSSIONS

Results of the Making of "Pegagan" Extract

The extract was made by maceration using ethanol 96% solvent, carried out with 2 repetitions. The results of the thick extract obtained can be seen in Table 1.

Table 1. Weight of "Pegagan" Extract

No	Powder Weight of Pegagan Simplicial (g)	Weight of Pegagan Extract (g)
1	100	70.8626
2	100	82.3571
3	100	64.1034

The experimental results showed that the weight of the thick extract obtained for each process was not the same. This may occur because of the uniqueness of "pegagan" simplicial, both the age of harvest, the proportion of leaves, stems, and roots of "pegagan", and resulting in the chemical content and weight of thick extract produced in the maceration process.

The Result of Making Pegagan Extract

The drying process of thick extract produced by maceration is done by adding a binder in the form of lactose flour and carried out with various comparisons. The comparison is as follows: half the weight of the heavy extract (2:1), equal to the weight of the extract (1:1), and one half of the weight of the thick extract (2:3). This is done to study the dynamics or character of the dried extracts, and lactose as a material used as a binder. The weight of dried extract flour is presented in Table 2.

Table 2. Dry Extract Flour Weight

No	Binder composition	Thick extract (g)	Dry extract flour (g)
1	2 : 1	70.8626	36.1150
2	1 : 1	82.3571	82.8579
3	2 : 3	64.1034	126.0557

In Table 2 the thick extract extraction process produces the highest dried extract flour obtained from the treatment of one half of the weight of extract (2:3), which is 126.0557 grams. The lowest is the proportion of binder (2:1), which is obtained by dry extract of 36.1150 grams. This illustrates that the dried extract of *Centella asiatica* which is dried carries out a reduction reaction to lactose by converting lactose flour into a volatile compound, or lactose is dissolved by a component of liquid which can be evaporated. It is water contained or volatile substances and alcohols that are used as solvents and are not able to be bound by a cylindrical substance.

Results of Characterization of Pegagan Extract Flour

The characterization of "pegagan" dried extract aims to get the best character from dried starch extract of "pegagan". Can the "pegagan" dry extract flour be classified as additive feed ingredients or not. Characterization of drying shrinkage aims shown the amount or large number of compounds lost in the drying process, in special cases identical to the water content, and determine the handling of the product produced. The results obtained, whether the product still needs preservatives or not, to avoid contamination of microorganisms. The characterization of total ash content aims to describe the internal and external mineral

content from the initial process to forming dried extract flour, and the determination of acid insoluble ash content aims to see the presence of metal content in the form of silicate salt. Examination of levels of water-soluble compounds and examination of the content of soluble organic solvent compounds aims to see the content of minerals and compounds that are efficacious as drugs (Departemen Kesehatan Republik Indonesia, 2013). The results of the characterization of "pegagan" dry extract are presented in Table 3.

Table 3. Results of Characterization of "Pegagan" Dry Powder Flour Test

No	Test Parameters	Binder composition		
		2 : 1	1 : 1	2 : 3
1	Drying shrinkage (% b/b)	1.5150	0.5730	0.5724
2	Total ash content (%)	1.1730	0.3930	0.0057
3	The level of acid dissolves ash (%)	0.9460	0.0360	0.0094
4	Water soluble compound level (%)	83.3215	86.0860	82.5756
5	Levels of soluble organic solvents (%)	13.0072	6.6690	5.9682

Table 3 illustrates that the best treatment is the addition of lactose or composition binder in a ratio of 2: 1. The characterization results obtained were: drying shrinkage value of 1.16%, total ash content of 1.17%, acid soluble ash content of 0.9%, water soluble compound content of 83.32%, and organic solvent soluble compounds of 13, 01%.

CONCLUSIONS

The results of this study concluded that the comparison of lactose (as a drying binder) with "pegagan" extract was half a part of lactose with one part of thick extract of "pegagan". The characterization test results obtained that the dried extract of "pegagan" (*Cantella asiatica*) has the properties and potential as feed additives which are rich in active ingredients in the form of water-soluble compounds and organic solvents.

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PIG PERFORMANCES FED WITH COCONUT WATER AND PULP

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Abstract

*Research was conducted to evaluate the utilization of coconut meat waste and water by-product in the diets related with pigs performances. The treatments involving 30 growing pigs were arranged in a 3*2 factorial design based on completely randomized design. The treatments of factor A were including levels of coconut meat waste product equivalent to 0% (A0), 22.5% (A1) and 45% (A2) substituting the rice bran in ration meal. The treatments of factor B were applying levels of coconut water equivalent to 0% (using fresh water = B0) and 100% (using coconut water = B1) substituting the fresh drinking water of animals. Each experimental unit of factor A and factor B was repeated using 5 pigs of crossbred castrated males (Yorkshire*Landrace*Local), each maintained individually in a pen with initial weight of 59.4±1.33 kg. Ration and drinking water were daily given ad libitum to the animals. Animals were weighed on each period of 14 days for data collection of animal growth rate. The result showed that proportions of coconut meat waste product were less than planned for treatments of 22.5% and 45%, while total of 100 percents of coconut drinking water were less than planned for 100% of fresh drinking water. Results also showed that utilization of coconut meat waste product up to 50% substituting rice bran in ration with the level of 22.5% was able to yield the optimum growth rate*

Key words*: coconut waste product, growing pigs.*

INTRODUCTION

This study on the utilization of coconut meat waste and water aimed to develop locally available feedstuff sources that promise to be viable alternatives to basal meals in diets for pigs. Detailed researches of foliage utilization from new Cocoyam (*Xanthosoma sagittifolium*) leaves (Rodríguez et al., 2006), sweet potato (*Ipomoea batatas*) (Le Van An et al., 2005; Sokha et al., 2007), cassava (*Manihot esculenta)* (Bui, 2006; Nguyen, 2006), mulberry (*Morus* alba) (Chiv et al., 2007) and water spinach (*Ipomoea aquatica*) (Chhay and Preston, 2006a,b) have been reported and well documented. Focused study is now being attended to coconut waste products which are widely distributed in tropical latitudes of Indonesia, often as abundant waste product of coconut plantation.

Value Added of Coconut Products has been used as staple part of the diets of almost all Polynesian and many Asian people for centuries. It is used as food, as flavoring and made into beverages. Today's young consumers depend heavily on conventional food. It is in this sector that coconut has a good chance to increase its use. The important products of coconut in almost all countries are whole coconut (tender and mature), copra, toddy, coconut palm candy, sugar, vinegar and other technological food products and few novel recipes like banana coconut cake. To use coconut in preparations, the consumer has to break the nut transversely into two parts (Photo 1c), to shred into gratings and then process further. On the other hand, if coconut gratings, its rich milk and even prepared foods are available as such on the shelves of the market, surely consumers, especially the younger generation will be generated to encourage to use them in preparations much more.

In a previous study by Berschauer et al. (1984), it was shown that the fat content of the animals fed ration containing 12% coconut fat was 15.9% and this was significantly lower ($p < 0.001$) than that of 21.1% measured in the animals fed rations containing 12% sunflower oil. The contents of myristic acid and linoleic acid were significantly different between sunflower oil and coconut fat; for the former values of 0.8% (sunflower oil) and 16.9%

(coconut fat) were determined, respectively, with corresponding values of 48.7 (sunflower oil) and 11.3% (coconut fat) for the latter. Rice bran is a by-product of rice grain production consisting primarily of the outer layers of the grain (Campabadal et al., 1976). The rice bran is removed during the process of milling to produce white rice for human consumption (Saunders, 1990). It usually includes the pericarp, seed coat and aleurone, as well as most of the germ (Kaufmann et al., 2005). Rice bran has highly available energy, and is generally economical. It is usually used in swine diets because it is widely grown in the East and South East of Asia. There are 40 to 45 million tons of rice bran produced annually (David, 1994). Some studies had been conducted to examine the feasibility of using rice bran as an ingredient in poultry (Hussein and Krarzer, 1982; Sayre et al., 1987; Warren and Farrell, 1990) and swine (Warren and Farrell, 1990).

Because of the large quantity of rice bran needs throughout the world, it is important to determine not only its function in term of chemical composition, but also the variation in chemical composition arising from different feed ingredients of the animals. The hypothesis for this study was that the rice bran was competitive ingredients of pig and poultry rations, in all regions and could be changed its function in animal ration with other alternative ingredients without competing human food. Therefore, the object of this study was designed to determine the utilization effects of coconut meat waste and water on growing performance and carcass quantity of pigs as a part replacement of the rice bran ration and a complete replacement of the drinking water in weaned pigs fed the same basal diets.

MATERIALS AND METHODS

The study was conducted at the swine farm in Tomohon, North Sulawesi Province of Indonesia between April and June 2013, divided into 14 days of preliminary treatment (treatment adaptation) and 56 days of data collection periods for growing variables and ending period for slaughtering animal and carcass observation.

The treatments involving 30 growing pigs were arranged in a 3*2 factorial design based on completely randomized design. The treatments of factor A were including levels of coconut meat waste product equivalent to 0% (A_0), 22.5% (A_1) and 45% (A_2)substituting the rice bran in ration meal

The treatments of factor B were applying levels of coconut water equivalent to 0% (using fresh water = B_0) and 100% (using coconut water = B_1) substituting the fresh drinking water of animals (Table 1). Each experimental unit of factor A and factor B was repeated using 5 pigs (marked at left ear of each pig) in a pen. The pigs were crossbred castrated males (Yorkshire*Landrace*Local) with initial weight of 59.4±1.33 kg.

The coconut meat waste products were dried under sunrise (uncompetitive product with human food) and used for substitution of rice bran in pig basal ration. Materials of basal feed ingredients were weighed with the compositions of yellow corn (45.8%), rice bran waste product (45%), fish meal (8.7%) and Top mix (0.5%).

Coconut meat waste product generally contains 7.73% of crude protein, 19.82% of crude fiber, 56.86% of fat, 0.06% of Ca, 0.14% of P, and 6,805 kcal/kg or 28.5 MJ/kg of gross energy (Laboratory Analysis of Sam Ratulangi University, Indonesia, 2013).

Rations fed to animals were formulated in the composition as shown in Table 1. In this study, growing pigs were weighed in the morning, before being fed, at the beginning of the trial and after each period of 14 days. Animals were weighed four times during experimental periods involving 56 days of study. Samples of ration were collected at the initial day and end of the day in each period of treatment. The left over ration feeding was collected daily. Daily difference between feed consumed and the left over ration feeding was defined as the animal feed consumption. Table 1 gives nutrient compositions of the trial animal ration.

In addition to feed consumption, animal daily drinking water using treatments of fresh water and coconut water waste product was also measured as the treatments. Daily difference between drinking water by the animal and the left over drinking water was defined as the animal daily drinking water (liter/animal/day).

Table 1. Nutrient compositions of ration ingredients of the treatments (%)

Ingredients	R_0	R_1	R_2
Yellow corn*	45.80	45.80	45.80
Fish meal*	8.70	8.70	8.70
Rice bran**	45.00	22.50	0
Coconut meat waste product**	0	22.50	45.00
Top Mix	0.50	0.50	0.50
Total	100.00	100.00	100.00
Nutrient content			
Protein (%)	13.13	12.10	11.72
Fat (%)	6.06	18.52	23.34
Crude fiber (%)	11.64	10.84	9.65
Calcium (%)	1.02	0.83	0.73
Phosphor (%)	0.93	0.54	0.52
Gross Energy (Kcal/kg)	3905.00	4325.00	4475.00

*Calculated on the bases of laboratory analysis at Feed and Nutrient Laboratory–Bogor Agricultural Institute, Indonesia (2013).
** Calculated on the bases of laboratory analysis at Sam Ratulangi University-Laboratory, Indonesia (2013).

Drinking water treatments were given as the first meal at 07.30h. After all first drinking water was consumed by animals the remainder of drinking water of treatments was given. The same procedure was repeated at 15.00h. Nutrient contents in drinking fresh and drinking coconut water were presented in Table 2.

Table 2. Nutrient compositions in fresh drinking water and coconut drinking water

Nutrient content*	Fresh water	Coconut water
Water (%)	98.81	96.63
Protein (%)	-	0.07
Fat (%)	-	0.09
Ash (%)	-	0.06
Sodium (%)	0.04	0.12
Potassium (%)	1.01	18.04
Calcium (%)	2.68	12.29
Magnesium (%)	0.64	1.96
Nitrogen (%)	-	1.14
Glucose (%)	-	1.14
Sucrose (%)	-	2.81

*Result of Chemical Laboratory Analysis at Sam Ratulangi University, Indonesia (2013).

Variables observed in the trial involved feed consumption of ration (g/animal/day); average daily gain (ADG) (g/animal/day), and feed efficiency was calculated as ratio between the ADG (g/animal/day) and feed consumption of ration (g/animal/day). Pig drinking water was calculated as daily difference between animal drunk water and the left over animal daily drinking water. Animal body weight at slaughter period was calculated as animal weight at the end of experiment (kg/animal).
Data were analyzed using Analysis of variance (ANOVA) (Steel and Torrie, 1980). Feed consumption, ADG, feed efficiency, pig drinking water, animal body weight at slaughter period were included as dependent variable, while three treatment levels of coconut meat waste product (factor A), two treatment levels of coconut water (factor B), and the interaction (AB) were included as independent variables in the ANOVA model (Steel and Torrie, 1980). Data were analyzed using the Insert Function Procedure of the related statistical category in datasheet of Microsoft Office Excel (2007). The significant difference in the model of treatments was tested using *honestly significant difference*, while differences between variable averages at levels of drinking water were tested using pair *t-test* (Byrkit 1987).

RESULTS AND DISCUSSIONS

There were significant differences between the treatment levels of substitution of rice bran by the coconut meat waste product as shown in Table 3.
In this study, attempts were made to control the intakes of coconut meat waste product substituting rice bran in ration meal and coconut water changing fresh drinking water so as to achieve the desired proportions of these three levels of ingredients in the diets. However, this was only partially successful with the result that proportions of coconut meat waste product were less than planned for treatments of 22.5 percents and 45 percents, while total of 100 percents of coconut drinking water were less than planned for 100 percents of fresh drinking water.
As a result the nutrient content of coconut meat waste product was 7.73% of crude protein compared with 13.35% of crude protein in the rice bran of the present experiment. This lower crude protein level being possible through use of coconut meat waste product would be considered in ration formulation as a protein source. This difference in levels of protein could the major reason found this study of growing pigs. In the present study with lighter pigs on the use of coconut meat waste product represented the diet of protein content in the treatment.

Table 3. Averages of feed consumption of each treatment using coconut meat waste product substituting rice bran in ration and animal drinking water using fresh water and coconut water in the experiment

Pig drinking water levels	Coconut meat waste product levels 0 %	22.5 %	45 %	Average
Average feed consumption(kg/animal/day):				
Coconut water 0 %	3.99	3.39	2.43	3.273 [y]
Coconut water 100 %	3.51	3.24	2.07	2.939 [z]
Average	3.75 [a]	3.32 [b]	2.25 [c]	3.106
Average consumption of drinking water (liter/animal/day):				
Coconut water 0 %	5.80	5.29	4.22	5.10[y]
Coconut water 100 %	8.47	7.08	5.19	6.91[z]
Average	7.14[a]	6.19[b]	4.71[c]	6.01
Average daily gain (ADG, gram/animal/day):				
Coconut water 0 %	773	777	470	674
Coconut water 100 %	630	673	410	571
Average	702[a]	725[b]	440[c]	662
Average of feed efficiency (ADG/feed consumption):				
Coconut water 0 %	0.190	0.230	0.190	0.200
Coconut water 100 %	0.180	0.210	0.200	0.190
Average	0.185	0.220	0.195	0.200
Average of body weight at slaughter period (kg/animal):				
Coconut water 0 %	106.33	108.67	90.83	101.94 [y]
Coconut water 100 %	99.00	101.67	88.33	96.33 [z]
Average	102.67 [a]	105.17 [b]	89.58[c]	99.14

[y,z] *Means within the same variables in each column are different at p<0.05;*
[a,b,c] *Means within the same variables in each row are different at p<0.05.*

There were also major differences in the apparent results of the fat content of the coconut meat waste product which was analyzed to be 56.86 % in this study compared with fat content of 32.5 in the rice bran at present study. However, the highest fat content in coconut meat waste product would be able to reduce the back fat thickness in growing pigs. The accumulation of fat in the pig body is related to the activities of adipose tissue (Rumokoy et al., 2014).It was shown that the fat content of the animals fed ration containing 12% coconut fat was 15.9% and this was significantly lower ($p<0.001$) than that of 21.1% measured in the animals fed rations containing 12% sunflower oil (Berschauer et al., 1984). The contents of myristic acid and linoleic acid were significantly higher of 48.7 and 11.3% in coconut fat, respectively; compared with 0.82% and 16.9% in sunflower oil (Berschauer et al., 1984).

The interested results indicated that higher level of the coconut meat waste product substituting rice bran would decrease feed consumption and drinking water consumption by animals. In contrast, utilization of the coconut drinking water of 100 percents would also increase animal drinking water consumption. This high coconut drinking water consumption might be due to stimulation by the nutrient contents in coconut water mainly dominated by high contents of Potassium, Calcium, Magnesium, Nitrogen, glucose and sucrose (Table 2) that were increasing water palatability.

The substitution of rice bran up to 100 percents by coconut meat waste product in ration would produce lowest ADG of the animals. However, the highest ADG was reached by ration containing a balance use of rice bran (22.5%) and coconut meat waste product (22.5%) in ration. This balancing use of rice bran and coconut meat waste product was also produce the optimum feed efficiency, although feed efficiency of the animals consuming ration containing all coconut meat waste products (45 percents) was higher than that of the animals consuming ration containing all rice bran (45 percents).

Different sources of drinking water of animals using fresh drinking water and coconut drinking water produced also different ADG of the animals. Nutrient contents in coconut drinking water (Table 2) might not be able to increase more ADG of animals, even though these could be able to equalize the feed efficiency due to low feed consumption by animals consuming coconut drinking water.

The significant relationships between coconut meat waste product intake and coconut drinking water imply that if the coconut meat waste product level in diet had been highly increased with changing fresh drinking water by coconut drinking water then the back fat thickness response in animals would also have

been lower. This hypothesis was approved in this experiment.

A related study to determine the use of coconut drinking water revealed the potential increase of carcass percentage, carcass length and the wide of loin eye area in growing pigs. However, the nature of the basal diet using coconut meat waste product substituting rice bran was quite limited to the present work in the component of carcass quantities.

There were significant differences in the average daily gain of the animals fed the higher contents of coconut meat waste product substituting rice bran in the diets.

The treatment with 100 % of coconut meat waste product substituting rice bran in ration had, however, 61 to 63% lower average daily gain (ADG) significantly when compared at similar ADG with the treatment using 50% of coconut meat waste product substituting rice bran in ration and control treatment without coconut meat waste product in ration, respectively.

As the lower protein concentration in diets and the growth rate were higher in correlation required per kg growth as shown for approximately protein content of 89% (R_1) and 83%(R_2) lower than that of the control treatment (R_0).

The efficiency of protein utilization combined with that of lower fat and crude fiber contents in ration containing coconut meat waste product of the animals was able to equalize the feed efficiency compared with that in control ration (R_0). The values of feed efficiency for the use of 50% coconut meat waste product substituting rice bran in ration were intermediate.

CONCLUSIONS

Utilization of coconut meat waste product up to 50% substituting rice bran in ration with the level of 22.5% was able to yield the optimum growth rate and carcass quantity of pigs.

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RESEARCHES REGARDING CANNON BONE PERIMETER AVERAGE PERFORMANCES IN ROMANIAN HUCUL HORSE BREED – PIETROSU BLOODLINE

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Abstract

Study of average performances in a population have a huge importance because, regarding 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. The biological material is represented by 91 Hucul horse from PIETROSU bloodline divided in 3 stallion families analyzed at 18, 30 and 42 months old, owned by Lucina hucul stood farm. The average performances for cannon bone perimeter was 17.20 cm. at 18 months, 18.04 cm. at 30 months old and 18.63 cm. at 42 months old. We can observe a good growth rate from one age to another and significant differences between sexes. The average performances of the character are between characteristic limits of the breed.

Key words*:horse, Hucul, Lucina, Pietrosu, bloodline.*

INTRODUCTION

The individual can no longer be a reliable source of information on genetic determinism or in mechanisms of phenotypic manifestation for the quantitative character considered, which makes the unit of study for these characters extend to the population level. Also, in order to study the nature of the quantitative differences regarding the manifestation of the same character in different individuals in different populations, measurements are required which generally do not express the character itself but its value.

The character's average performances, in a population, have a great value because it can offer an overview of the genotypic value. All this is possible because, regarding to a population, the average phenotypic values is equal with the average of genotypic values (Maftei, 2015). More than that, the study of average values of characters, in a population, can offer an idea about populational genetic level.(Maftei, 2015).

Tracking of body growth can be done by periodic determination of body weight and body dimensions. As a rule, there is a direct relationship between the weight of an animal and its volume, which means that the dynamics of the weight will, indicate also the dynamics of the dimensions. Determining only the body weight can not always indicate the clearest picture of the evolution of the growth process, as it may happen when the weight remains the same between two determinations (Popescu – Vifor, 1978; 1985).

The growth process can be followed by: growth energy, growth rate, growth intensity, and growth coefficient.

Perhaps more than in other species of economic interest, in horses, phenotypic characters occupy an important place in the breeding programs, as they play an essential role in the expression of production characters.

In this group of characters, the characters expressing the growth process (height, cannon bone perimeter, thoracic perimeter) and those expressing the body conformation specific to the production specificities (running, sports, jumping, recreation, traction) are predominantly included. These characters belong to the group of morphological characters and are determined by somatometry. Somatometry is the most objective method of assessing the exterior of the horses. In principle, it consists in direct measurement, on

the live animal, of the dimensions of the different body regions, or even the characteristic size of the species. In this study we use the cannon bone perimeter values.

MATERIALS AND METHODS

The purpose of using somatometry in assessing the exterior of the horses is to determine, first of all, body development, but also to establish the overall harmony of the specimen (Marginean et al., 2005).

In this study we analyze the cannon bone perimeter, measured with the ribbon and representing the circumference of the middle third of the whistle.

Body size judgments (valid for both young and adult animals) are usually based on the scales set for the standard of each breed, or according to the scales set by the breeding program.

The characteristic limits for each character are different from a breed to another and also between the two sexes. To reach the maximum limit, note 10 is given. For the minimum limit and below this limit, note 4 is given. The exceeding of the maximum values is penalized by subtraction of the note.

For realising purposed objectives, the biologic material became from Lucina stood farm. It is a sample of 91 horses from Hucul breed – Pietrosu bloodline, divided in 3 stallions families: Pietrosu VIII, Pietrosu IX and Pietrosu X, presented in Table 1. It was 44 males and 47 females analyzed at three different ages: first grading at 18 months old, second grading at 30 months old and third grading at 42 months old. After the third grading the individual will be tested for energetic capacity. The sample of 91 horses was extracted from population in according with registered performances, for all three ages, in order to have one balanced experimental plan (Popa, 2009).

The individuals was studied at three different ages:18, 30 and 42 months old.

We had calculate statistics like Average, variant, average error, standard deviation, and coefficient of variability. We applied significance tests like Student. The Fisher test was applied to the case of several samples, preceded by a variance analysis. The calculated F value was obtained by reporting the average squares value between the samples at the average squares from sample. The Tukey test involves calculating a statistic, noted

$$w = q_{(p;GL_e;\alpha)} \times s_{\bar{X}}$$

where q represents the standardized amplitude read from the table at the desired significance level (α), p being the number of groups, and GLe - degrees of freedom from the intragroup component of the variance analysis table. The value is obtained by the fact that MPe is the intragroup squares average value, and n is the average size of the groups. Applying Fisher or Tukey tests had the advantage to highlight, to allows us to see between which families we recorded significant differences.

Table 1. Analyzed biological material

PIETROSU families	Individuals	Males	Females
Pietrosu VIII	6	2	4
Pietrosu IX	65	31	34
Pietrosu X	20	11	9
TOTAL	**91**	**44**	**47**

RESULTS AND DISCUSSIONS

The average performances for cannon bone perimeter, in Pietrosu bloodline, is presented in Table 2, and the dynamics of the same character can be observed in Figure 1

Analyzing the data presented, there is a more pronounced variability of the cannon bone perimeter, in the Pietrosu bloodline at the first ranking (18 months old), in males. This is most likely due to the environment, or possibly intangible factors. From the analysis of the data can notice the existence of differences with a high degree of significance between individuals belonging to the two sexes.

The calculated Fisher test scores reveal distinctly significant differences between half sibs (males and females) families, in the Pietrosu bloodline for the cannon bone perimeter, but only at the age of 42 months old (F = 6.53).

Tukey's test calculated values show that at the age of 42 months old there are significant differences between the performance of the Pietrosu VII and Pietrosu X families, as well as between the performance of the Pietrosu IX and Pietrosu X families.

Table 2. Average performances for cannon bone perimeter in Pietrosu bloodline

Family	Sex	Age (months)											
		18				30				42			
		n	$\overline{X} \pm S_{\overline{X}}$	s	v%	n	$\overline{X} \pm S_{\overline{X}}$	s	v%	n	$\overline{X} \pm S_{\overline{X}}$	s	v%
P VIII	M	2	18	0	0	2	18.5 ± 0.5	0.71	3.84	2	18.5 ± 0.5	0.71	3.84
P IX		31	17.42 ± 0.13	0.75	4.31	31	18.29 ± 0.11	0.63	3.44	31	18.82 ± 0.12	0.68	3.61
P X		11	17.41 ± 0.22	0.74	4.25	11	18.64 ± 0.24	0.78	4.18	11	19.45 ± 0.17	0.57	2.93
Total M		**44**	**17,44 ± 0,11**	**0.73**	**4.19**	**44**	**18.39 ± 0.1**	**0.67**	**3.64**	**44**	**18.97 ± 0.11**	**0.70**	**3.69**
P VII	F	4	16.75 ± 0.15	0.29	1.73	4	17.75 ± 0.15	0.29	1.63	4	17.63 ± 0.24	0.48	2,72
P IX		34	17.04 ± 0.1	0.59	3.46	34	17.72 ± 0.09	0.52	2.93	34	18.32 ± 0.13	0.73	3,98
P X		9	16.83 ± 0.22	0.66	3.92	9	17.72 ± 0.26	0.79	4.46	9	18.56 ± 0.15	0.46	2,48
Total F		**47**	**16,98 ± 0,09**	**0.59**	**3.47**	**47**	**17.72 ± 0.08**	**0.56**	**3.16**	**47**	**18.31 ± 0.1**	**0.70**	**3.82**
Total		**91**	**17,20 ± 0,07**	**0.70**	**4.07**	**91**	**18.04 ± 0.07**	**0.70**	**3.88**	**91**	**18.63 ± 0.08**	**0.77**	**4.13**
Significance of observed differences		3.54 ***				5.58 ***				4.71 ***			

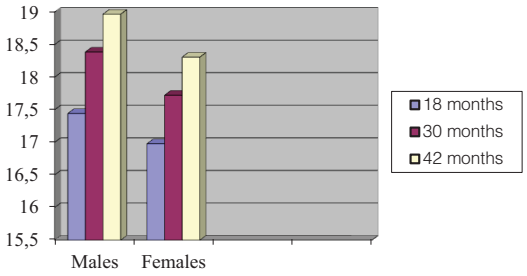

Figure 1. Dynamics of cannon bone perimeter

CONCLUSIONS

The calculated values for Fisher test reveal the existence of some distinctly significant differences between half sibs families from Pietrosu bloodline, for cannon bone perimeter, but only at 42 months old (F=6.53). Values of Tukey test shows that at 42 month old are significant differences between performances of families Pietrosu VII and Pietrosu X, and also between Pietrosu IX and Pietrosu X families. From the data presented in for the cannon bone perimeter whistle, it is observed that, at this three ages, the average values of the character are approximately equal, at all the genealogical lines, for both sexes

Table 3. Observational and causative components of variance at18 months old

Cannon bone perimeter	Components of variance	Observational			Causative		
		S_F^2	S_I^2	S_i^2	V_A	V_D	V_M
	Abs. val.	0.4659	0.0064	0.4595	0.0256	0.0554	0.3849
	%	100.00	1.37	98.63	5.49	11.89	82.62

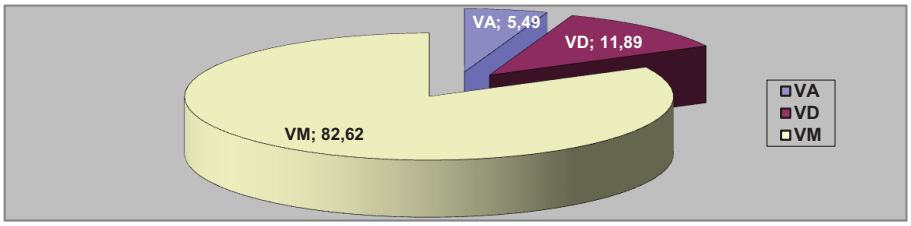

Figure 2. Percentage of causal components of variance for cannon bone perimeter at 18 months

Table 4. Observational and causative components of variance at 30 months old

Cannon bone perimeter	Components of variance	Observational			Causative		
		S_F^2	S_I^2	S_i^2	V_A	V_D	V_M
	Abs. val.	0.4414	0.0012	0.4402	0.0048	0.1194	0.3172
	%	100.00	0.27	99.73	1.09	27.05	71.86

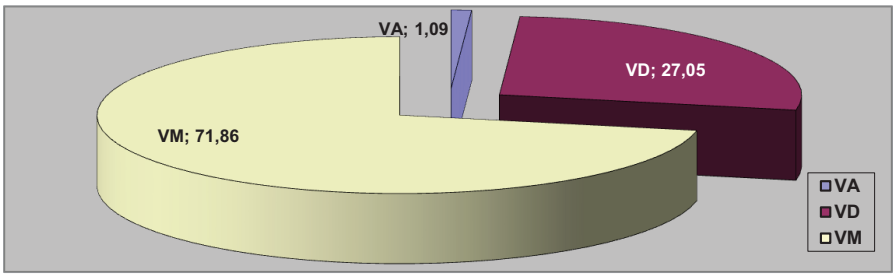

Figure 3. Percentage of causal components of variance for cannon bone perimeter at 30 months

Table 5. Observational and causative components of variance at 42 months old

Cannon bone perimeter	Components of variance	Observational			Causative		
		S_F^2	S_I^2	S_i^2	V_A	V_D	V_M
	Abs. val.	0.6177	0.0656	0.5521	0.2624	0.0238	0.3315
	%	100.00	10.62	89.38	42.48	3.85	53.67

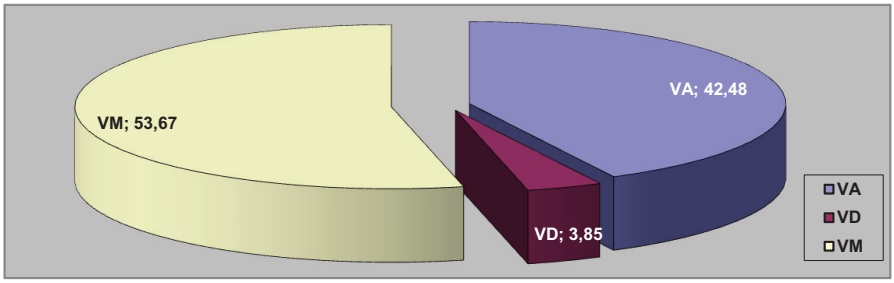

Figure 4. Percentage of causal components of variance for cannon bone perimeter at 30 months

The results obtained in the analysis of the causal components of the variance, presented in Tables 3, 4 and 5 and in Figures 2, 3 and 4, for the cannon bone perimeter at this three ages, lead to the following additions:

The perimeter of the whistle is a character that shows a very poor genetic determinism at the first two ages, the share of the additive genetic variance in the total phenotypic variance being very small: 5.49% 1.5 years and 1.09% 2.5 years.

At 3.5 years, the situation changed radically, the additive genetic variation accounting for a significant share of the phenotypic variance (42.48%).

At 2.5 years, there is a significant share of the variance due to dominance (27.05%).

The environmental variation also has significant weightings at the three studied years, but it shows a decreasing trend (82.62% at 1.5 years, 53.67% at the 3.5 years ranking).

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THE DYNAMICS OF MILK PRODUCTION IN MONTBELIARDE BREED ON A FARM IN SOUTHERN ROMANIA

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Abstract

The Montbeliarde breed became known in Romania after 2009 when several animals from France were imported. This breed is robust, has easily adapted to the growing conditions in Romania and the milk has a special quality. This study was conducted in the Didactic Farm Moara Domnească on a number of 25 dairy cows during 2017-2018. The quantitative and qualitative parameters of milk production were analyzed, based on records on the farm, as well as periodical analysis reports.

Key words*: milk, Montbeliarde breed, farm.*

INTRODUCTION

Milk is a very important food product due to its complex chemical composition, biological value and high digestibility. It contains more than 100 substances needed by the human body: all 20 amino acids, 10 fatty acids, 25 vitamins and 45 mineral elements.

This is a product of great socio-economic importance, essential for the physical and intellectual development of the individual, as well as for maintaining the health of the population.

Beside ensuring the smooth growth and development of mammals, milk is a perfect antidote for adults who perform work activities in toxic environments. Regular milk consumption allows the body to dispose of any and all toxic wastes that may have been deposited in the human body during the hours of exposure tothe toxic environment.

Expressed in calories, the nutritional value per one liter of milk is equivalent to: 400 g pork; 750 g beef; 7-8 eggs (Georgescu et al., 2000, 2009).

Currently and in perspective, as a result of the growth rate of the human population and the surging preferences for food products of animal origin, the main purpose of cattle breeding is the continuous and sustained increase of milk and meat production.

The history of the Montbeliarde breed is linked to the beginning of the 18th century, namely when a series of farmers from the present-day territory of Switzerland settled in France, in the France Comté region, bringing along their cows. As a result of a methodical process of selection of specimens from this cattle population, they began to participate in various exhibitions under the name of Montbeliarde (after the name of the main city in the department of Doubts, in the region of France Comté). Montbeliarde is a mixed breed, being deemed the most advanced breed for milk production within the Simmental family.

The Montbeliarde breed is part of the Simmental breed group, specializing in dairy production. Formation of this breed has gone through a comprehensive process improvement from one generation to another (Vidu et al., 2014). In these successive stages, Red Holstein participated (below 25%) in improving the amount of milk in the Montbeliarde breed. This breed is characterized by: large body development, differentiated according to sex (body weight, 600-750 kg cows and 1000-1200 kg bulls) (Bugeac et al., 2013).

24% of respondents toa survey conducted in 2012, in Western France, said that the Montbeliarde breed is robust and its health problems are reduced compared to the two other breeds in the region, namely Prim

Holstein and Normande. This breed has a low predisposition to metabolic disorders, such as milk fever and displaced abomasum, as well as mastitis.
The share of mastitis in the three breeds studied in the region has shown that it doubles in the Holstein and Norman breeds, compared to Montbeliarde. Costs for veterinary services are 34% lower in the Montbeliarde breed.
In terms of the breeding activity, the Montbeliarde breed recorded the lowest consumption of semen doses per one gestation (1.8), compared to the Norman breed (2 doses of semen per gestation) and Holstein (2.2 doses /gestation).
The average Calving Interval is 402 days, 38 days less than in the Holstein breed (www.montbeliarde.org).
In Romania, almost all species of farm animals have been and are bred. Of all these, cattle breeding is a highly important production branch of agriculture, as it provides the highest volume of products of animal origins that humans need, respectively the highest share of raw material for the food industry.
In recent years, there has been an intensification of Romania's participation in international trade, so that the area of utilization of cattle breeding is gradually expanding so as to provide as large quantities as possible, demanded by both the internal and external markets.
As for our country, Montbeliarde breed became known in Romania after 2009 when several specimens from France were imported. This breed is robust, has easily adapted to the conditions in Romania and the resulting milk is highly qualitative.
In 2010, the Moara Domneasca Didactic Farm of the University of Agronomic Sciences and Veterinary Medicine in Bucharest imported from France 20 heifers, which calved in the following 3-4 months on the farm. The cows adapted to the conditions of our country and performed very well, especially in milk production, considering the difference of habitat between France and Romania. The imported primiparous cows exceeded by far the performance of local cows and were comparable to cattle breeds such as Holstein, red or black variety. The studies conducted on the first lactation highlighted the following results: the average duration of lactation was 289 days, and the average milk production was 6921 liters of milk, compared to France's national average of 6671 liters of milk (Vidu et al., 2010).

MATERIALS AND METHODS

This study was conducted in the Moara Domnească Didactic Farm on a number of 27 dairy cows during 2017-2018. The quantitative and qualitative parameters of milk production wereanalyzed, based on the farm records, as well as on the periodical test reports.
Located in the Plain of Vlasia, the territory of the Moara Domneasca farm is a subunit of the Romanian Plain. The locality of Moara Domneasca is located in the Southeastern part in the transition zone from steppe to forest steppe, latitude 44°30' North and longitude 29°31' East, at an altitude of 90 m above sea level. The climate is temperate with harsh winters, when the average temperature of the coldest month drops below -3°C, and the warmest month has an average temperature higher than + 10°C; summers are hot, with the average temperature of the hottest month exceeding 22°C.
The dairy cows are housed during the stalling period in a barn updated in 2010, with free stalls. The animals benefit from a sleeping area fitted with a rubber mat. During summers or when the weather is fine, the animals are taken to the pen.
The feeding technology is not seasonally differentiated, each age category receiving feed ratios in accordance with physiological and production needs.
The milk produced by farm is sold directly via 2 milk vending machines or is used for the preparation of fresh cheese.

Figure 1. Image of the Moara Domneasca Cattle Farm

Table 1 shows that dairy cows represent 43% of the total cattle number. Heifers are used for the selective replacement, and the redundancy is capitalized at various farmers.

Table 1. Herd structure in the Moara Domnească farm

Age category	Heads	Percentage of the total number of cattle
Dairy cattle	27	42.86
Heifers	8	12.7
Young females 12-18 months	4	6.35
Young females 6-12 months	0	0
Young females 3-6 months	5	7.94
Young male 3-6 months	4	6.34
Young females 0-3 months	2	3.17
Young male 0-3 months	5	7.94
Beef	8	12.7
Total	63	100

RESULTS AND DISCUSSIONS

Dairy cows farm University of Agronomic Sciences and Veterinary Medicine of Bucharest are kept in loose housing, are milked twice a day herringbone milking parlor. Cows receive one type of ration every season. A cow receives 6 kg alfalfa hay, 10 kg of corn silage, 5 kg concentrate feed and 6 kg straw.

In 2017 the farm produced 121.19 tons of milk, which was used as follows: 64.5% was sold as fresh milk through dispensers, 9.2% was used for the feeding of calves, 25.4% turned into cheese and 0.8% was distorted because the quality was not good (Table 3).

Figure 2. Image from the milking stalls

Table 2. Table title example that should be replaced with your information

Parameter	n	X±*Sx*	V%
Duration of lactation (days)	25	295.14±2.41	8.5
Quantity of milk per total lactation (kg)	25	5916±172.01	18.2
Quantity of fat (kg)	25	261.12±5.11	18.54
Quantity of protein (kg)	25	223.57±5.02	18.75
Fat %	25	3.81±0.03	15.11
Protein %	25	3.23±0.1	17.21

The analysis performed on 25 lactations completed in the period 2017-2018 revealed the following: the total lactation duration was, on average, 295 days, with a relative low variability of 8.5%.

The total lactation in the analyzed cows was 10 days shorter than normal lactation. The amount of milk per total lactation reached an average value of 5,916 kg of milk, with a slightly higher coefficient of variability, respectively 18.2%.

This variability is explained by the fact that the study analyzed lactations in cows during various lactation stages.

Figure 3 presents the dynamics of milk production per each cow for one year. The graphical representation illustrates that milk productions are lower in July, August and October. This lower value of milk production is caused by the fact that several cows are at the end of their lactation period, when the total production decreases physiologically. The highest average monthly production is obtained in December-January, when cows are in the ascending phase of lactation.

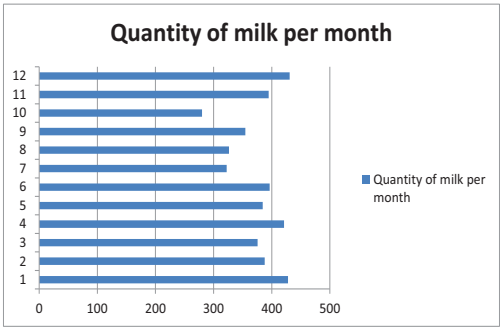

Figure 3. Milk quantity per cow / month in 2018

As for the supply of milk dispensers, Table 3 shows that consumption is lower during summertime, a significant share of the milk production being processed into cheese. As for the consumption of whole milk for feeding of calves, a sinuous evolution is noticed, depending on the suckling phase they are in. It is worth noting that the calves on the farm are fed with whole milk, not with milk substitute. Furthermore, 972 kg of milk are not intended for human consumption as a result of drug residues that are present in this milk.

Table 3. Capitalization of milk in 2017

Month	Produced milk	Milk fordispenser	Milk forcalves	Milk for cheese	Altered milk
01	11308	6743	2469	2096	
02	10812	8041	1302	1469	
03	13067	7977	1440	3576	332
04	12093	7779	1386	2928	
05	11874	8135	459	3280	
06	11437	7735	482	3220	
07	10810	5938	786	4086	
08	10482	5003	1189	3970	320
09	9849	5056	868	3925	
10	8694	6065	400	1909	320
11	5380	5062	42	276	
12	5380	4535	437	408	
Total	121186	78069	11260	31143	972

A higher quality of milk is a permanent concern of farmers because it is base for setting the purchase price of raw material milk.

Table 4. Physical properties of milk

Indicator	Average values in milk
Density la 20 °C	min. 1.030 g/100g
Dry substance	min. 12.4 g/100g
Specific heat	0.93 cal/g.degrees
Freezing point	-0.555 °C
PH	6.6-6.8
Acidity (Thorner degrees)	16
Index of refraction (20 °C)	1.35

The physical properties of milk are important mainly for processing (Table 4). For example, the freezing point is important to control the counterfeiting of milk by addition of water, to detect milk-soluble impurities, to indirectly estimate milk's fat content (a high percentage of fat determines the decrease of the freezing point).

The microbiological analysis of milk can offer valuable information on the hygiene conditions in stalls, respectively during milking, on the conditions under which the primary treatment of the milk is made and on the health of the animals.

Figure 4. Housing of dairy cows in the Moara Domneasca farm

The experience of farmers and researchers in the industry has proven, without any doubt, that where the teats of cows are not cleaned before starting milking (washed and mandatorily dried with a single-use towel), where the milking teat cups and the tubes through which milk flows inside the milking device, as well as the containers used to collect milk (collection tank, buckets, etc.) are not washed first with cold water, then with hot water and detergent and mandatorily disinfected with water and chlorine, the total number of germs (TNG) has a steep increase in fresh milk. If such milk, which has been contaminated with germs during milking, is kept at room temperature (20ºC), then the number of germs will increase to such extent that it will shortly exceed the

standard of milk quality. Since a very large share of the germs collected from teats or skin and especially from the milking tools are germs who like the milk nutrients (especially lactose), their activity will cause the physical and chemical alteration of milk components to such a large extent that it can no longer be used into processing high quality dairy products.

Table 5. Evolution of TNG and NCS in 2018 in the milk produced in the Moara Domneasca farm

Month	TNG (ufc/ml)	NSC (/ml)
01	$9.0x10^4$	214000
02	$8.5 x10^4$	134166
03	$8.2 x10^4$	141666
04	$8.5 x10^4$	136000
05	$8.5 x10^4$	129666
06	$8.3 x10^4$	133833
07	$8.4 x10^4$	144000
08	$8.1 x10^4$	132000
09	$8.5 x10^4$	135000
10	$8.7 x10^4$	440000
11	$8.1 x10^4$	170000
12	$8.8 x10^4$	123000
Media	$8.46x10^4$	169444

Table 5 shows that the total number of germs was not exceeded during the year of 2018, which brings additional safety to milk quality, especially since it is also marketed in a non-pasteurized form by milk vending machines.
As for the number of somatic cells (NSC), we find that the maximum permitted value (400000 nsc/ml) was exceeded in one month only (October 2018). Figure 5 illustrates the evolution of the number of somatic cells in 2018.

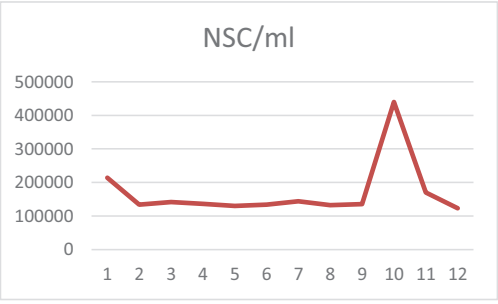

Figure 5. Evolution of the number of somatic cells in 2018

CONCLUSIONS

The Montbeliarde breed has universal importance, especially for milk production, but can also produce good results in meat production. The state of health of the Montbeliarde breed is very good, the breed adapts easily to environmental conditions and does not require special care. The quality of milk is high, consumers preferring milk from this breed.

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STUDY OF HOOF TRIMMING IMPORTANCE FOR TRANSITION PERIOD COW

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Abstract

Management of lameness in dairy cows became a big problem for specialists, leading to legislative rules. Lameness has an major impact on health and cow comfort, affecting also the fertility and milk production. While in many instances pain may be difficult to recognize in an animal, lameness is a clear sign that an animal is experiencing pain and discomfort. This study has been performed in Agroserv Mariuta dairy on 50 cows in transition period. In this study was include factors who influence the hoof heath, prevention tips, treatment and benefits.

Key words*: lameness, hoof trimming, health, dairy, production, fertility.*

INTRODUCTION

Hooves are painful diseases with costly treatments, which affect cow productivity and breeding performance. Worldwide, clinical estimates of laminitis prevalence range from 20 to 30%. Estimates of the prevalence of foot lesions found at the cut of the hoof are much higher, ranging from 40 to 70% of cows (Solano et al., 2016). Types of lameness due to foot injuries can be broadly categorized into infectious (digital dermatitis (DD) heel horn erosion, foot rot) and hoof horn (ulcers, white line disease, hemorrhage). Although infectious lesions are the most common type of lesions in most herds, hoof horn lesions are far more costly due to their effects on milk production and culling (Cramer, 2018).

The actual identification and management of the foot diseases of the animals in production is a growing concern of the breeders which has brought to the legislative attention worldwide. All aspects of food production are subject to increased consumer control, as well as animal welfare and rights. While most of the pain is difficult to identify in animals, foot disorders are a clear sign that the animal is suffering and has a discomfort. Because of this at the industrial level, farms are committed to reducing the incidence and severity of foot-and-mouth disease. The result of this activity will materialize at the farm level by increasing milk production, reducing reformed animals from pedal causes and increasing profit (Burgi, 2016).

Foot conditions are a big challenge, being a problem of the same magnitude as the problems at the level of the udder. Specialized studies can identify the main factors that cause this problem.

The high density in the stable, if there are too few cushions for the number of cows in that group, the cow will not find a clean place to rest, and then she will choose to sit on an unpaved, dirty place which may increase the incidence of cows. There were problems with the tabs. Group exchange increases the risk of foot infections. Also in this category we include the poor quality of the area.

Although it is desirable for the cow to have a large production, it is precisely her effort to produce much milk that leads to combined problems of the udder and the tabs. The pressure on the cow's body to produce milk creates a metabolic stress that will decrease the immunity (Baraitareanu and Vidu, 2019).

One of the main factors is the genetic one, the podal problems are inherited, it is desirable to eliminate these cows from the herd, as well as their descendants. Too high the protein-energy level in the feed ratios increases the incidence of interdigital disorders.

Foot baths made incorrectly or rarely will affect the health of the feet. Incorrect treatment of problems and poor management of a regular trimming will lead to an increase in the number of animals suffering. The biggest problem is not the treatment but the identification, the farmers underestimate the problem, a good management can lead to the significant reduction of reformed animals due to the pedal problems.

More specifically, body condition score (BCS) and the thickness of the digital cushion (DCT) have been identified as risk factors for lameness. Studies conducted in the UK have shown that a decrease in BCS, or a low BCS, increases the risk of lameness (Randall et al., 2015). Studies done in a single herd in New York, USA have shown that there is an association between DCT and lameness (Machado et al., 2011; Cramer, 2018).

In order to identify the laminites we will have to visualize how the cow goes, so its gait and body position during the gait is framed in a scale. In order to facilitate the work of the farmer, different devices appeared on the market that estimates the activity, the corporal position, announcing the farmer of the possible problems. (4d4f).

Burgi (2016) explains that during the transitional period the cow goes through hormonal, metabolic and behavioral changes, which can influence the predisposition for foot conditions. Integration after calving in a new group or in a new shelter (another type of floor, another type of couch, etc.) can be a stress factor, which causes it to spend more type standing. Therefore, this position can exert additional pressure on the limbs as well as on the bloodstream.

Also, during the pre-partum period in the body of the cow a complex of hormones is elaborated, which have the role of relaxing the ligaments of the pelvis, including those of the hind limbs.

Particular attention should be paid to the period of close-ups, because the decrease in dry matter intake during this period leads to the deterioration of the health condition during the fresh cow period. For example, ketosis and abomasum dysplasia may occur.

Most cases of floor-laminate disorders occur from 45 to 70 days after calving or during the transitional period. The diseases overlap with the increase of milk production, it is difficult to treat, and prevention is recommended. It is recommended that each cow be evaluated 8 weeks before calving, and during each year, each cow is evaluated 2-3 times (Burgi, 2016).

MATERIALS AND METHODS

This work aims to bring to the farmers an authentic problem and that by treating it will significantly increase milk production. Nowadays due to a massive demographic increase and the increasing degree of poverty, the main food sources increase their volume. In the case of dairy farms, the desire of the farmer everywhere is to increase milk production, the economic variant being to increase the quantity of milk per cow head, a main factor leading to this is the creation of animal welfare. This paper, by the method presented aims to raise awareness of the importance of trimming in milk cow farmers during the post-partum transition period.

For the present experiment, 50 cows were selected during the second lactation, 25 of them were sent in the first 21 days after the fetus and the other 25 were sent when they had problems at the foot level. Both groups of animals were treated by the same technician using the same containment stand and the local treatments.

The animals chosen for genetic study are similar, their mothers performed on average with a production at 305 days of 12,500 kg milk. The experiment was conducted within the dairy cow farm SC AGROSERV MARIUTA SRL from Ialomita county, Romania. The study started in March 2018 and was completed in March 2019, the animals analyzed were monitored during the second lactation.

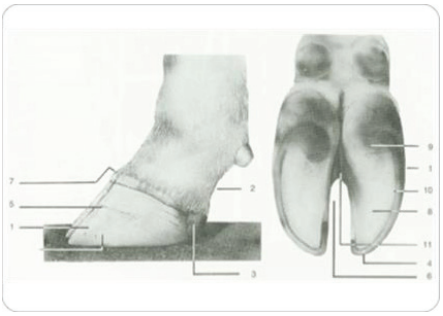

Figure 1. Conformation of the foot (veepro.nl)

The conformation of the foot:
1 .The horny wall of the claw.
2. The pastern.
3 .The heel or bulb.
4. The weight-bearing border of the wall.
5 .Growthrings.
6 .The interdigital space.
7 . The coronet.
8. The sole; if the claw is healthy, the thickness is 5 to 7 mm.
9. The soler part of the heel; the weight-bearing part of the heel.
10. The white line; the horny connection between the weight-bearing border and the sole.
11. The interdigital skin.

The working protocol was as follows:
The animals entered in the protocol of those controlled in the first 21 days after feeding, is a well-organized protocol so the animals between 15-30 days after feeding will enter the contention stage daily and will be subjected to the first clearing of the lactation. Those found with problems and needing a check-up will return to the stand within 5 days of diagnosis. The process will be repeated from 5 to 5 days until the area is healed.
The product used mainly for the cows studied is PODODERMIN. It involves a local treatment that acts with the specific, synergistic bactericidal effect of oxytocin, Nitrofan, Iodofor and bismuth, which acts against gram positive and gram negative germs, as well as by the keratolytic and scarring effect of salicylic acid. The specific smell removes insects.
The drug is of a creamy, solid consistency. Due to the chemical composition, it is used with gloves. The product is placed on a sterile phase in an amount of 5-10g, then this phase is placed on the affected area at the level of the tabs and then for fixing them will bandage the tab.
This treatment will be repeated every 5 days so the phase and bandage will be replaced and the operator will be able to review the affected area. If this is healed then no further action will be taken.
Depending on the severity of the tab problem, the operator may attach prosthesis. This prosthesis is made of wood and with the help of special glue it sticks to the healthy nail thus helping the diseased and deep cleaned to regenerate (Figure 2).

Table 1. Rations used differentially for the transition period

RATION STRUCTURE	CLOSE UP	AFTER CLOSE UP
CORN SILAGE	18	30
ALFALFA SILAG	6	14
STRAW	0.5	
SOYBEAN MEAL	1	3
CORN	2.8	5
PREMIX 1		
PREMIX 2	0.2	0.3
PREMIX 3	1	
ALFALFA HAY	1.5	1
WHEAT	1.5	3.2
LIN SEED	0.4	1
CORN GLUTEN	1	1
DEXTROZA	0.3	
RUMEN PROTECTED FAT	0.3	0.4
RUMEN PROTECTED SOYBEAN MEAL	1	0.5
MONOCALCIC PHOSPHATE	0.1	0.1
Mg OXIDE	0.05	0.08
SODIUM BICARBONATE	0.2	0.2
MONOPROPILEN GLLYCOL	0.3	0.3
SOYA HULLS	1	1.5
SALT		0.1
CALCIUM CARBONATE		0.1
WATER		2

Figure 2. Wood prosthesis

RESULTS AND DISCUSSIONS

Foot conditions most often occur during the transitional period, when the animal is under very strong pressure. During this period the milk cow is in the phase of ascension of the lactation or of the mobilization of the corporal reserves.
From the observations I found that there is a decrease in body weight by 5-8% compared to the one born at birth (weight loss occurs on account of body fat and less protein); appetite is low and capricious (the intake is lower by 45%), slow growth dynamics (4-5 months), in the case of poor quality feed and more

pronounced (2-3 months) in the case of good quality feed.

Also, it is known that the nutritional balance especially the energetic one is negative, in the first two months after calving (-0,23 UN); the lactation curve is ascending (40% of the milk production is done); the reproduction activity is null in the first 2 months, with the gestation settling towards the end of the phase.

Table 2 presents the trimming interventions for 25 cows in the first days after calving. It can be observed that the average of the intervention interval is 18.4 days. In 56% of cases, a second intervention was needed in a range of 3 to 150 days, with an average of 61.71 days.

Tabel 2. Podotechnical events appeared for the group sent in the first 21 days after the facade

Animal	Birth date	Trimming date	Days in milk	Trimmimg control	Quantity of milk from the trimming day	Milk quantity after 15 days from trimming
1	**5/11/18**	5/30/18	19	9	40,9	42,1
2	**9/15/18**	10/4/18	19	77	41,5	42,7
3	**1/23/19**	2/3/19	11	3	41,1	40,2
4	**5/25/18**	6/13/18	19	55	41,3	40,5
5	**7/5/18**	7/17/18	12	150	41,3	42,1
6	**8/3/18**	8/25/18	22		41,3	41,2
7	**8/11/18**	9/6/18	26	105	41,4	41,9
8	**1/8/19**	1/31/19	23		41,5	37,3
9	**11/11/18**	11/26/18	15	24	41,7	40,3
10	**12/12/18**	12/24/18	12		41,8	41,3
11	**2/1/ 18**	2/22/18	21		41,9	40,9
12	**9/13/18**	10/6/18	23	54	42,1	42,1
13	**9/17/18**	10/8/18	21	112	42,3	42,3
14	**1/31/19**	2/21/19	21		42,4	41,0
15	**10/18/18**	11/8/18	21		42,5	40,4
16	**9/3/18**	9/21/18	18	136	42,5	41,3
17	**11/5/18**	11/25/18	20	25	42,5	41,7
18	**12/5/18**	11/25/18	19		42,9	41,9
19	**12/7/18**	12/19/18	12		43,0	41,2
20	**1/16/19**	1/25/19	9	11	43,0	41,1
21	**1/30/19**	2/14/19	15		43,3	39,4
22	**12/30/18**	1/16/19	17		43,4	41,0
23	**12/9/18**	12/29/18	20		43,6	41,0
24	**10/27/18**	11/20/18	24	29	43,9	42,4
25	**9/16/18**	10/7/18	21	74	43,7	42,9
		X=18,4 days		X= 61,71 days	X=42,23 kg milk/day	X=41,18 kg milk/day

The genetic potential of all the animals chosen for the experiment is similar, as shown in 43.3. The animals in Table 3 were treated after the problem was identified. Thus podotechnical operators from human error could omit certain animals.

„Thus, animals with foot problems may have difficulty walking and also all of them lead to a gradual decrease in milk production. Foot problems draw down the genetic potential of animals, which cannot manifest their true genetic value.

Tabel 3. Podotechnical events that appeared for the trimmed group when problems occurre

Animal	Birth date	Trimming date	Days before calving untill first trimming	Quantity of milk from the trimming day	Milk quantity after 15days from trimming
101	**11/9/18**	12/13/18	34	24,1	32,7
	1	12/19/18			
	2	1/18/19			
	3	1/22/19			
	4	1/26/19			
	5	1/30/19			
	6	2/5/19			
102	**3/2/18**	4/10/18	39	17,7	32,1
	1	4/16/18			
	2	4/19/18			
	3	5/29/18			
	4	8/7/18			
	5	12/01/18			
	6	1/8/19			
103	**1/23/19**	2/3/19	11	17,0	32,1
	1	2/8/19			
104	**7/11/18**	8/17/18	37	15,9	32,1
	1	8/2/18			
	2	8/6/18			
	3	10/17/18			
	4	2/8/19			
105	**10/25/18**	12/18/18	54	17,2	33,2
	1	1/7/19			
	2	1/11/19			
	3	1/15/19			
	4	1/19/19			
	5	1/30/19			
	6	2/5/19			
106	**11/28/18**	12/29/18	31	18,3	30,1
107	**8/15/18**	11/22/18	99	17,5	32,5
	1	9/6/18			
108	**11/11/18**	12/22/18	41	15,8	35,1
	1	12/19/18			
	2	2/2/19			
	3	2/6/19			
109	**1/15/18**	2/28/18	44	19,2	32,1
	1	3/6/18			
	2	5/30/18			
	3	8/7/18			
	4	8/12/18			
	5	8/16/18			
110	**6/2/18**	8/13/18	72	18,8	32,8
	1	1/20/19			
	2	1/29/19			
	3	2/4/19			
111	**12/19/18**	3/10/19	81	16,3	32,8
		1/15/19			
112	**8/31/18**	11/18/18	79	16,1	35,4
	1	9/13/18			
	2	9/19/18			
	3	12/20/18			
	4	2/2/19			
113	**12/2/18**	1/25/19	54	17,0	33,7
	1	12/14/18			
	2	12/19/18			
	3	2/2/19			

114	**1/11/18**	2/27/18	47	18,8	35,1
	1	3/29/18			
	2	4/3/18			
	3	5/29/18			
	4	6/8/18			
	5	7/18/18			
	6	7/30/18			
	7	8/10/18			
115	**1/12/18**	5/29/18	137	17,6	33,3
	1	1/14/19			
116	**3/17/18**	6/27/18	102	20,3	33,5
	1	6/3/18			
	2	8/14/18			
	3	2/4/19			
	4	2/8/19			
117	**1/25/19**	5/7/19	102	17,7	33,5
	1	1/17/19			
	2	1/21/19			
	3	2/5/19			
118	**1/6/19**	4/6/19	90	19,4	33,7
	1	1/26/19			
	2	2/2/19			
119	**9/15/18**	12/17/18	93	19,4	33,7
	1	12/20/18			
120	**3/17/18**	5/21/18	65	17,5	35,0
	1	3/23/18			
	2	3/27/18			
	3	3/31/18			
	4	5/22/18			
	5	6/5/18			
	6	8/13/18			
	7	10/17/18			
121	**11/17/18**	1/18/19	62	19,6	31,2
	1	2/2/19			
	2	2/6/19			
122	**8/3/18**	11/5/18	94	19,0	33,9
	1	10/17/18			
	2	12/20/18			
	3	1/31/19			
123	**11/28/18**	1/4/19	37	17,0	35,6
	1	1/9/19			
	2	2/2/19			
124	**8/20/18**	12/13/18	115	19,1	35,4
	1	12/22/18			
	2	12/25/18			
125	**6/19/18**	10/10/18	113	20,1	40,1
	1	7/30/18			
	2	8/13/18			
	3	8/24/18			
	4	9/26/18			
	5	10/2/18			
	6	10/4/18			
	7	10/19/18			
	8	11/2/18			
	9	12/20/18			
				X=18,22 kg milk/day	X=33,79 kg milk/day

If for the first experimental group the trimming was compulsory after calving, in the first month, for the experimental group 2 (Table 3) the trimming intervention was performed when a problem was observed in vacuum; in most cases difficulty walking.

In Table 2 we can see that the cows that were sent in the first 21 days required between 1 and 2 check visits, while the group sent when the animal already had pain had an average of 4.44 controls. The visits to the podiatry stand were more frequent, some requiring only 2 visits, while others of 9 visits to cure the problem.

The dynamics of milk production for cows that gave birth during the same month (March 2018), and the identification of acute pod problems was carried out in a period of 25-40 days. Each cow returned to control 3 times. Thus, the average daily quantity at the time of identification of the problem was 24, 1 kg milk, after tartar at control 1 the production increased to 47 kg, but also against the background of the normal ascending production curve. At the second control, the average quantity of milk increased to 52 kg, and at the last control the production was in the downstream phase (38 kg milk per day). Careful treatment of sprouts has limited their influence on milk production, cows following the normal lactation curve.

To prevent the occurrence of foot disorders, measures are required, such as:

- hygiene of the shelters, by keeping the surfaces of the shed clean and dry;
- bathing the feet of the cows at the milking room, by maintaining the same bath for a period of 2-3 days;
- periodic trimming, at least 2-3 times a year;
- the rations of the dairy cows during the transition period are energy-protein balanced, the frequency of their modification is reduced, the content of good quality fats is ensured according to the nutritional requirements, in the breast rest period (the dry period) the ration content in concentrates are reduced, even until the total removal of ration, and after calving the content of concentrates gradually increases, the addition of Zn in ration has favorable effects on the skin and its resistance to diseases at its level; the improvement of the animals in the direction of obtaining some animals with strong structure, with correct aplomb and resistant to the foot afflictions.

CONCLUSIONS

1. The animals from the first experimental group entered the mandatory trimming protocol in the first 21 days after feeding, respectively an average per group of 18, 4 days, which led to the rapid identification of the pedal problems, the preventive treatment and the reduction of costs with late treatments, with veterinary staff called for intervention. Also, the interval of return to trimming was reduced, being 61-71 days, a period that falls within the current recommendations of the specialists.

2. The animals from experimental group 2 entered the trimming protocol as needed, which

led to an increase in the number of interventions up to 9 during the second lactation.

The average milk production in the 25 cows was 18.22 kg milk, and after 15 days from the intervention the average milk production stood at 33, 79 kg milk per cow.

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INFLUENCE OF HEAVY METALS ON COCOON *BOMBYX MORI* L.

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Abstract

The silky butterfly with its high sensitivity can successfully be used as a bioindicator to detect environmental pollution. A major source of heavy metal pollution is large industrial plants for ferrous and non-ferrous metals, as well as ore extraction and ore mining companies. In such an area, the mulberry garden is a source of food for the experimental groups. Bombyx mori L are fed with mulberry leaf with a high content of heavy metals reared in the most polluted area of the Non-ferrous metals Plovdiv. This study is part of many years of scientific research in the field. The high content of heavy metals inhibits the development of traceable biological features. Significant differences in results between control and trial groups were found.

Key words*: Bombyx mori L., heavy metals, feeding, dirty leaves, silk cocoons.*

INTRODUCTION

The species *Bombyx mori* L. is hypersensitive to chemical contamination. *Bombyx mori* L suffered huge losses from poisoning by pesticides and heavy metals. Larvae can hardly overcome chemical poisoning, and most of them die.

Mamedova (1971) experiments with leaf-powered silkworms pretreated with copper and phorone salts and their mixtures, and establish an increase in yields of cocoons from one can of seed and an increase in the length of the silk thread. Positive impact on butter fly reproductive capacity and larval vitality is observed. The same author found a decrease in larval vitality, a reduction in the weight of cocoons and pupae, and a significantly lower consumption of iodine-treated mulberry leaf. Macro and trace elements are important for the development of such processes as maintaining a constant pH in the body. Deviating in one direction or another causes disturbances in the function of the enzymes (Babenko, 1965).

Populations of *Bombyx mori* L. of mono- and bi-voltinal origin are relatively higher in productive potential, but with lower tolerance to adverse environmental conditions (Murakami and Ohtsuki, 1989).

Macya (1983) found age differences in inhibitory doses of first to fifth larvae for the Co and Ni contents at 200 to 800 ml for these elements. In larvae that have taken larger amounts, a lethal effect caused by intoxication is observed. Thangavel (1990) finds that when larvae of *Bombyx mori* L. are fed with copper, magnesium and iron-treated leaves and their salts from rainwater, the metabolic activity of the larvae is stimulated, vitality and rotation increase. Increase the number of eggs laid in one piece. The percentage of silk increases by 2-4%.

In silkworms there is age sensitivity to heavy metals and pesticides. With increasing age, resistance increases. They are resistant are in the last fifth age.

According to Miyoshi (1971, 1978), high concentrations of heavy metals in food have a toxic effect on the silkworm. According to the same authors, the toxicity of heavy metals depends on the extent of their absorption in the intestine.

The silkworm (*Bombyx mori* L.) has the ability to accumulate large quantities of heavy metals in its organs without significantly affecting their development.

Our country owns one of the richest genetic resources of a silk butterfly. High-productive breeds and hybrids have been created in the SAES – Vratza. Our country has deep traditions in this direction. In the past, the cultivation of silk larvae was mainly directed towards the production of silk and silk products.

In the twentieth century, larvae were used as laboratory animals in a number of countries,

and in the present century as effective autotransplants, biosensors, silk chips and surgical sutures in medicine.

These facts reinvigorate the cultivation of silk larvae not only for the production of expensive silk fabrics but also as a producer of valuable products for other branches of the light industry and the high technologies in medicine and pharmacy.

Each stage of the metamorphous development of *Bombyx mori* L. - from egg to butterfly gives products that can be used and used in other industries. Even the butterfly itself is used in the pharmaceutical, cosmetic and food industries.

Silk larvae have been successfully used as a bioindicator and to identify environmental pollution with insecticides because of the insect's high sensitivity to them (Zlotin 1995).Literary sources indicate as good bio-indicators of environmental status of mulberry trees and silkworm larvae. The silk larva has been successfully used as a bioindicator and for detecting environmental pollution with insecticides due to the high sensitivity of the insect to them (Zlotin 1995).

Zhou (2015) in his studies tracks the accumulation of Pb in the feed chain soil, mulberry, larva. Heavy metals enter the leaves, and from there in the body of the fed silk larvae.

Harmful elements accumulate in the body where they are retained, some of them are excreted with excrement without passing into the final product - raw silk.

Mamedova (1971) conducted an experiment with leaf-treated silk larvae treated with copper and pine salts, and established an increase in the number of cocoons from one box of eggs and an increase in the length of the silk thread.

A positive influence on the butterfly reproductive capacity and the vigor of the pupation rate.

The same author finds a decrease in pupation rate, a reduction in the weight of cocoons and pupae, as well as a significantly lower consumption of iodine-treated mulberry leaf.

Shoukat (2014) monitors the accumulation of chromium (Cr) with a concentration (100 mg / l) and the toxic effects on the life cycle and growth of the larvae. It accumulates in the silk gland, the cuticle and the digestive canal. The author concludes that high chromium (Cr) inhibits the proper development of larvae and reduces silk yield. These results are close to that found by Khan (2009). According to the author, the accumulation of lead (Pb) in the body of the larvae reduces the production of silk.

Arnandova and Grekov (2003) also reported increased mortality with increasing accumulation of heavy metals in the bodies of insects.

MATERIAL AND METHODS

The study was conducted in the experimental base of the Agrarian University of Plovdiv. For controlled feeding of the larvae, leaves from the garden of the Agrarian University - Plovdiv, which is located outside the pollution zone (more than 10 km).

The experimental groups were fed with leaves of mulberry garden near. Experimental groups are fed with heavy metals contaminated leaves.One of the most polluted heavy metals in the country.

Objects of experimental work

The subject of the study is industrial hybrids of *Bombyx mori* L. and mulberry (*Morus alba* L.) as the main feed for larvae grown on highly contaminated soils.

The larvae of the hybrids (Super 1 x Hessa 2 and Baxa 1x Svila 2) were grown under controlled regime and observing technology for different ages. Observation of the larvae was performed using the standard technology (Grekov et al., 2005), during the spring season - May. All hybrids were grown in 4 replicates of 200 larvae counted after a second sleep. The information on the values of the main productive signs is taken according to commonly accepted methods (Grekov et al., 2005).

The larvae of the mulberry silk butterfly are fed three times a day with equal amounts of contaminated mulberry leaves.

We followed these quantitative characters:

- cocoon weight (mg);
- fresh cocoon weight(mg);
- filament length (m);
- filament size, g/denier,
- silk ratio (%).

High-performance hybrids C1xX2 and B1x2 were used, characterized by all the values of the main biological features. For the purposes of

the experiment, the cocoons obtained from the two hybrids (ClxX2 and Blx2) were analyzed for the main features determining the yield of silk worms. A medium sample of dried cocoons was formed for the technological characteristic of the cocoon and the silk thread of each iteration. The weight characteristics of the silk envelope and the cocoons are determined by weighing with an electronic scale (precision 0.001 g).
The length of the silk thread (m) is determined by cocoon drawing. Laboratory yield of raw silk (%) is determined individually for each cocoon:

RESULTS AND DISCUSSION

From Figure 1, it can be seen that the values of the investigated signs between the test and the control groups differ significantly.

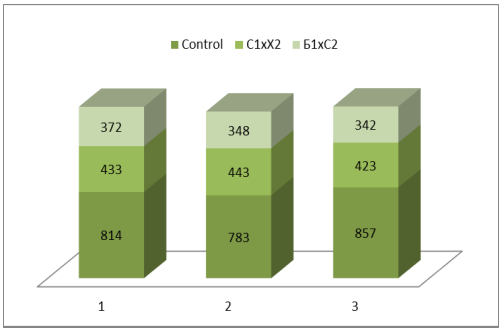

Figure 1. Cocoon weight (mg)

The cocoon weight (Figure 1) and the weight of the cocoon thread (Figure 2) are actively involved as elements of productivity. For these signs, the values are lower than the control.

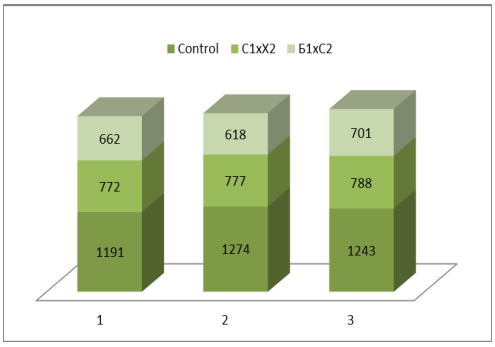

Figure 2. Fresh cocoon weight (mg)

In larvae fed with pure leaves, the cocoon mass values are from 783 to 857 mg and signify-cantly lower in the variants fed with contaminated leaves.
The technological features of the silk thread have greatly contributed to forming the quality of raw silk.
Here again, the values are comparatively lower than those observed in control groups fed with pure mulberry leaves.
Larvae fed with dirty leaves, turn cocoons with a lower weight, which affects the thread.

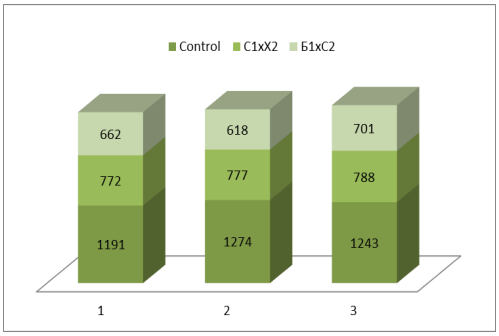

Figure 3. Filament length (m)

The most significant differences between the groups were reported in the length and mass of the cocoon thread Figures 3 and 4. In the test groups, the attribute values ranged from 618 to 788 m of thread length and significantly longer in the control groups from 1191 to 1274 m. Signs is heavily influenced by the presence of heavy metals in the mulberry litsa.

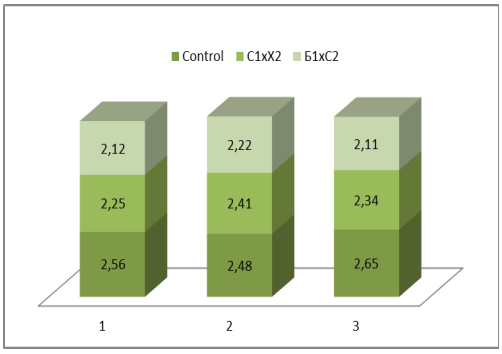

Figure 4. Filament size, g/denier

No significant differences in the values between the groups are observed in the Filament size.

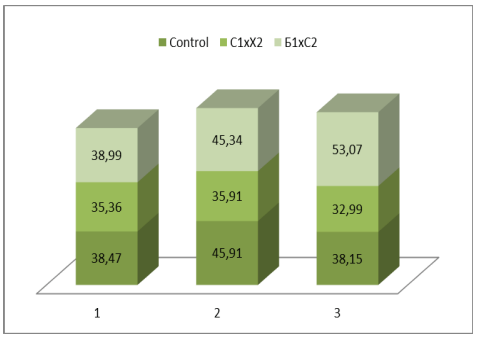

Figure 5. Silk ratio (%)

The signs of filament size and silk ratio are less affected by the presence of heavy metals in the diet. Even so, there were observed differences from the adverse effects of heavy metals.

CONCLUSIONS

From the results obtained, the following conclusion can be drawn:

The high content of heavy metals inhibits the development of trace able biological features. Significant differences in the results between the test and control groups were found for some of the signs, others were poorly influenced.

Significantly influence the presence of heavy metals had on the length and weight of the filament, and the less in the thickness of the thread and in the silk ratio. The results we have come close to those of authors such as Miyoshi (1978); Masui and Matsubara, (1984), Grekov (1995), Cenov (1997) and Petkov (1999).

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SOME CORRELATIONS BETWEEN ENVIRONMENTAL PARAMETERS AND THE FORAGING BEHAVIOUR OF HONEYBEES (*APIS MELLIFERA*) ON OILSEED RAPE (*BRASSICA NAPUS OLEIFERA*)

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Abstract

The climate changes of recent years are affecting the foraging behaviour of bee colonies. We present correlations found between flight time of foraging worker bees, the quantity of food material harvested expressed in terms of hive weight, and meteorological factors (temperature and humidity). Bee colonies were monitored hourly throughout each 24 hour period using the BeeWatch Professional recording system. Monitoring was carried out during the oilseed rape (Brassica napus oleifera) collection period over a 13 day interval in 2018 and 2019. Results show positive correlations statistically significant ($p<0.01$) between flight time of foraging workers and temperature. The influence of humidity on flight time shows a negative correlation at the same level of significance ($p<0.01$). Hive weight is postively correlated with a mean temperature of 20.7˚C and negatively with a mean humidity of 65%.

Key words: *honey bee, foraging behaviour, statistical correlations, meteorological factors.*

INTRODUCTION

Oilseed rape (*Brassica napusoleifera*) has an important place in Romanian agriculture, an area of 632,000 hectares being devoted to its cultivation in 2018. It is also a major source of nectar and pollen, since it is a melliferous plant which supports good bee colony dynamics and significant honey production. Although some varieties of oilseed rape are self-pollinating studies have demonstrated the importance of bees as pollinators. (Cambó et al., 2011; Rosa et al., 2011; Carruthers et al., 2017; Lindström, 2017). The quantities of nectar and pollen harvested by honeybees from oilseed rape may be influenced by the hybrid variety under cultivation, soil type and meteorological conditions (Farcas and Zajácz, 2007).

Studies have shown the influence of a number of factors on the harvest of nectar and pollen by worker bees. Abou-Shaara (2014) grouped these into those interior to the colony (state of the queen, colony vigour, colony health and genetic strain of the colony) and external ones (availability of forageable material, meteorological conditions). Siegel et al. (2012) showed that the ovulation rate of the queen plays an important role in the behaviour of nectar-collecting worker bees.

Clarke and Robert (2018) consider that meteorological conditions influence the entire activity of the bee colony, from its development to the generation of swarms and the build-up of overwintering food stores. Poor weather conditions can also affect pollinating behaviour due to the fall in nectar quality (Corbet, 1990; Lawson and Rands, 2019; Herman et al., 2018).

Temperature is one of the most important environmental factors for bees for their activities both within the hive (nursing the brood) and outside it (gathering nectar and pollen, the nuptial flight of the queen).

The survival rate of honeybees is influenced by external temperature and is also strongly correlated with the subspecies of bee (Ghamdi and Alattal, 2015).

Honeybees are capable of regulating the temperature within the hive in the face of fluctuations in outside temperature (Szopek et al., 2013), a characteristic which allows them to survive through cold spells and to begin raising new brood as early as the middle of winter (Seeley and Visscher, 1985).

Plant nectar secretion is influenced by external temperature, with the optimum time window for secretion depending on species (Pătruică et al., 2017).

Studies have shown close correlations between temperature, solar radiation and the behaviour of foraging worker bees. Results have brought to light the fact that the bees constantly monitor these external factors and that this affects whether they leave the hive on collecting forays (Clarke and Robert, 2018).

The climatic changes the whole world has been facing in recent years may have a direct influence on the behaviour and physiology of honeybees, with implications for the foraging capacity and state of health of colonies. (Le Conte and Navajas, 2008). Climate changes could also be affecting the process of pollination and the ecological equilibrium (Hegland et al., 2009; Rami Reddy et al., 2012; Tanasoiu et al., 2015). Such considerations require a constant monitoring of bee colonies the identification of correlations between their behaviour and environmental factors with an eye to the discovery of solutions to the problems that have appeared.

MATERIALS AND METHODS

The study was carried out at the apiary belonging to the King Michael I of Romania Banat University of Agricultural Science and Veterinary Medicine, Timişoara, Romania in two successive years. The twenty colonies of *Apis mellifera* were transported to Chişoda (Timiş county) to work the oilseed rape (*Brassica napus oleifera*) hybrid *ESTORM*, for the period21 April - 4 May 2018 and in the period to 20 April -3 May 2019 to Sânandrei (Timiș county). The bee colonies were moved when the plants were 20% in flower and the hives were placed about 1 km from the foraging areas.

During the period of study monitoring was carried out of climatic factors (temperature, humidity), intensity of flight activity of the bees and hive weight, using a BeeWatch Professional 45726158 monitoring system placed under a Dadant hive. The bee colony chosen for monitoring was an average one for the apiary, of median vigour with eight frames of brood and working bees arranged in two hive sections. Statistical processing of the data obtained during the two study period was effecting using the IBM SPSS Statistics 23 package and bivariate Pearson and Spearman correlations were calculated.

RESULTS AND DISCUSSIONS

Climate changes impact not only the secretion of nectar in oilseed rape (*Brassica napus oleifera*) but also the behaviour of foraging bees, both of these being correlated with the productive potential of the colony. With a view to establishing the correlations between hive weight, flight time, exterior temperature and humidity during the period in which the bees work the oilseed rape, each colony in the study was monitored on an hourly basis throughout the 24 hours for the whole 13 day period. Taking into account the fact that the meteorological pattern, even for the same calendrical time period, can be very different in different years, the observations were carried out in two years, 2018 and 2019, making it possible for the correlations that exist with respect to the foraging behaviour of worker bees to be analysed under different conditions.

Some authors have held that worker bees start intensively visiting oilseed rape flowers at 08.00, with a peak of visiting between 11.00 and 14.00 (Mesquida et al., 1988; Iordache, 2009) but flight intensity is directly correlated with atmospheric conditions (Pătruică et al., 2017).

The research undertaken in this present study shows a statistically significant ($p<0.01$) positive correlation between hour of flight of foraging bees and temperature. There is also a statistically significant ($p<0.01$) negative correlation between hour of flight and humidity. These interpretations are valid for all the days of the 2018 period of study, when the mean temperature was 20.7˚C and the mean relative humidity was 65.8% (figure 1). A positive correlation ($p<0.01$) was observed between hive weight and hour of flight, a pattern valid for all the days of 2018 studied (table 1). Foraging bees were observed to take flight in large numbers between 11.00 and 12.00, data comparable with the reported finding of Iordache (2009). Temperatures over 13˚C have a positive influence on nectar secretion and the foraging behaviour of worker bees.

Table 1 shows that the feeding behaviour of bee colonies is influenced by atmospheric humidity, with negative correlations (significant, $p<0.01$) between this and the amount of food collected each day.

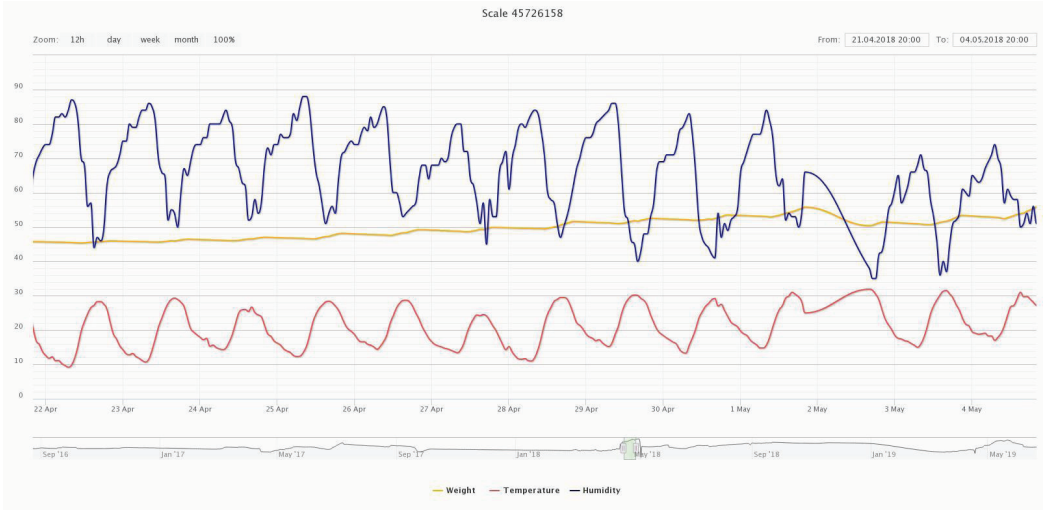

Figure 1. Changes in meteorologicalfactors and hive weightduring oilseed rape nectar harvest 2018

Table 1. Some correlations between hive weight, hour of flight of bees, temperature and humidity during oilseed rape nectar harvest 2018

Day	Hive weight (kg)	Flight time		Temperature (°C)		Humidity (%)	
		Pearson correlation	Spearman's rho	Pearson correlation	Spearman's rho	Pearson correlation	Spearman's rho
1	45.64	0.666**	0.481*	0.253	0.296	-0.306	-0.395
2	45.95	0.755**	0.630**	0.439*	0.496*	-0.399	-0.582**
3	46.43	0.770**	0.640**	0.302	0.387	-0.491*	-0.578*
4	47.16	0.804**	0.635**	0.478	0.491*	-0.416*	-0.608**
5	48.23	0.802**	0.642**	0.381	0.478*	-0.544**	-0.620**
6	49.19	0.741**	0.595**	0.287	0.365	-0.561**	-0.643**
7	50.23	0.847**	0.691**	0.565**	0.574**	-0.455**	-0.625**
8	51.64	0.802**	0.686**	0.358	0.457*	-0.576**	-0.657**
9	52.52	0.733**	0.534**	0.317	0.333	-0.421*	-0.327
10	53.72	0.572**	0.643**	0.736**	0.841**	-0.604*	-0.834**
11	51.08	0.891**	0.762**	-0.851**	-0.762**	0.740*	0.683
12	51.62	0.789**	0.646**	0.341	0.485*	-0.215	-0.496*
13	53.50	0.747**	0.578**	0.689**	0.705**	-0.705**	-0.718**

* significant at the 0.05 level
** significant at the 0.01 level

In 2019 the average temperature recorded for the period studied was 14.5°C and the average relative humidity was 73%. This period was characterised by night-time temperatures below 10°C and spells of heavy rain during the daytime (Figure 2). The meteorological conditions were similar to those of 2017 (Pătruică et al., 2017) but abnormal for this period of the year. Positive correlations were observed between hour of flight of foraging bees and temperature and negative ones between hour of flight and humidity. The correlation coefficients were statistically significant for days when average night temperatures were above 10°C and average humidity above 65% (Table 2). Cold nights with temperatures between 4.8°C and 6.9°C caused the bees to cluster tightly in order to maintain the temperature needed for the brood. On the days after such cold nights changes in food collection behaviour were observed, with negative correlations between hour of flight and temperature ($p<0.01$), and a negative influence on hive weight was also observed (Table 2).

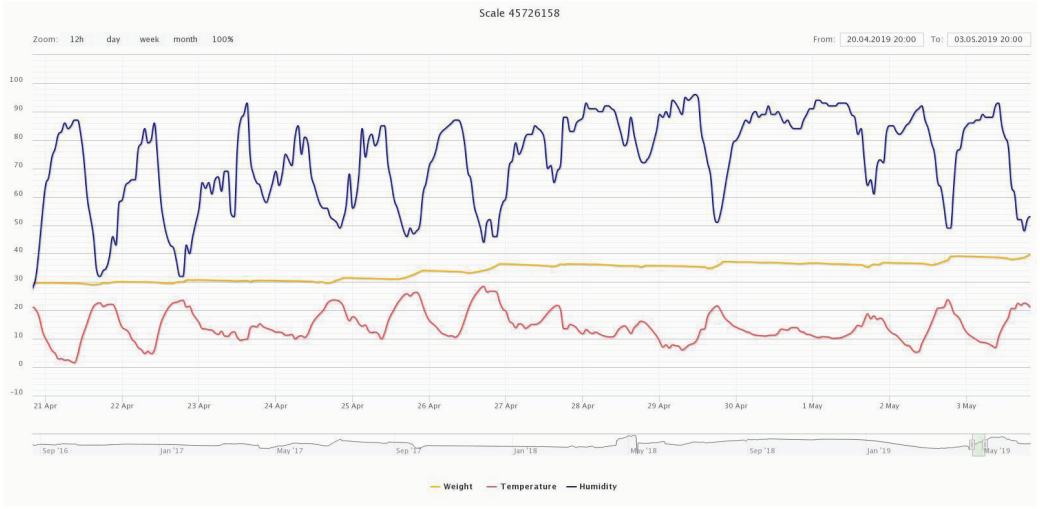

Figure2. Changes in meteorologicalfactors and hive weightduring oilseed rape nectar harvest 2019

Table 2. Some correlations between hive weight, hour of flight of bees, temperature and humidityduring oilseed rape nectar harvest 2019

Day	Hive weight (kg)	Flight time		Temperature (°C)		Humidity (%)	
		Pearson correlation	Spearman's rho	Pearson correlation	Spearman's rho	Pearson correlation	Spearman's rho
1	29.64	0.026	-0.037	-0.208	-0.101	0.157	0.034
2	30.08	0.344	0.057	0.084	0.080	-0.144	-0.117
3	30.55	-0.519**	-0.549**	0.346	0.420*	-0.346	-0.347
4	30.43	0.333	0.034	-0.001	-0.128	-0.062	0.024
5	31.78	0.742**	0.442*	0.479*	0.527**	-0.414*	-0.521**
6	34.18	0.632**	0.370	0.364	0.381	-0.516**	-0.491*
7	36.08	0.010	0.101	-0.580**	-0.484*	0.274	0.304
8	35.94	-0.534**	-0.477*	-0.602**	-0.479*	0.786**	0.695**
9	35.83	0.497*	0.117	0.223	0.013	-0.306	-0.225
10	36.82	-0.929**	-0.939**	-0.232	-0.189	0.047	0.081
11	36.35	-0.264	-0.315	-0.422*	-0.406*	0.412*	0.416*
12	37.18	0.707**	0.415*	0.470*	0.432*	-0.444*	-0.481*
13	38.80	0.009	0.033	-0.218	-0.137	0.141	0.090

* significant at the 0.05 level
** significant at the 0.01 level

CONCLUSIONS

The meteorological factors studied influence the foraging behaviour of worker bees, hour of flight and hive weight.

Foraging bee flight and hive weight are positively correlated with temperature, with statistically significant differences ($p<0.01$), if this does not fall below 12°C.

Temperatures below 7°C have a strong negative effect on food-collecting behavior from oilseed rape the following day and for those days a negative correlation was found between flight time, hive weight and temperature ($p<0.01$).

High humidity (heavy or prolonged spells of rain) has a negative influence on foraging, with a statistically significant ($p<0.01$) correlation coefficient being found between hour of flight, hive weight and average humidity levels of greater that 60%.

With the effects of climate change becoming more serious each year, we need to ask whether bee colonies will be able to adapt to these and at what cost. This question merits continued close monitoring.

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HOMOLOGATION OF SHEEP BREEDS - EUROPEAN AND NATIONAL LEGISLATION

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Abstract

Homologation of a new sheep breed represents the appreciation of the genetic potential of an animal population obtained from the application of the amelioration process. Homologation aims to recognize new populations as breeds, or populations that are already existing and are non-homologated, in order to increase the biological diversity of animal breeds and to adapt the animal breeding directions to market requirements. The homologation process involves research and testing of animals of high production value, and approval is done by the national competent authority in animal breeding that certifies the performance of the population under investigation. The study was conducted with the main objective to describe the procedures and conditions for the homologation of sheep breeds, as well as the presentation of existing legislation at national and European level, the regulations and directives repealed so far on the homologation of new sheep breeds. Regulation (EU) 1012/2016 is to be applied in national legislation by amending it in accordance with the updated European provisions which entered into force on 1 November 2018. In this context, with regard to recognition of breed societies and breeding operations, the competent authority must ensure that they update the breeding programs of breeds in accordance with Regulation 1012/2016 and that the breeding programs already submitted will be checked in accordance with the requirements of the legislation and the established principles of breeding so that all breeding programs of sheep breeds must to comply the conditions, criteria and rules contained in the updated European Regulation.

Key words: *sheep, breeding program, homologation, legislation, testing of performances.*

INTRODUCTION

Sheep breeding, along with other livestock-specific sectors, has notably contributed to the development of the economy and the living standard of people in growing areas. Due to the diversity of products resulting from sheep breeding and their special biological value, sheep breeds continue to enjoy a special appreciation and attention from breeders (Pascal, 2007).

The biological potential for all sheep livestock production and exploitation can be increased by paying greater attention to the selection and amelioration, as well as the use of the existing genetic fund, while taking into account the diversity of the structure of indigenous and imported breeds (Mochnacs et al., 1978).

Amelioration of sheep represents the modification of the genetic structure of a population by improving its morpho-productive and reproductive attributes. It aims to influence the evolution of these populations from one generation to the next in order to quantify the effects of the improvement process (Ivancia, 2005). The genetic structure of an animal population is the set of genotypes and specific genes of the individuals that make up this population. Being dynamic, the genetic structure can be changed at every change in the population by eliminating or adding an individual in the population, since each individual has its own genetic configuration that can affect the characteristics of the population (Mochnacs et al., 1978). By developing clear, accurate and effective programs of genetic amelioration, for each morpho-productive type, by applying the science of amelioration, remarkable results are achieved in zootechnical interest species. On the other hand, the methods of breeding applied to sheep have remained traditional, and are currently very little changed compared to the past.

As a result of amelioration processes, it is intended to obtain morpho-productive and reproductive performance of sheep populations with superior characteristics to their ascendants. By applying the processes of

improvement and selection of sheep populations, the genetic structure of this population changes and higher animal productions are obtained (Pascal, 1998). Following these developments, it is intended to recognize and approve new sheep populations. The purpose of homologation is to recognize populations as breeds in the direction of increasing the biological diversity of animal breeds and adapting the growth directions to market requirements (Taftă et al., 1997).

The homologation process is regulated by legislation, both at national and European level. Thus, homologation is done under the authority of the national competent authority in animal breeding that certifies the morpho-productive and reproductive performance of the population under investigation.

In the presented context, the aim of this paper is to describe the procedures and conditions for the homologation of sheep breeds, in order to have a thorough knowledge of them. Also, research on existing national and European legislation, regulations and directives repealed so far, as well as their modification, will seek to obtain a complete picture of legislation on the homologation of new sheep populations.

MATERIALS AND METHODS

The main objectives of this paper are theoretical and aim at acquiring specific knowledge on the subject discussed, namely notions regarding the homologation of sheep breeds. It also seeks to highlight the basic legislation on the amelioration process, performance testing, homologation process and other reference procedures in this area.

The aim is to project an overview of the current legislation, both at national and European level, on working methods and how sheep breeds can be homologated.

The methodology used in this research is a qualitative one by analyzing some documents of interest. Thus, detailed legislative documents, regulations and operational procedures of the competent authorities implementing the legislation were analyzed. Among the legislative documents used, the most important will be mentioned below.

European Legislation: (1) *Regulation (EU) 2016/1012 of the European Parliament and of the Council of 8 June 2016* on zootechnical and genealogical conditions for the breeding, trade in and entry into the Union of purebred breeding animals, hybrid breeding pigs and the germinal products thereof and amending Regulation (EU) No 652/2014, Council Directives 89/608/EEC and 90/425/EEC and repealing certain acts in the area of animal breeding ('Animal Breeding Regulation'); (2) *Commission Implementing Regulation (EU) 2017/717 of 10 April 2017* laying down rules for the application of Regulation (EU) 2016/1012 of the European Parliament and of the Council with regard to the model forms of zootechnical certificates for breeding animals and their germinal products; (3) *Commission Implementing Regulation (EU) 2017/716 of 10 April 2017* laying down rules for the application of Regulation (EU) 2016/1012 of the European Parliament and of the Council with regard to the model forms to be used for the information to be included in the lists of recognised breed societies and breeding operations.

National legislation: (1) *Law no. 32 of 16 Januaru 2019 for animal breeding*; (2) *Government Decision no. 1188/2014*onthe organisation and functioning of the National Agency for Animal Breeding "Prof. dr. G. K. Constantinescu; (3) *Government Decision no. 77/2019*for modifying and completing the Government Decision no. 1188/2014 on the organisation and functioning of the National Agency for Animal Breeding "Prof. dr. G. K. Constantinescu; (4) *Order no. 383/2009* on the approval of technical regulations for the homologation of breeds, lines, hybrids and new biological creations for domestic animals; (5) *Order no. 22 of 20 January 2006* for approving the appreciation norms for reproduction sheep and goats.(6) *Order no. 1045 of 14 May 2018*for modifying and copleting thethe appreciation norms for reproduction sheep and goats, approved through the Ministry of agriculture, forests and rural development Order no. 22/2006; (7) *Order no. 332 of 16 October 2017* for modifying and copleting the the appreciation norms for reproduction sheep and goats, approved through the Ministry of agriculture, forests and rural development Order no. 22/2006; (8) *Law no. 191 of 30 October 2012*for approving the Government

Emergency Ordonance no. 23/2010 regarding the identification and registration of swine, sheep and goats, as well as for the modification and completion of some normative acts.

RESULTS AND DISCUSSIONS

Homologation of sheep breeds – general principles. Homologation of a new sheep breed represents the appreciation of the genetic potential of an animal population obtained from the application of the amelioration process.

Homologation aims at recognizing new populations as breeds or populations that are already existing and non-homologated, in order to increase the biological diversity of animal breeds and to adapt the growth directions to market requirements.

In order to homologate a sheep population, it is necessary to apply an official control of performances, which involves developing and recording some measurements and establishing the productive performances, including the quality of the animal products obtained.

The process of homologation of sheep populations, to the same extent as other species of animals that can be homologated (sheep, goats, etc.), is a procedural one, which is done through a competent authority. In order to have official character, performance control must be evaluated by a neutral and impartial state institution, or an organization empowered for that purpose.

At national level, according to *Government Decision no. 1188/2014,* in Romania the competent authority responsible for supervising the homologation processes is the National Agency for Animal Breeding (ANZ). The powers of the competent authority for animal husbandry are stipulated in *GD no. 1188/2014*, in accordance with the *Law no. 32/16 January 2019* of animal breeding, regulating the breeding and exploitation, amelioration, reproduction and feeding, the conservation of the genetic resources in animal species of interest (GD no.1188/2014). According to the mentioned law, the Ministry of Agriculture and Rural Development elaborates, promotes and coordinates the policies for the whole activity in the animal breeding field through the competent authority (Law no. 32, 2019).

Homologated sheep breeds. In the last 10 years, in Romania were homologated 4 sheep breeds, recognized according to *Order no. 383/2009*. In 2010, the *Palas Milk Breed* (Homologation Certificate no. 2/22.03.2010), the *Blackhead Sheep of Teleorman* (Homologation Certificate no. 3/11.03.2010), and the *Brown KarakulLine* (Homologation Certificate no. 4/24.11.2010)were approved, followed by *Palas Meat Breed* (Homologation Certificate no. 6/20.08.2012)in 2012. These breeds have been homologated by the Research and Development Institute for Sheep and Goat Breeding Palas - Constanta (*Palas Milk Breed, Palas Meat Breed*), the Research and Development Station for sheep Popauti - Rachiti (*Brown Karakul Line*) and the Teleorman Sheep and Goat Breeding Association (*Teleorman Blackhead Sheep*).

Homologation process. In order to establish the homologation process and the standards to be complied with, the Animal Breeding Competent Authority has drawn up the *Operational Procedure regarding the homologation of breeds, lines, hybrids and new biological creations for domestic animals/31.01.2012,* according to the regulations stipulated in *Order no. 383/2009*.

The purpose of this procedure is to establish the steps in the homologation process to be followed by the competent authority specialists, both at central and territorial level (Order no. 383/2009).

Recognition of a breed for homologation can be done at the initiative of organizations, associations and sheep farms, which ask the competent authority to test the new breeds. The association/breeders' organization is any legally constituted associative form whose members have animals or other breeding species of interest registered in the National Farm Register, in the Agricultural Register and / or in another national database recognized by the competent authority. In order for the initiation of the homologation process to be approved, it is necessary to have an amelioration plan for the animals tested (Law no. 32, 2019).

According to *Article 8 of Regulation (EU) 1012/2016*, the amelioration program must be evaluated and approved by the competent authority. Breeding programs for pure-bred breeding animals shall be carried out by amelioration societies recognized by the competent authority or, in the absence of any

breeding company carrying out a breeding program with pure-bred breeding animals, the competent authority may decide to carry out an amelioration program for the breed concerned if the conditions laid down in the Regulation are met (Regulation (EU) 2016/1012).

The amelioration society is any breeders' association, amelioration organization or public body recognized by the competent authority, for the purpose of carrying out an amelioration program with pure-bred breeding animals entered in the breeding book established or maintained by it, including species of zootechnical interest (Law no. 32, 2019).

Annex 1 to *Regulation (EU) 1012/2016* (Part 1 and Part 2) contains information on the conditions for recognition of breeding societies, as well as the criteria for approval of amelioration programs.

Regulation (EU) 1012/2016 specifies that breeding animals included in the breeding program must be entered in a breeding book containing livestock information, that is administered by an amelioration society.

In order to be approved, the amelioration program must

For approval, the breeding program must pursue one or more of the following goals: breed improvement, breed conservation, breed creation, breed reconstitution (Regulation (EU) 2016/1012).

Also, in pursuit of the breeding program's objectives, the animals are subjected to performance testing or any other assessment and data on the characteristics in relation to the objectives of the breeding program in question are recorded in the breeding book. Annex 2 to *Regulation (EU) 1012/2016* provides the main criteria for the registration of breeding animals in the breeding book.

Regarding the initiation of the homologation process, and given the above, the organizations and associations involved require to the competent authority accreditation to establish and administer the breeding book of the newly created breed or of the already existing and non-homologated breeds (Law no. 32, 2019).

Testing of performances. The genetic potential of the breeds is assessed by testing the performance in the growing area of the tested sheep population.

In the *Operational Procedure regarding the homologation of breeds, lines, hybrids and new biological creations for domestic animals/31.01.2012* it is stipulated that the testing is based on an experimental plan and the processing of the obtained statistical data. The experimental plan includes the characters on which the population value is established, the methods of recording and primary testing of the control data, the duration of the test, the size of the sample, the number of generations, the standard exploitation conditions for each type of exploitation and production (ANZ, 2019).

The breed testing is based on a request and a preliminary study of the breeds to be tested, drawn up in accordance with *Annex 1* and *Annex 2* of *Order no. 383/2009.*

Performance testing is performed in test stations and farms approved by the competent authority. According to its operational procedure, the competent authority draws up a technical guidance on the testing methodology, and the testing organization is the one that establishes the testing methodology together with the Research and Development Institute, which is subsequently approved by the competent authority. Resorts and farms for breeds performance testing have the obligation to provide the material basis and the means of production to achieve a level of growth and exploitation that allows highlighting the productive genetic potential(Order no. 383/2009). *Article 27 (1) of Regulation (EU) 1012/2016* provides that breeders or breeding societies conduct performance testing or designate third parties to carry out the testing (Regulation (EU) 2016/1012).

In accordance with the *Law 32/2019*, animal breeding principles are used to test performance and take into account the rules and standards established by the European Union reference centers or the principles agreed by the International Committee for Animal Recording (ICAR). ICAR is the world organization for standardizing animal registration and productivity assessment. Its purpose is to promote improved animal registration and assessment by formulating guides, standards and certificates worldwide. ICAR Guidelines provides general principles that are updated regularly, the last update of Section 1 - General

Instructions being made in October 2018(ICAR Guidelines, 2018).

According to *Article 25* and *Annex 3* of *Regulation (EU) 1012/2016*, performance testing shall be performed on the basis of one or more of the following performance testing systems: (a) individual performance testing of breeding animals themselves or of breeding animals based on their progeny, siblings or collaterals at test stations; (b) individual performance testing of breeding animals themselves or of breeding animals based on their progeny, siblings, collaterals and other relatives on farms; (c) performance testing through survey data collected by farms, points of sale, points of slaughter or other operators; (d) performance testing of contemporary groups of breeding animals(Regulation (EU) 2016/1012).

The competent authority operational procedure specifies the conditions for introducing new breeds for testing in order to be homologated: the minimum number of animals will be calculated with a view to ensuring the evolution of the breed or line after approval; the level of productive attributes must significantly exceed the production of existing breeds or have other special morphological or physiological attributes.

Zootechnical certificate. *Law no. 32/2019* of animal breeding describes the zootechnical certificate as being *a breeding certificate, attestation or commercial documentation issued on paper or in electronic format for breeding animals and biological material thereof, which provides information on pedigree, identification and, if available, the results of performance testing or genetic evaluation.* For breeding animals to be introduced into an improvement program and to be subsequently tested for approval, they must have a zootechnical certificate (Law no. 32, 2019).

Animal breeders participating in an improvement program require zootechnical certificates for their breeding animals. Zootechnical certificates are issued by the breeding company or breeding farm carrying out the breeding program for each breeding animal, containing mainly the identification data of the animal, according to the *Law no. 191 of October 30, 2012 for the approval of GEO no. 23/2010*, as well as information about the company and the breeding book to which it belongs (Law no. 191, 2012).

The zootechnical certificate must be issued by each racial register in accordance with the model provided for in *Regulation (EU) 717/2017*.

Homologation certificate. After testing the performances of breeding animals included in a breeding process, it is intended to obtain the homologation certificate for the recognized breed.

According to *Operational Procedure regarding the homologation of breeds, lines, hybrids and new biological creations for domestic animals/31.01.2012,* in order to obtain the homologation certificate, the initiator of the homologation shall draw up a documentation containing the morphological characters, production attributes, data on the exploitation technology and the livestock breeding area.

In the documentation submitted, the initiator of the homologation process must describe the reasons for the approval of these new breeds, specifying the scope and geographical area for which it is intended, the problem that led to the creation of the new breed, the objectives considered in the breeding plan, the presentation of the solution and an indication of differentiation elements from other existing breeds (ANZ, 2019).

According to the regulations stipulated in *Order no. 383/2009*, the documentation for approval will be analyzed by the Technical-Scientific Council of the National Agency for Animal Breeding „Prof. Dr. G. K. Constantinescu". Following the verification of the documentation submitted for approval, the board draws up a homologation certificate under which ANZ issues the approval certificate for the new recognized breed (Order no. 383/2009).

CONCLUSIONS

Homologation of sheep breeds is a subject of great zootechnical interest, as sheep breeding represents a branch with great economic potential in Romania. The sheep breeding sector has seen a positive trade balance in recent years, therefore the proper capitalization of sheep breeds would lead to the development of the livestock sector.

Homologation of sheep breeds requires in-depth studies and performance tests that can be conducted over extended periods of time. Thus, in the last 10 years, in Romania were approved 4 breeds of sheep, recognized according to *Order no. 383/2009*.

Recognition of a breed for homologation is carried out in accordance with national and European legislation, the regulations being implemented by the competent authority in animal breeding, respectively the National Agency for Animal Breeding.

National legislation on animal breeding and approval is the *Law no. 32 on animal breeding*, updated in January 2019, in accordance with *Regulation (EU) 1012/2016*. However, following the analysis made in this study on national legislation in this field, it has been observed that most of the regulations and procedures for animal breeding and approval have not been updated according to the latest regulations, and changes will be implemented in the next period.

Thus, in view of these limitations, the present study was carried out taking into account the updated European provisions, which entered into force on 1st of November 2018. At national level, *Order no. 383/2009 on the approval of technical regulations for the homologation of breeds, lines, hybrids and new biological creations for domestic animals* will also be updated in line with the new European provisions.

Regarding recognized breeding societies, the competent authorities must ensure that they update the breeding programs in accordance with *Regulation (EU) 1012/2016*.

Also, the leading associations of breeding books in Romania, recognized as breeding societies under *Regulation (EU) 1012/2016*, have submitted to the National Agency for Animal Breeding updated programs before 1st of November 2018. They will be evaluated by the competent authority, in accordance with the requirements of the legislation and the established breeding principles, so that all breeding programs for sheep breeds meet the selection and amelioration objectives, in compliance with the conditions, criteria and rules stipulated by *Regulation (EU) 1012/2016*.

Therefore, the information presented in this study can be considered as guidance to the main legislation on breeding and sheep breeding processes. However, it can be improved and supplemented according to legislative changes both at European and national level.

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MONTHLY CHANGES OF BEHAVIORAL CHARACTERISTICS IN HOLSTEIN-FRIESIAN, BROWN SWISS AND SIMMENTAL BULLS

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Abstract

Behaviors of Holstein-Friesian (HF), Brown Swiss (BS) and Simmental (SIM) bulls were determined under the Mediterranean conditions for a period of six months. A total of35 bulls were fed in two groups (10 HF and 8 BS in group I and 10 SIM and 7 HF bulls in group II). At the time of high feed consumption, the tendency to drink water from all breeds was also high and HF bulls had higher drinking and elimination behavior rates than those of BS and SIMbulls especially in hot summer months. All breeds preferred to perform locomotor activities late in the evening during the hot summer months. The bulls decreased feeding, standing and locomotion activities during hot hours at a lower rate or postponed these behaviors to the cooler hours of the day, but they increased lying and rumination activities in those hours. While HF bulls were more affected by higher temperatures than SIM and BS bulls, taking precautions against high temperature on farms level would lead to increase the fattening performance and also the welfare of the bulls.

Key words**:** *behavioral changes, cattle breeds, fattening, heat stress.*

INTRODUCTION

In beef production, more importance has been given to the genetic improvement and nutrition, however, environmental factors and animal welfare aspects were generally pushed into the secondary plan. It is admitted that the studies on the behaviors of fattening cattle have been neglected for a long time but, there has been an increasing interest on the behaviors of fattening cattle (Brown-Brandl et al., 2006). In earlier studies, under hot environmental conditions, the behavioral changes in fattening bulls (Dikmen, 2013; Rosselle et al., 2013; Zgur et al., 2014), in heifers (Mitlöhner et al., 2001; Mitlöhner et al., 2002), in steers (Tapki, 2012) and in dairy cows (Tapki and Sahin, 2006) were determined. In these studies, the changes of losing appetite and decreasing feed intake, activity (Brown-Brandl et al., 2006), increasing water intake (Mitlöhner et al., 2002, Dikmen, 2013) and spending more time for standing (Cook et al., 2007) were mentioned. However, no studies were conducted to determine the behaviors of the three most common cattle breeds, Holstein-Friesian (HF), Brown Swiss (BS) and Simmental (SIM) bulls. Therefore, in this study the effects of environmental factors on the behaviors of fattening bulls and behavioral differences among HF, BS and SIM bulls under the Mediterranean climatic conditions by using scan sampling technique were aimed to be determined.

MATERIALS AND METHODS

This study was performed with the ethical permission (IX. Session held on October 8, 2013) of ADU-HADYEK. The study was carried out at a farm located at 37°46'55.2''N and 28°4'9.12''E in Turkey. Temperature Humidity Index (THI) was calculated by using the temperature and relative humidity records (HOBO U10) in the barn (Kibler, 1964).

In group I, 10 HF and 8 BS, in group II, 10 SIM and 7 HF bulls aged 8-12 months old were fed in two paddocks. Each paddock area was 120 m^2. Rumination, standing, lying, walking, feeding, drinking, mounting, agonistic, defecation and urination behaviors of the bulls were monitored every Monday for one hour at 06:00, 09:00, 12:00, 14:00, 17:00, 20:00 and 23:00 for 10 min period from February to August by using scanning sampling technique (Mitlöhner et al., 2001; Mitlöhner et al., 2002; Dikmen, 2013). The animals were fed with

wheat straw, tomato meal (24% DM), barley flakes and concentrates. The feed intake, nutrient components of ration, fattening performance, carcass and beef quality of the bulls were reported in another study by Çatıkkaş and Koç (2017).

Prior to the statistical analysis of the data, an arcsine-square root transformation was performed on the behavioral data (Mitlöhner et al., 2001). Statistical analysis of data was performed with using PROC GLM procedure of Statistical Analysis System (SAS, 1999). The differences between LSMEANS of the fixed factor levels were taken into account to be statistically significant at $P<0.05$ (2-tailed) based on Tukey's adjustment type I error rate. Statistical model used for the analysis of data is given in Equation I as follow:

$$y_{ijkl}=\mu+a_i+b_j+c_k+(ab)_{ij}+(ac)_{ik}+(bc)_{jk}+e_{ijkl} \quad (1)$$

where μ is the overall mean, y_{ijkl} is the observation of the behavior, a_i is the breed effects (i=HF, BS and SIM), b_j is the month effects (j=February, March, …… and August), c_k is the observation hour effects (k=06:00, 09:00, 12:00, 14:00, 17:00, 20:00 and 23:00), $(ab)_{ij}$ is breed (x) month and $(ac)_{ik}$ is breed (x) observation hour, $(bc)_{jk}$ is month (x) observation hour interaction effects and e_{ijkl} is the residual random errors.

RESULTS AND DISCUSSION

Climatic conditions: From June till the end of fattening period, the THI values (Figure 1) were over the threshold level (THI=72) of heat stress in cattle (Ravagnolo and Misztal, 2000; Gantner et al., 2011) and the behaviors of the bulls were affected more or less from the heat stress.

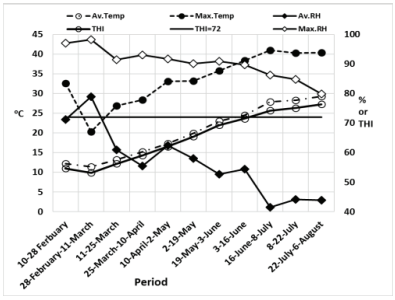

Figure 1. Average and maximum temperature (°C), relative humidity (RH, %) and temperature humidity index (THI) during fattening.

It can be said that the bulls in the last three months of the fattening were under thermal comfortless conditions and the physiological, biochemical and behaviors of them could be significantly affected (Rosselle et al., 2013; Umpapol et al., 2014). To decrease the effect of heat stress on the farm level some precautions like providing cool water, changing the ration formulation, establishing evaporative cooling system and etc. need to be taken on this farm and in all the farms of the region.

Behavioral characteristics in group I and II are given in Table 1 and 2, respectively. The daily activities in both groups were mainly similar. The highest daily activities in both groups were lying with about 35% and standing for more than 30% (Figure 2).

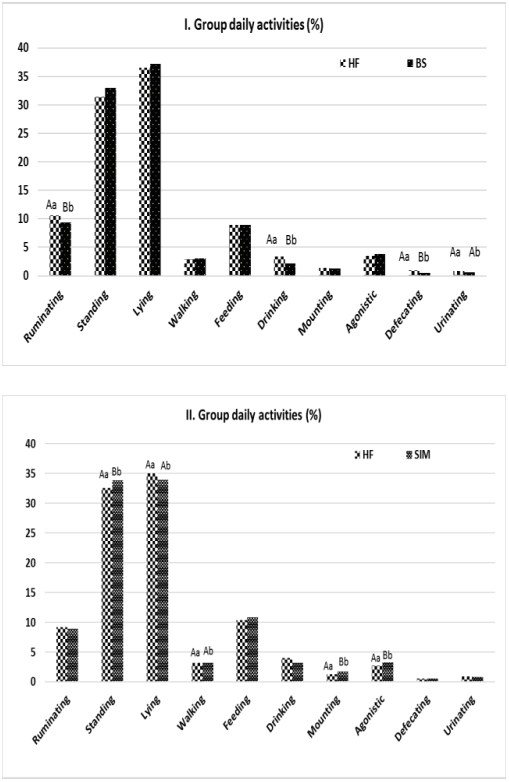

Figure 2. Daily activities (%) of HF, BS and SIM bulls. Different letters show differences between the breeds, A, B for $P<0.01$; a, b for $P<0.05$.

Table 1. Behavioral characteristics in group I

Factor	Feeding	Ruminating	Drinking	Walking	Lying	Standing	Mounting	Agonistic	Defecating	Urinating
Breed	NS	**	**	NS	NS	NS	NS	NS	**	*
HF	0.089±0.003	0.106±0.003^{Aa}	0.034±0.002^{Aa}	0.030±0.002	0.365±0.007	0.314±0.006	0.014±0.001	0.035±0.002	0.009±0.001^{Aa}	0.008±0.001^{Aa}
BS	0.089±0.003	0.094±0.003^{Bb}	0.022±0.002^{Bb}	0.031±0.002	0.372±0.007	0.330±0.006	0.013±0.001	0.038±0.002	0.005±0.001^{Bb}	0.006±0.001^{Ab}
Hour	**	**	**	**	**	**	**	**	**	**
06:00	0.045±0.006^{ACa}	0.178±0.006^{Aa}	0.010±0.003^{a}	0.010±0.003^{Aa}	0.580±0.014^{Aa}	0.153±0.011^{Aa}	0.004±0.002^{ABac}	0.004±0.004^{Aa}	0.005±0.002^{Aac}	0.004±0.002^{Aa}
09:00	0.199±0.006^{Bb}	0.043±0.006^{Bb}	0.044±0.003^{Bb}	0.039±0.003^{BDbd}	0.133±0.014^{Bb}	0.471±0.011^{Bb}	0.015±0.002^{ADbe}	0.038±0.004^{BCbd}	0.010±0.002^{Ab}	0.007±0.002^{ABab}
12:00	0.065±0.006^{AEce}	0.134±0.006^{ACc}	0.031±0.003^{Bbc}	0.034±0.003^{BCDb}	0.222±0.014^{Cc}	0.434±0.011^{BDbd}	0.013±0.002^{ABEab}	0.046±0.004^{Bb}	0.010±0.002^{Aab}	0.008±0.002^{ABab}
14:00	0.024±0.006^{Cd}	0.126±0.006^{Cce}	0.017±0.003^{ACad}	0.018±0.003^{ACac}	0.635±0.014^{Aa}	0.143±0.011^{Aa}	0.003±0.002^{Bc}	0.023±0.004^{ACc}	0.005±0.002^{Aabd}	0.003±0.002^{Aa}
17:00	0.150±0.006^{De}	0.069±0.006^{Dd}	0.030±0.003^{BCbc}	0.030±0.003^{BCb}	0.281±0.014^{Cc}	0.391±0.011^{Cc}	0.006±0.002^{ABac}	0.029±0.004^{BCcd}	0.007±0.002^{Aabd}	0.003±0.002^{Aa}
20:00	0.080±0.006^{Ec}	0.042±0.006^{Bb}	0.032±0.003^{Bbc}	0.046±0.003^{Dd}	0.272±0.014^{Cc}	0.409±0.011^{CDcd}	0.029±0.002^{Cd}	0.077±0.004^{De}	0.004±0.002^{Aabd}	0.007±0.002^{ABab}
23:00	0.057±0.006^{AEae}	0.100±0.006^{CDe}	0.027±0.003^{BCcd}	0.038±0.003^{BDbcd}	0.453±0.014^{Dd}	0.247±0.011^{Ee}	0.023±0.002^{CDEde}	0.036±0.004^{Bbd}	0.004±0.002^{Acd}	0.012±0.002^{Bb}
Month	NS	**	**	**	NS	**	**	**	NS	NS
February	0.102±0.006^{Aa}	0.125±0.006^{Aa}	0.024±0.003^{ABab}	0.015±0.003^{Aa}	0.372±0.015	0.321±0.013^{ACa}	0.010±0.002^{Aa}	0.018±0.004^{ADad}	0.008±0.002	0.007±0.002
March	0.094±0.004^{Aa}	0.095±0.004^{Aa}	0.023±0.002^{Aa}	0.031±0.002^{BCb}	0.347±0.010	0.344±0.008^{ABa}	0.012±0.002^{Aa}	0.038±0.003^{BCEbc}	0.008±0.001	0.009±0.001
April	0.082±0.004^{Aa}	0.102±0.005^{Aa}	0.016±0.002^{Aa}	0.042±0.002^{Bbc}	0.376±0.011	0.306±0.009^{ACa}	0.017±0.002^{ABab}	0.047±0.003^{Bbc}	0.005±0.001	0.005±0.001
May	0.085±0.004^{Aa}	0.076±0.004^{Bb}	0.033±0.002^{BCb}	0.042±0.002^{Bc}	0.383±0.010	0.309±0.008^{ACa}	0.022±0.002^{Bb}	0.043±0.003^{Bb}	0.004±0.001	0.004±0.001
June	0.086±0.004^{Bb}	0.058±0.005^{Bc}	0.042±0.002^{Cc}	0.040±0.002^{Bc}	0.226±0.011	0.367±0.008^{Bc}	0.011±0.002^{Aa}	0.057±0.003^{ACac}	0.008±0.001	0.006±0.001
July	0.085±0.009^{Aa}	0.141±0.009^{Aa}	0.028±0.004^{ABCab}	0.016±0.005^{ACa}	0.407±0.022	0.283±0.018^{Ca}	0.010±0.003^{Aa}	0.017±0.006^{DEd}	0.008±0.002	0.009±0.002
BreedxHour	NS	NS	NS	**	**	NS	NS	**	NS	NS
BreedxMonth	NS	NS	**	NS	NS	NS	NS	NS	*	NS
HourxMonth	**	**	**	**	**	**	**	**	*	*

HF: Holstein-Friesian, BS: Brown-Swiss, NS: not significant, *: p<0.05, **: p<0.01; A,B,C,D,E: Same letter in the column show insignificance for P<0.01, a,b,c,d,e: Same letter in the column show insignificance for P<0.05.

Table 2. Behavioral characteristics in group II

Factor	Feeding	Ruminating	Drinking	Walking	Lying	Standing	Mounting	Agonistic	Defecating	Urinating
Breed	NS	NS	NS	*	*	**	**	NS	NS	NS
HF	0.104±0.003	0.092±0.003	0.040±0.002	0.031±0.002^{Aa}	0.350±0.007^{Aa}	0.326±0.005^{Aa}	0.013±0.001^{Aa}	0.027±0.002^{Aa}	0.006±0.001	0.009±0.002
SIM	0.108±0.003	0.089±0.003	0.032±0.002	0.032±0.002^{Ab}	0.339±0.007^{Ab}	0.338±0.005^{Bb}	0.017±0.001^{Bb}	0.033±0.002^{Bb}	0.006±0.001	0.008±0.002
Hour	**	**	**	**	**	**	**	**	**	**
06:00	0.067±0.006^{Aa}	0.124±0.005^{Aad}	0.027±0.003^{Aa}	0.023±0.003^{Aa}	0.481±0.013^{Aa}	0.239±0.010^{AEa}	0.009±0.002^{Aa}	0.013±0.003^{Aa}	0.007±0.001^{ABac}	0.010±0.001^{ABad}
09:00	0.232±0.006^{Bb}	0.033±0.005^{Bb}	0.047±0.003^{Bb}	0.035±0.003^{Bb}	0.110±0.013^{Bb}	0.482±0.010^{Bb}	0.016±0.002^{Bb}	0.035±0.003^{Bbe}	0.006±0.001^{ABac}	0.006±0.001^{ACac}
12:00	0.041±0.006^{Cc}	0.129±0.005^{Aa}	0.035±0.003^{ACac}	0.038±0.003^{Bb}	0.352±0.013^{Cc}	0.339±0.010^{Cc}	0.012±0.002^{ABa}	0.028±0.003^{BCbc}	0.011±0.001^{Aa}	0.016±0.001^{Bb}
14:00	0.017±0.006^{Dd}	0.117±0.005^{Aad}	0.039±0.003^{ACc}	0.021±0.003^{Aa}	0.544±0.013^{Dd}	0.227±0.010^{Ad}	0.008±0.002^{Aa}	0.021±0.003^{Aa}	0.002±0.001^{Bb}	0.004±0.001^{Cc}
17:00	0.202±0.006^{Ee}	0.059±0.005^{Cc}	0.038±0.003^{BCbc}	0.024±0.003^{Aa}	0.287±0.013^{Ee}	0.353±0.010^{Cc}	0.010±0.002^{Aa}	0.019±0.003^{ACac}	0.004±0.001^{BCbc}	0.004±0.001^{Cc}
20:00	0.121±0.006^{Ff}	0.062±0.005^{Cc}	0.032±0.003^{ACac}	0.050±0.003^{Cc}	0.213±0.013^{Ff}	0.426±0.010^{De}	0.026±0.002^{Cc}	0.062±0.003^{Dd}	0.005±0.001^{BCbc}	0.007±0.001^{ACacd}
23:00	0.063±0.006^{ACac}	0.110±0.005^{Ad}	0.034±0.003^{ACc}	0.033±0.003^{Bb}	0.423±0.013^{Ag}	0.260±0.010^{Ea}	0.026±0.002^{Cc}	0.034±0.003^{Be}	0.007±0.001^{ACac}	0.011±0.001^{ABd}
Month	**	**	**	**	**	**	**	**	**	**
February	0.111±0.006^{ABab}	0.116±0.006^{ADade}	0.021±0.003^{Aa}	0.023±0.004^{Aa}	0.360±0.014^{ABab}	0.326±0.011^{Aa}	0.016±0.003^{ABacd}	0.010±0.004^{Aa}	0.009±0.002^{ABa}	0.009±0.001^{ABac}
Marc	0.099±0.004^{ABa}	0.110±0.004^{Aa}	0.022±0.002^{Aa}	0.033±0.002^{Bb}	0.383±0.009^{Aac}	0.297±0.007^{Aa}	0.011±0.002^{Ab}	0.030±0.002^{Bb}	0.009±0.001^{Aac}	0.006±0.001^{ACac}
April	0.098±0.005^{ACac}	0.100±0.004^{ADad}	0.019±0.002^{Aa}	0.039±0.003^{Bb}	0.363±0.010^{ACac}	0.306±0.008^{Aa}	0.019±0.002^{BCac}	0.049±0.003^{Cc}	0.004±0.001^{BCb}	0.004±0.001^{Aa}
May	0.094±0.004^{ABa}	0.074±0.004^{Bb}	0.040±0.002^{Bb}	0.039±0.002^{Bb}	0.381±0.009^{Aa}	0.297±0.007^{Aa}	0.023±0.002^{Ba}	0.046±0.003^{Cc}	0.003±0.001^{Cb}	0.006±0.001^{ACac}
June	0.075±0.005^{Bb}	0.059±0.004^{Cc}	0.035±0.002^{Bb}	0.053±0.003^{Cc}	0.327±0.010^{Bb}	0.371±0.008^{Bb}	0.015±0.002^{ACce}	0.055±0.003^{Cc}	0.005±0.001^{BCcd}	0.008±0.001^{ACac}
July	0.128±0.005^{CDcd}	0.096±0.004^{ADde}	0.049±0.002^{Cc}	0.021±0.003^{Aa}	0.307±0.010^{BCb}	0.353±0.008^{Bb}	0.012±0.002^{ACbde}	0.013±0.003^{Aa}	0.008±0.001^{ADa}	0.013±0.001^{Bb}
August	0.136±0.009^{Dd}	0.082±0.008^{Bdbe}	0.066±0.005^{Dd}	0.018±0.005^{Aa}	0.289±0.021^{ABbc}	0.376±0.015^{Bb}	0.010±0.004^{ACbc}	0.009±0.005^{Aa}	0.003±0.002^{BCDbd}	0.011±0.002^{BCbc}
Breed x Hour	NS	**	**	*	NS	NS	**	*	**	**
Breed x Month	*	**	**	**	NS	*	NS	**	NS	NS
Hour x Month	**	**	**	**	**	**	**	**	**	**

HF: Holstein-Friesian, SIM: Simmental, NS: not significant, *: p<0.05, **: p<0.01; A,B,C,D,E: Same letter in the column show insignificance for P<0.01, a,b,c,d,e,f: Same letter in the column show insignificance for P<0.05.

These behaviors were followed by nutritional activities. In both groups, feeding, ruminating and drinking activities occupied more than 20% of the bulls' time.

Nutritional, standing and lying behaviors of HF, BS and SIM bulls were mainly similar. The feed intake behavior increased at 09:00 and 17:00 and the bulls preferred drinking at the time when they had higher feeding rates. Ruminating behavior rate was lower at 09:00 and 17:00 and 20:00 due to higher feeding activities at this time (Figure 3). Except for August in group II, HF bulls had lower standing rates than those of BS and SIM bulls. HF bulls had higher lying rate in July and in August than those of the BS and SIM bulls and unlike the group I, the lying rates in group II were decreased from May to August for HF and SIM breeds.

As the ruminating and feeding behaviors decreased gradually from February to July, except for BS bulls in July, the drinking activity was increased in hot summer months for all breeds. Similar to Dikmen (2013) a higher drinking rate for HF bulls than those of BS bulls was detected.

The lower ruminating behavior rate at 09:00 and 17:00 and 20:00 due to feeding activities at this time (Figure 3)agree with Zgur et al. (2014) with the study about Sloven Cika and SIM bulls and Dikmen (2013) with the study about HF and BS bulls. The higher ruminating rates found for HF and BS in group I than those of HF and SIM bulls in group II could be due to longer fattening time of group II in hot weathers. During this time, the animals tend to decrease feed intake especially forages to decrease the heat load. The higher feeding rate found in the morning and in the evening in this study was also similar to Mitlöhner et al. (2001).

The lower standing rate found early in the morning and late in the evening and higher standing rates at 09:00 and 17:00-20:00 agree with Dikmen (2013).Similar to Platz et al. (2007)the lying behavior is the highest daily behavior and similar to the Dikmen (2013) the different lying behavior rates between HF and BS breeds were detected.

Locomotor behaviors in all breeds decreased significantly in July and August (Figure 4), due to higher temperature seen in the region. In these months because of THI >72, the bulls might have heat stress and in order to decrease heat load on their bodies, they reduced their locomotor activities. Similar to Mitlöhner et al. (2001) and Dikmen (2013), the locomotor activities of the bulls were intense during the evening hours.

Eliminating behaviors: For almost all hours the defecation and urinating rates for HF bulls in group I were higher than those of BS bulls ($P<0.05$). In group II, only in the evening the elimination rates were obviously higher in HF bulls than that of SIM bulls. In hot summer months, the elimination rates were higher in HF bulls than those of BS and SIM bulls. The higher eliminating behavior found for HF bulls than BS bulls disagree with the results of Dikmen (2013).

CONCLUSIONS

The increase in THI in hot summer months showed that the animals were exposed to heat stress and as a response to heat stress the bulls performed some of their behaviors like decreasing feeding, standing and locomotion and increasing drinking and eliminating behaviors or postponing these behaviors to the cooler hours of the day. In terms of drinking, defecation and urination, BS bulls could be more resistant to higher environmental temperatures and relative humidity than those of HF bulls, however the behavioral differences were not obvious between HF and SIM bulls.

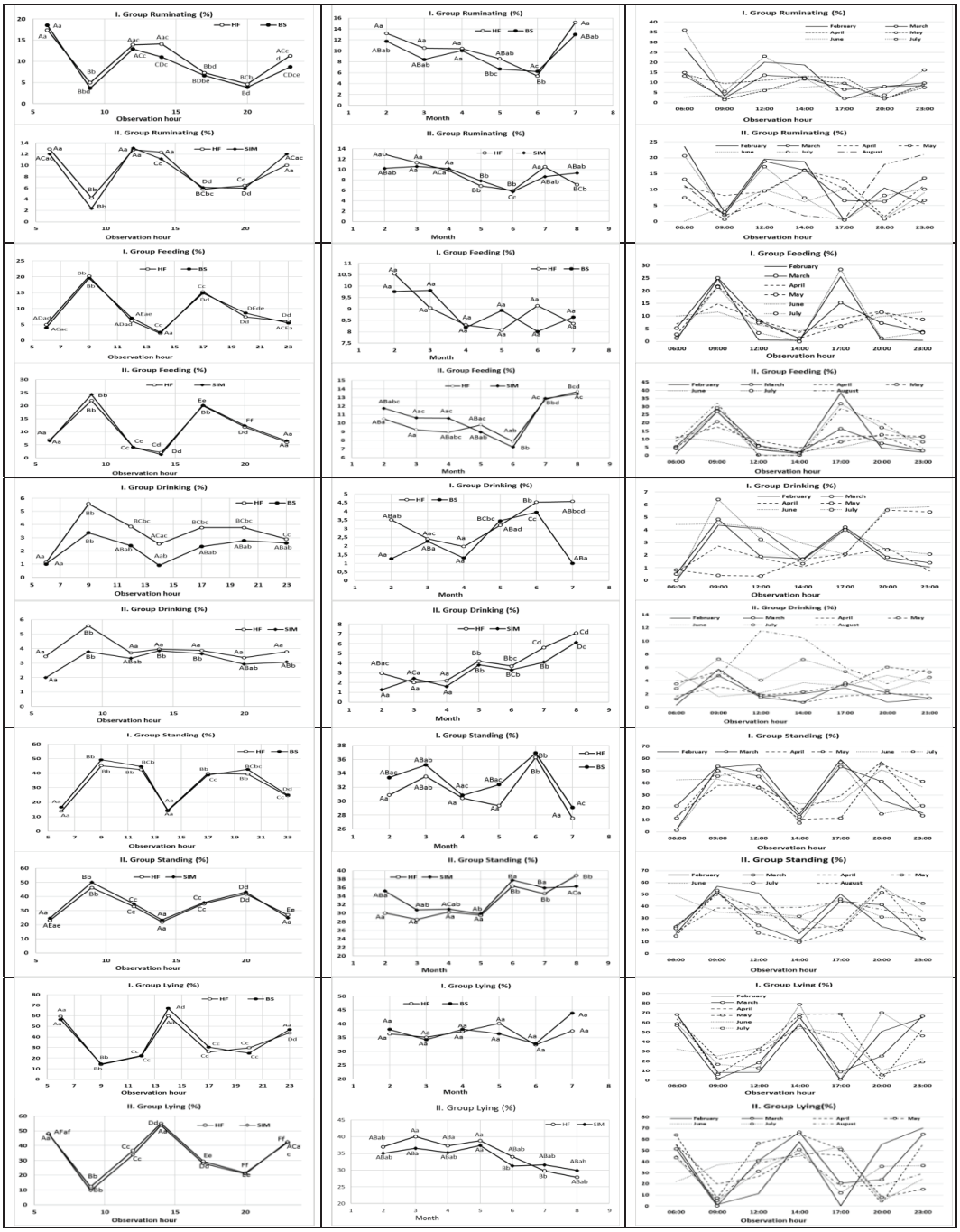

Figure 3. Changes of nutritional, standing and lying behaviors of HF, BS and SIM depending on observation hour and month.

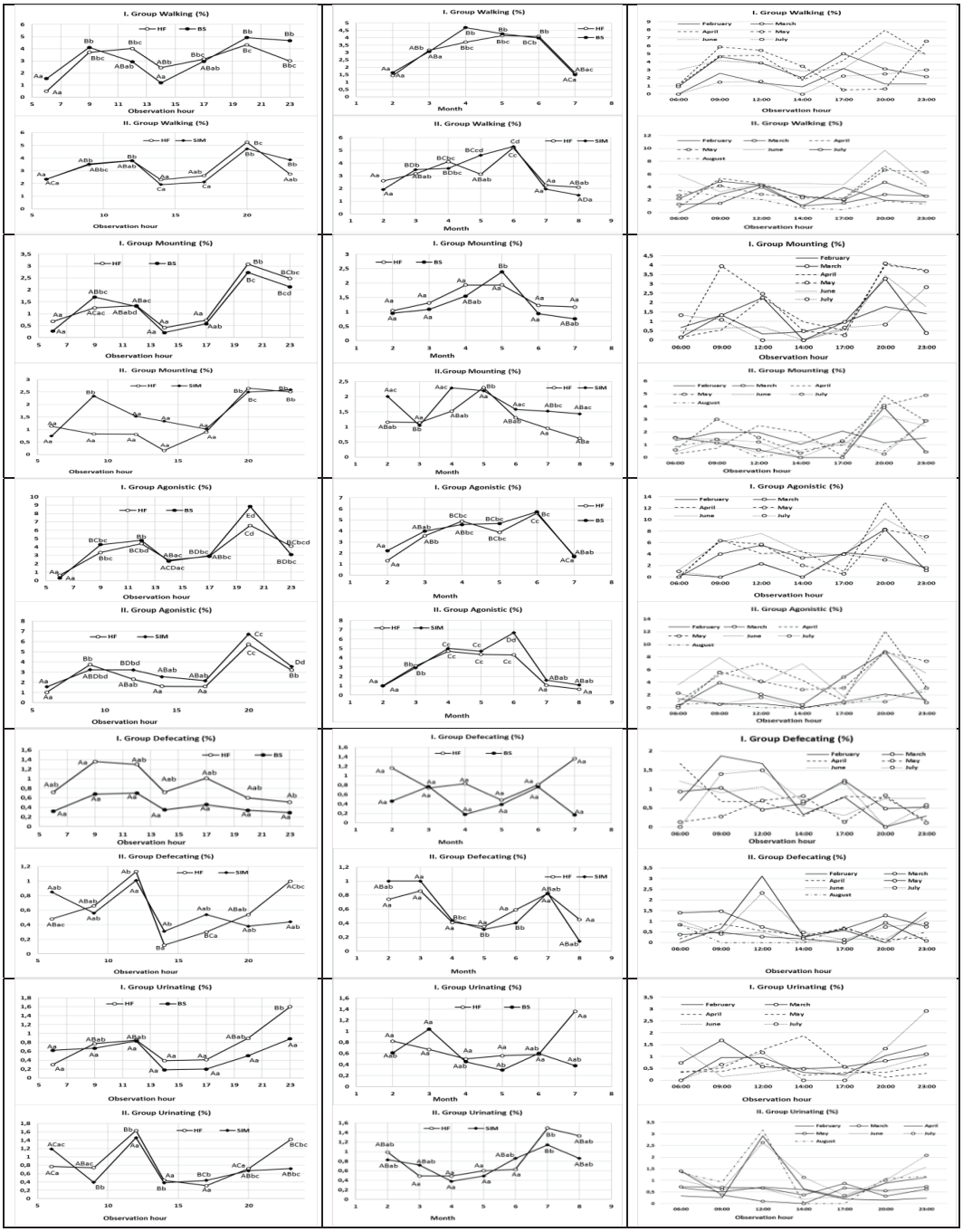

Figure 4. Changes of locomotion and eliminating behaviors of HF, BS and SIM depending on observation hour and month.

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RESEARCH ON THE EVOLUTION OF THE GROWTH PROCESS AT THE TZURCANA SHEEP WITH THE PATERN BREEDS VENDEEN AND WHITE OF CENTRAL MASSIF

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Abstract

Research on growth performance, weight, daily average enrichment recorded on hybrids of the native Tzurcana breed with the two paternal breeds of French Vendeen and White of Central Massif were the objective of study and analysis of this paper. The study was conducted from the spring of 2018 on the sheep private farm of the County - Olt on the 180 females sheep, of which 30 were of the Vendeen breed to track the performance of the pure breed in our country. The other 100 females were cross with the two races to obtain F1 hybrids with Vendeen and BMC, and 50 were considered a control group of the pure Tzurcana native breed. All animals were subjected to control of calf weight W_0 at 30 days of age W_1 at 70 days W_2 and W_3 at 98 days of age. The results high lighted both the net superiority of the meat and the adaptability of the Vendeen paternal breed to the semi-intensive exploitation system towards Tzurcana; the prolificity was 140% versus 103% and a net average net increase of 72 g/day in females and 94 g/day in males in the first month of 90 g/day in both sexes in the 2nd month of growth respectively 91 g/day in females and 87g/day in males in the third month of growth. It is evident both the superiority of the pure paternal breed and the crossbreed obtained from the breeds with the breeds mentioned, regarding the assimilation-conversion capacity and the different growth rate in stages compared to the native breed, but between the crossbreeds the results are relatively similar to the small difference.

***Key words**: Vendeen and Tzurcana hybrid, White of Central Massif and Tzurcana hybrid, body weight, average daily gain.*

INTRODUCTION

World production of meat was 45 million tonnes in 1950 and in 2010 it exceeded 300 million tonnes over 6 times and in only 16 years reached 317 million tonnes more than 7 times as market demand. Is it only a sign of the well-being of the modern world, or only a demand for the demise of the explosion? World average per capita consumption is 34.6 kg/head, while the US dollar is over 126.6 kg/head (according to FAO 2009 and OECD 2016).

The situation of sheep flocks worldwide is different due to a series of criteria related to the geo-climatic conditions of the vegetation consumption of the products of meat especially being considered at the moment the most volatile due both to the very high price and to the quite fluctuation large as production but also macro-amplitude.

In Romania, a sheep population of 10150 thousand heads is ranked 3rd at EU 28 level, with an upward trend in livestock after the 10 years of post accession MS, compared to other member states where the number of Tzurcana sheep is over 70% and for the meat product requires a selection of a rustic breed and hybridization (after INS 2018).

Thus also imported meat breeds from different states with tradition to improve performance at F1 cross of the native breed. The growing requirement for export of sheep's youth has led to the need for domestic sheep flocks with meat breeds such as Vendeen and White of Central Massif.

MATERIALS AND METHODS

We have followed and analyzed the results of the crossbreeding between the native Tzurcana

native mutts of the native breed with two paternal breeds of sheep imported from the spring of 2018 from France by Vendeen and Alba from the Central Massif, but also of a 30-breed Vendeen females of pure breed, in the private farm that was the subject of this study. Imports were made at the level of the Federation of Mountain Sheep Breeders of Romania F.O.R. with the aim of improving the meat performance of the Tzurcana and Tsigaia breeds at the level of regional associations. Thus, a total of 50 dc animals were monitored. 25 females and 25 male Vendeen cross with Turcana, 50 animals, from which 25 females and 25 males with Alba of the Central Massif, of a control lot of 50 and 25 plus 25 pure Turcana but also of a lot of only 30 heads of 15 females and 15 males of the pure Vendeen breed, exploited under the same conditions of the semi-intensive system on the farm.

They were recorded once with the control weighing scale from the breeding and the earning of the products and subsequently the control over the three major growth phases, respectively; 30 days, 70 days and 98 days or 14 weeks. All records and calculations were determined and statistically assured to determine the differences between the cross F1 groups and the pure breeds on the growth trend and the homogeneity of the groups by age group.

RESULTS AND DISCUSSIONS

Numerous studies have been conducted on both the local Turcana breed by numerous researchers and many other paternal breeds for milk and especially for meat made to improve the performance of obtaining a better classing that can fit into the European classification grille. Only for the past thirty years have we witnessed massive imports of males especially for meat such as Norwegian White, German Blackhead, Texel, Hampshire Down, Dorper, Charollaise, White of the Central Massif, Vendeen, Suffolk, but also for milk Friza, Lacaune, Sarda, Assaf, Awassi, which, unfortunately, may have been empirically used but also exploited without a specific result, which only brought to a certain mosaic of mating without respecting a certain program of improvement and planning according to the zoning of the breeds at national level or with a specific improvement goal.

However, in the last two decades, in some private units and in the resorts, some good results of the breeding of our Tzurcana breed, which holds more than 72% of the herd, with various other breeds meat or meat whose authors have shown better hybrid performance with good skills in the exploitation of the cross of these breeds as (Gavojdian et al., 2011).

They were recorded. to capture different stages of milk feeding that are more of a lactogenic potential for product growth potential, with a major maternal effect after the weaning transition and switching to ruminant feeds here, with the *degree of precocity* and last close to delivery date to about 100 days for meat performance whose most important parameters are the average daily increase.

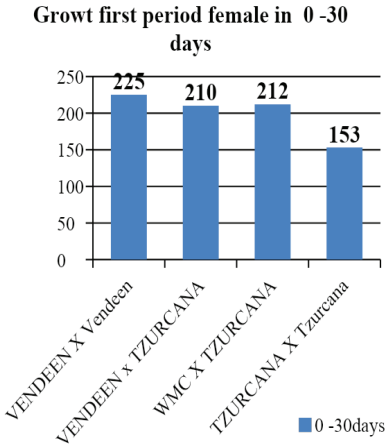

Figure 1. Evolution of a.d.g. in first period of young females sheep.

Although we are talking about different gender differences of different prolificities not typical of the breeds in the country of origin except for the Vendeen to which the graves of the twins produced, although not in the majority or with the same parity in all the metiers, have been traced, these few specimens were added to observe the differences in weight between single and twin meats since the breeding in both variants of the crossbreeding crosses.

The prolificity obtained in purebred Vendeen females was 140% while in Turcana only 102% and the Tzurcana female that were pooled are not much bigger than the differences of only

106% in the Vendeen females and 104% to those with WMC (White of Central Massif), but we still talk about different sexes or genres of both cross and race. Thus, in the meadows with the WMC breed the sexual parity is 6% higher in favor of the males, 56-44 and in the meadows with Vendeen there were only 52 males in 48 females, and in the pure Vendeen race the parity was still in favor of males from 54 to 46 females.

Average body weight at birth and differences between the crosss obtained. In the Tzurcana breed, we can not speak of female fetuses in the present case, so that we can consider the weights of the maternal race almost similar; of 2.96 ± 0.1 kg with a CV of 15.02% in females, and in males of 3.2 ± 0.19 kg having a 21% CV being quite high, comparable to those of the cross with Vendeen of 2.88 kg of twin and male twins of 3.16 kg or in the White of Central Massif (WMC), where the weights of the lambs from the fetuses are the following 2.85 ± 0.14 kg in the females twin and 3.24 ± 0.12 kg in twin males (Table 1).

We can say that the breeds are more homogeneous in both breeds, without much variability within the group, by sexes comparable to the maternal breed, which in males for example exceeds 20 percentage points, typical of the rustic breed without a more rigorous selection.

The calf weights of single cross of the Tzurcana and Vendeen breed are differentiated between genres or sexes where females have an average weight of 4.34 ± 0.12 kg with a CV of 6.89% in males of 4.81 ± 0.15 kg with a 7.81% CV, and in WMC (White of Central Massif), we recorded a single weight for single product in females of 4.39 ± 0.14 Kg with a CV of 8.2 % and in males of 4.92 ± 0.15 kg with a 5.91% CV with relatively homogeneous groups and single products.

Although there are very few differences between race groups between grammars in contrast to Vendeen cross and the native breed are considered very significant differences of 1.35 kg in females and 1.61 kg in males and those with WMC of 1.43 in females and 1.7 in males, results that show extra performance from the start of the calving.

The elements of differentiation of body weights between the Tzurcana native breed and the pure Vendeen breed exploited under the same conditions demonstrate the net superiority of a specialized breed that had in the group of females in females of 3.39 ± 0.1 kg and in males 3.7 ± 0.04 kg and in simple breeds of 5.15 ± 0.12 kg in females and 5.39 ± 0.13 kg in males with a coefficient of variation below 10%, good homogeneity within the group but and a lesser differentiation on sexual dimorphism (Table 1).

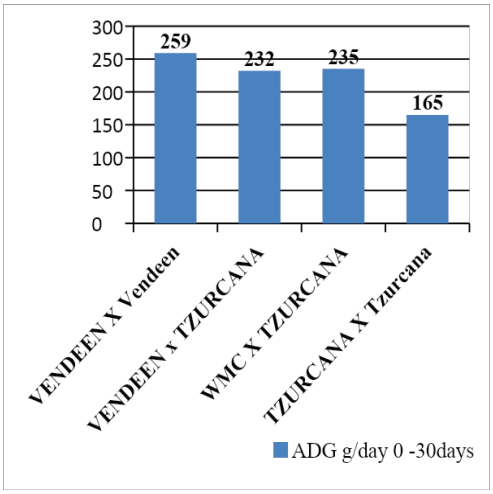

Figure 2. Evolution of ADG in first period of young males sheep

Figure 3. Vendeen pure breed young sheep at 30 days

In Fig. 4 all the results of the cross between Tzurcana with Alba of the Central Massif and Vendeen, as well as the products of the pure Tzurcana and Vendeen breed, are presented in a synthetic way to compare the degree of combinability but also the way they respond to the degree of acclimatization and how they respond to the operating conditions native.

Table 1. Evolution of weight sheep pure breed to Vendeen and Tzurcana and cross Tzurcana with WMC and Vendeen

BREED/ CROSS F1	Number of animals	Weight to Parturition W0				Weight 1 0-30 days	Weight 1 0-30 days	Weight 2 30-70 days	Weight 2 30-70 days	Weight 3 70-98 days	Weight 3 70-98 days
	Parity by sex	Twin ♀	Twin ♂	Single♀	Single♂	Single♀	Single♂	Single♀	Single♂	Single♀	Single♂
VENDEEN x Vendeen	15♀	3,39		5,15		11,87		22,40		30,15	
Pure Breed	15♂		3,70		**5,39**		**12,37**		**23,44**		**31,48**
The Av. Daili Gain						**225**	**259**	**265**	**275**	**276**	**285**
TZURCANA x Tzurcana	25♀			2,96		7,56		14,57		19,87	
Pure Breed	25♂				3,20		8,21		15,77		21,40
ADG						**153**	**165**	**175**	**185**	**185**	**198**
VENDEEN x Tzurcana	25♀	2,88		4,34		10,58		19,83		26,40	
Cross F1	25♂		3,16		4,81		10,98		21,33		28,71
ADG						**210**	**232**	**231**	**238**	**240**	**260**
WMC x Tzurcana	25♀	2,86		4,39		10,78		20,43		27,63	
Cross F1	25♂		3,24		4,92		11,82		21,88		29,37
ADG						**212**	**235**	**240**	**250**	**255**	**265**

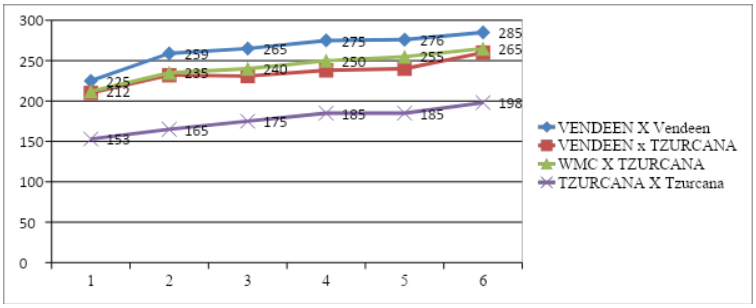

Figure 4. Evolution of ADG in growth period of young sheep

Average body weight at birth and differences between the metods obtained. In the Tzurcana breed, we can not speak of female effect in the present case, so that we can consider the weights of the maternal race almost similar; of 2.96 ± 0.1 kg with a CV of 15.02% in females, and in males of 3.2 ± 0.19 kg having a 21% CV being quite high, comparable to those of the cross with Vendeen of 2.88 kg of twin and male twins of 3,16 kg or in the White of Central Massif (WMC) where the weights of the lambs from the fetuses are the following 2.85 ± 0.14 kg in the females twin and 3.24 ± 0.12 kg in twin males. We can say that the breeds are more homogeneous in both breeds, without much variability within the group, by sexes comparable to the maternal breed, which in males for example exceeds 20 percentage points, typical of the rustic breed without a more rigorous selection. The calf weights of single cross of the Tzurcana and Vendeen breed are differentiated between genres or sexes where females have an average weight of 4.34 ± 0.12 kg with a CV of 6.89% in males of 4.81 ± 0.15 kg with a 7.81% CV, and in WMC (White of Central Massif), we recorded a single weight for single product in females of 4.39 ± 0.14 kg with a CV of 8.2% and in males of 4.92 ± 0.15 kg with a 5.91% CV with relatively homogeneous groups and single products (Fig. 5).

Although there are very few differences between race groups between grams in contrast to Vendeen cross and the native breed are considered very significant differences of 1.35 kg in females and 1.61 kg in males and those with WMC of 1,43 in females and 1,7 in males, results that show extra performance from the start of the calving (Fig. 6).

The elements of differentiation of body weights between the Tzurcana native breed and the pure Vendeen breed exploited under the same conditions demonstrate the net superiority of a specialized breed that had in the group of

females in females of 3.39 ± 0.1 kg and in males 3.7 ± 0.04 kg and in simple breeds of 5.15 ± 0.12 kg in females and 5.39 ± 0.13 kg in males with a coefficient of variation below 10%, good homogeneity within the group but and a lesser differentiation on sexual dimorphism.

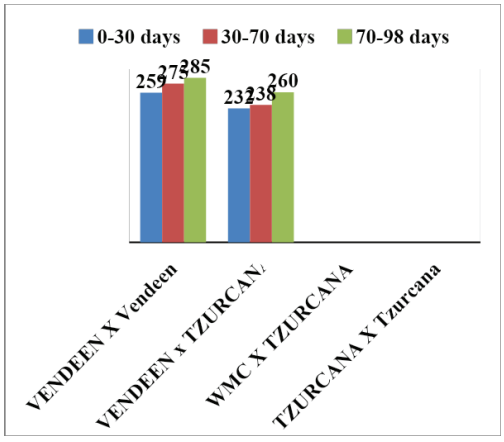

Figure 5. Evolution in a.d.g. of cross and pure young sheep of Tzurcana and hybrid with Vendeen

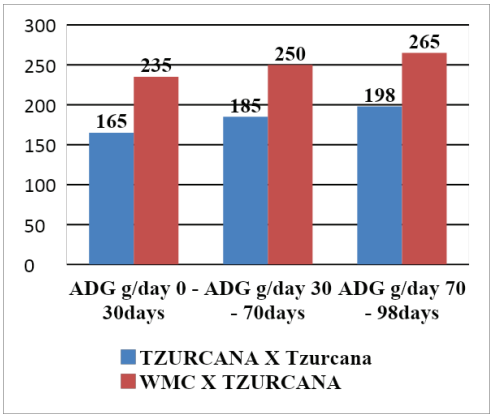

Figure 6. Evolution in a.d.g. of cross and pure young sheep of Tzurcana and hybrid with WCM

Average body weights at first control W1 weights at 30 days and differences between cross obtained of young sheep reveal the onset of lambs growth as a primordial genetic effect, but especially the maternal effect, especially on maternal lactogenic capacity in the first month of life in Vendeen young who had in females an average weight 11.87 ± 0.35 kg with a CV of 11.83% and in males of 12.37 ± 1.13 kg with a 15.8% CV and in the Vendeen meadows with Tzurcana at the first control in females registered a weight of 10.58 ± 0.15 kg with a 7.3% CV and in males of 10.98 ± 0.22 kg with a 8.64% CV and pure breed Tzurcana of only 7.56 ± 0.14 kg with a CV of 9.3% in females and 8.21 ± 0.12 kg with a 7.4% CV.

The differences between Ven x Tzu hibrid with pure paternal race Vendeen in the first month of life were -1.3 kg for females and -1.4 kg for males, and for pure Tzurcana the females are 3.02 kg in females and 2.77 kg males with extra performance for the production of meat.

Daily average spores are and are the primary and important indicator of selection, so after other authors the same types of crosses performances are different in manners; In the first 21 days at the Tzu x Vendeen cross, Borzan et al. in 2017 registered a 330 g increase quite high in total compared to the one registered by us on the whole lot of only 220 g a day. The average daily gains are more synthetic in the Figures 4 and 5 below with the differences between the paternal breed and its hibrid with Tzurcana and in the figure 6 there are also the differences of the domestic breed with another paternal race of White of Central Massif for meat. Making comparisons of the finds found by the same author, Borzan et al. in 2017 in the WCM with Tzurcana, where we recorded average spores of 250 g/day/group in the first 21 days and in our study we found average values of 224 g/day in the first 30 days of growing different from other authors such as Vlaic et al. in 2009 (cited by Gavojdian et al., 2011), 2010, 2012 with the hybrid between Tzurcana and Norwegian White.

The second W2 weighing at 70 days has a double significance related to the phenomenon of young sheep's growth of the maternal lactogenic potential but also of the precocity of the paternal races and cross, namely a particularity of how the transition to the dry feed and the precocity printed by the imported breed, the way of adapting to them in our case of the Vendeen breed in front of the exploitation conditions on the farms and the national farms.

Weight weights in female Vendeen ovine females averaged 22.4 ± 0.29 kg with a CV of 5.15% and in males of 23.44 ± 0.45 kg with a CV of 7,5%, and Vendeen cross with Tzurcana at the first control in females weighing 19.83 ±

0.35 kg with a 8.7% CV and in males 21.33 ± 0.17 kg with a 4.6% CV and a pure breed Tzurcana of only 14.57 ± 0.3 Kg with a CV of 10.8% for females and 15.77 ± 0.26 kg for a 5.8% CV, and in the second stage of growth, the paternal breed is detached with another growth rate, the increases of which are increased over the cross with Tzurcana maternal races over 27 g/day in the first stage with 37 g/day in the second and over 25 g/day in the last up to 98 days, the aspect is found in previous charts no. but also through a good adaptation of the differences between native breeds.

Average weights recorded by Borzan et al. (2017) on the same group of cross by Ven x Tzurcana in females at 50 days, were 18.6 ± 0.86 kg and in males 19.03 ± 0.71 kg while at WMC X Tzurcana at the entire 50 - day group it was 19.98±4.48 kg, compared to our results at Ven X Tzurcana in females at 70 days were 19.83 kg and in males of 21.33 kg and WMC x Tzurcana in females at 70 days was 20.43kg and in males of 21.88 kg, where our results are much weaker as performances compared to periods of time or stages at similar.

At the last weighting scale W3 performed on the farm, the following ovine youth weight gains are recorded, the differentiations of which are somewhat similar to those of other authors who tracked both the Tzurcana breeding phenomenon, especially for meat (Gavojdian et al., 2011; Borzan et al., 2017) as well as racial combining modalities for certain imports, but especially for meat hybrids.

The study of average enrollments over periods of 0 to 50 days - 70 days and 98 days performed by many authors such as Borzan et al. (2017) on the same groups of cross by Ven X Tzurcana in females at 90 days were 25.42 ± 1.39 kg and in males 26.31 ± 1.66 kg while at WMC x Tzurcana for the whole lot at 90 days it was 28.48 ± 4.38 kg versus the results recorded at Ven x Tzurcana in females at 98 days were 26.4 ± 0.35 kg with a CV 4.6% and in males of 28.71 ± 0.32 kg with a CV of 5.2% and WMC x Tzurcana in females at 98 days was 28.73 ± 0.25 kg with a CV of 4.8 and in males of 21.4 ± 0.34 kg but a 6.72% CV with fairly homogeneous lots considering a CV below 10% in general.

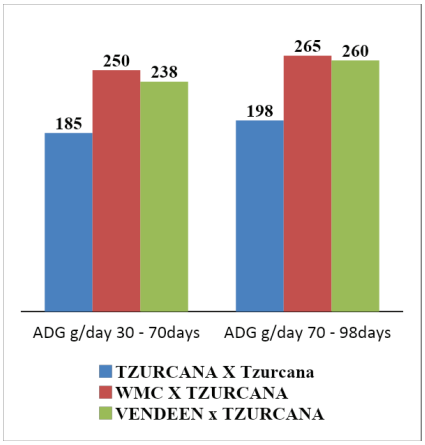

Figure 7. Evolution in ADG of cross and pure young sheep of Tzurcana and hybrid with Vendeen

Studies by Gavojdian et al. (2011) reported an increase of 229.46g (0-28 days) and 168.5 g at (0-240 days) in the German Blackhead cross with Tzurcana and in the Tzurcana cross with the Norwegian White obtained the average growths between 0-60 days 0-120 days and 0-160 days of 199,89g, and 181,18g and 168,18 g of the pure Tzurcana breed of only 167,65g of 159,35 g and 151.1 g/day, and our maternal outcomes were 159.4 g/day in the 0-30 days of lactation period of 175.6 g/day in the 0-70 days and 178 g/day in the 0-98 days considered as much better growth results but also with some homogeneities regarding the degree of variability within the analyzed groups.

The results according to the increases, followed by the groups of cross but also the two major stages that take into account the maternal effect, gave the following values considered as general averages that outline not just the visible decrease of the growth rate but a better adaptation through clear results the growth performance from the first stage to the 70 days and 100 days respectively for all cross but especially for the Vendeen breed are 246,34 g females with 255.12 g and males 257.14 g with 266.22 g at Vend x Tzurcana in females 221.41 g with 225.2 g in male 236.14 g with 244.8 g, at WMC x Tzurcana are 230.4 g females with 236.8 g, in 242.5 g males with 249.6 g, and in pure breed native breeds in females 161.12 g with 172.14 g and in males of 179.57 g with 185.7 g mentioned above lot.

CONCLUSIONS

Mean values recorded at control weights and calculation on average mid-point increment estimates from one to 30 days, at 70 days, and at 98 days of the Tzurcana sheep young and the Vendeen cross and WCM have highlighted both the superiority of the pure Vendeen breed on the degree of adaptation of its prolificity and the weight to calving and other meat performances to all parameters analyzed.

Also, the same superior performance to the Tzurcana native breed was also demonstrated in the Tzurcana cross with the Vendeen breed and the White of Central Massif breed which, through the hardships recorded and the increases of the last two stages, clearly demonstrates a continuous evolution of almost constant growth and very good possibility of crossing or combining with the two French breeds, with the specification and requirement to ensure balanced nutrition conforming to the requirements of the food reglementations and not the extensive type.

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PHYSICOCHEMICAL PARAMETERS AND SPECTRAL STRUCTURE (FT-IR) OF HONEY FROM IASI COUNTY (NORTH-EASTERN ROMANIA)

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Abstract

The aim of this study was to investigate some physicochemical parameters of honey from Iasi County (North-Eastern Romania). Twenty-four samples of four floral honey types (rapeseed, acacia, linden, polyfloral) were collected in 2016 from beekeepers. All analyses were performed according to both Romanian and EU standards. The results for colour, refractive index, total soluble solids, moisture and density varied between 1.12-69.70 mm Pfund, 1.4886-1.4948, 80.84-83.28 ºBrix, 16.72-19.16% and 1.423-1.439 g cm^{-1}, respectively. The pH, free acidity, electrical conductivity and ash ranged between 3.84-4.75, 17.6-48.6 meq kg^{-1}, 166-794 µS cm^{-1} and 0.05-0.20%, respectively. The total phenolic content ranged from 16.10 mg GAE/100 g to 31.44 mg GAE/100 g while total flavonoid content ranged from 0.97 mg Q/100 g to 2.59 mg Q/100 g. For all honey samples the results revealed strong positive correlation between colour and total phenolic content with total flavonoid content, and between ash and electrical conductivity, respectively. The spectral structure (FT-IR) of studied honey samples showed the presence of various functional groups.

Key words*: honey, physicochemical, FT-IR, NE Region, Romania.*

INTRODUCTION

According to EU legislation (European Commission, 2002) honey is defined as the natural sweet substance produced by *Apis mellifera* bees. Honey is a complex natural foodstuff and the only sweetening agent that can be used by humans without processing. The variety of the environmental forms, different weather conditions and a large variety of melliferous flora make Romania an important producer of honey in European Union (Popescu, 2017).

The composition and the properties of this product of beehive are considerably influenced by floral source, geographical and environmental factors (Halouzka et al., 2016; Mărghitaş et al., 2009). The highest amounts of components in honey consists of a complex mixture of sugars and water. Honey also contains other chemical compounds which, even at low concentrations, contribute to its high nutritional and curative properties (Arawwawala and Hewageegana, 2017; Ferreira et al., 2009). Polyphenolic compounds, such as flavonoids have been proved to give the antioxidant and antimicrobial activities of honey (Bridi et al., 2017; Ferreira et al., 2009; Kurtagić et al., 2013).

The main objective of this study was to investigate the quality of honey from some sites of Iasi county. Some parameters, such as moisture, total soluble solids, acidity, electrical conductivity can give information about the honey quality in terms of standard regulations (Isopescu et al., 2014).

The infrared spectroscopy technique was used to determine the spectral structure of the analysed samples.

MATERIALS AND METHODS

Twenty-four samples (three sub-samples from each type of honey) of four floral honey types (rapeseed - R1, R2, acacia - S1, S2, linden - T1, T2, polyfloral - P1, P2) were collected in 2016 from beekeepers at different sites of Iasi county (Figure 1).

Honey samples were stored in the dark in laboratory at 20-24°C. Three laboratory replicates were analysed for each sub-sample, according to Romanian standards (Standard Roman, 2009), harmonised methods of the International honey commission (Bogdanov,

2009) and European Union Honey Directive (European Commission, 2002).
The color was determined spectrophotometrically at 635 nm (Shimadzu UV-mini-1240) for a 50% honey aqueous solution (w/v).
The honey samples were classified according to the Pfund scale (Table 1) after conversion of the absorbance values (Ferreira et al., 2009; Pontis et al., 2014; Rebiai & Lanez, 2014; Sant'ana et al., 2014).

Figure 1. The honey sampling sites

The refractive indices of honey samples were measured using an ABBÉ Kruss AR 2008 refractometer and corrected for the temperature of 20°C (Bogdanov, 2009; Rebiai & Lanez, 2014; Standard Roman, 2009). The moisture content, the total soluble solids and density were determined by the refractometer method using the values of refractive indices (Bogdanov, 2009; Crane, 1979; Popescu & Meica, 1997; Standard Roman, 2009; USDA, 1985).
pH was measured on a 10% (w/v) honey solution and the electrical conductivity on a 20% (w/w) honey solution (dry matter basis) with WTW MULTI 3320 multiparameter (Bogdanov, 2009; Popescu and Meica, 1997; Sereia et al., 2017).
Free acidity was determined by the titrimetric method. A 10% (w/v) honey solution was titrated with 0.1 N NaOH using TITRONIC universal-SCHOTT Instruments (Popescu and Meica, 1997; Standard Roman, 2009).
The ash content was determined by calcination of samples in a muffle furnace (SUPERTHERM) at 550°C (Cantarelli et al., 2008; Popescu and Meica, 1997).
The total phenolic content (TPC) was determined by using Folin-Ciocalteu method, modified from Bobiş et al. (2008) and Sereia et al. (2017). The absorbance was measured at 742 nm against a blank (UV-1400 SHIMADZU Spectrophotometer). Gallic acid was used as standard to obtain the calibration curve (5 calibration points; $y=0.0993x+0.0737$; $R^2=0.9991$). The results were expressed in mg of gallic acid equivalents (GAE)/100 g.
Total flavonoid content was determined by using a method with minor changes developed by Bobiş et al. (2008), Özkök et al. (2010) and Pontis et al. (2014).
The absorbance was measured at 430 nm against a blank (UV-1400 SHIMADZU Spectrophotometer). A standard calibration curve of quercetin was obtained in 5 calibration points ($y=0.1364x-0.0131$; $R^2=0.9997$). The results were expressed in mg of quercetin (Q)/100 g.
The structural identification of some compounds in honey samples was made by FTIR spectroscopy method with an Identify IR–Portable FT-IR spectrometer within a range from 4000 cm^{-1} to 650 cm^{-1}.
The statistical analyses were performed with the Statistica 12 and the FT-IR spectra were processed using Origin 8 software.

RESULTS AND DISCUSSION

Color of honey is not considered a quality parameter but has a direct visual impact on consumers and on the price.
The color variation is directly related to floral origin, mineral content, product and storage of honey, flavonoids content (Sereia et al., 2017).
The results showed that color of rapeseed honey samples is light amber and that of acacia honey is water white.
The color of linden honey samples (T1) is extra light amber and of T2 samples is white (Table 2).
The polyfloral samples also had different colors: P1 samples color was between extra light amber and light amber and P2 color samples was much lighter - white color.
The determination of moisture content is the most frequent analysis to evaluate the quality of honey. Any excess of water affects the physical properties of honey.

Table 1. Pfund scale for determining color*

Color	Pfund scale (mm)
Water white	1 to 8
Extra white	8–17
White	17–34
Extra light amber	34–50
Light amber	50–85
Amber	85–114
Dark amber	More than 114

*Sereia et al., 2017

Water content is very important to avoid fermentation and increase storage time (Soares et al., 2017). National and international legislation recommend the water content in honey to be less than 20% (European Commission, 2002; Standard Roman, 2009). The moisture content in the analyzed honey samples ranged between 16.72% (polyfloral sample P1) and 19.16% (polyfloral sample P2) (Table 2). For all samples moisture content was below 20%. Stihi et al. (2016) reported four acacia honey samples from Romania with moisture content between 20% and 22.8%.

Total soluble solids are represented by sugars and depend on moisture content. When values are higher than 80°Brix (20% moisture) honey can be considered of high grade and stable during storage because as total soluble solids increase, moisture drops (Nyau et al., 2013, USDA, 1985). In honey samples values of total soluble solids ranged between 80.84-83.28°Brix (Table 2).

Density of honey is a parameter of practical significance, that could indicate the optimum amount of honey to be stored (honey with high water content is less dense). Polyfloral samples P2 were of the lowest density (1.423 g cm^{-1}) and the polyfloral honey samples P1 are the densest (1.439 g cm^{-1}).

Usually, the pH of honey varies between 3.5 and 5.5 (Popescu and Meica, 1997). The pH values of the analyzed honey samples were between 3.84 and 4.75 (Table 3). At low pH values the microbial activity is inhibited and the shelf life is extended (Krishnasree and Ukkuru, 2017).

Honey is an acid product. The free acidity is related to the freshness of honey. High acidity could indicate a possible fermentation. International legislation (European Commission, 2002) requires the highest limit value of 50 meq kg^{-1} free acidity.

The lowest values of free acidity were determined on acacia honey samples: S1 with 17.6 meq kg^{-1} and S2 with 18.9 meq kg^{-1} average value, respectively.

The polyfloral honey samples (P2) had the highest free acidity value (48.6 meq kg^{-1}).

Table 2. Physico-chemical parameters of honey samples

Type	n	Descriptive statistics	Color (mm Pfund)	RI	TSS (°Brix)	Moisture (%)	Density (g cm^{-1})
R1	3	Min-Max	67.52-72.35	1.4907-1.4914	81.68-81.96	18.04-18.32	1.429-1.431
		Mean±SD	69.70±1.69	1.4910±0.001	81.78±0.11	18.22±0.11	1.429±0.001
		CV	2.42	0.02	0.14	0.61	0.05
R2	3	Min-Max	55.63-61.58	1.4928-1.4943	82.52-83.08	16.92-17.48	1.434-1.438
		Mean±SD	58.15±2.26	1.4937±0.001	82.84±0.19	17.16±0.19	1.436±0.001
		CV	3.88	0.03	0.23	1.11	0.09
S1	3	Min-Max	3.27-4.01	1.4917-1.4929	82.08-82.56	17.44-17.92	1.432-1.434
		Mean±SD	3.51±0.32	1.4924±0.001	82.35±0.16	17.65±0.16	1.433±0.001
		CV	9.15	0.03	0.19	0.9	0.06
S2	3	Min-Max	1.04-1.41	1.4893-1.4903	81.08-81.52	18.48-18.92	1.425-1.428
		Mean±SD	1.12±0.16	1.4899±0.001	81.33±0.15	18.67±0.15	1.426±0.001
		CV	14.61	0.02	0.18	0.79	0.07
T1	3	Min-Max	37.06-40.41	1.4888-1.4897	80.92-81.28	18.72-19.08	1.424-1.426
		Mean±SD	38.80±1.02	1.4893±0.001	81.11±0.12	18.89±0.12	1.425±0.001
		CV	2.62	0.02	0.14	0.61	0.05
T2	3	Min-Max	29.64-31.86	1.4902-1.4915	81.46-82.00	18.00-18.54	1.427-1.438
		Mean±SD	31.04±0.85	1.4907±0.001	81.67±0.21	18.33±0.21	1.430±0.003
		CV	2.73	0.03	0.25	1.13	0.24
P1	3	Min-Max	48.21-51.92	1.4943-1.4952	83.11-83.44	16.56-16.89	1.438-1.440
		Mean±SD	49.81±1.27	1.4948±0.001	83.28±0.13	16.72±0.13	1.439±0.001
		CV	2.56	0.02	0.15	0.75	0.06
P2	3	Min-Max	29.64-31.86	1.4874-1.4903	80.36-81.54	18.46-19.64	1.420-1.428
		Mean±SD	30.87±0.83	1.4886±0.001	80.84±0.38	19.16±0.38	1.423±0.002
		CV	2.69	0.07	0.48	2.01	0.18

n-no. samples; RI-refractive index; TSS-Total Soluble Solids; SD-standard deviation; CV-coefficient of variation

For all honey samples the free acidity values are below the required limit of 50 meq kg^{-1} (Table 3).

Electrical conductivity depends directly on the ash content and has been replacing the ash content in the international standards. The electrical conductivity values in the analyzed honey samples varied in range of 166-794 µS cm^{-1}, and do not exceed the recommended limit value (0.8 mS cm^{-1}) (European Commission, 2002). The lowest values were observed for acacia honey samples (Table 3).

The mineral content of honey depends on the quality of the nectar collected by bees. The number and the content level of honey minerals and trace elements depend on botanical and geographical origins. The lowest ash content of 0.05% was found on acacia honey samples (S1). In honey samples from different regions of Romania, Mărghitaș et al. (2009) reported similar lowest values of 0.03-0.28% on acacia honey samples.

Polyphenolic compounds are mainly responsible for the antioxidant properties of honey. The content of these compounds depends on season, climatic conditions and mostly on the botanical origin of honey (Soares et al., 2017). The lowest value of total phenolic content was 16.1 mg GAE/100 g for acacia honey samples (S1) and the highest value of 31.44 mg GAE/100 g was recorded for polyfloral samples (P1) (Table 4).

Table 3. Characteristic parameters (pH, free acidity, electrical conductivity, ash) of honey samples

Type	n	Descriptive statistics	pH	Free Acidity (meq kg^{-1})	EC (µS cm^{-1})	Ash (%)
R1	3	Min-Max	3.98-4.08	24.8-26.2	239-253	0.09-0.11
		Mean±SD	4.01±0.03	25.5±0.51	245±4.51	0.10±0.008
		CV	0.73	1.98	1.84	7.93
R2	3	Min-Max	4.08-4.12	22.3-23.5	222-234	0.07-0.08
		Mean±SD	4.10±0.01	23.0±0.38	230±4.04	0.07±0.005
		CV	0.33	1.65	1.76	6.32
S1	3	Min-Max	4.00-4.10	17.1-18.2	160-173	0.05-0.06
		Mean±SD	4.06±0.04	17.6±0.37	166±3.82	0.05±0.004
		CV	0.88	2.12	2.3	6.74
S2	3	Min-Max	3.75-3.91	18.5-19.3	249-262	0.10-0.13
		Mean±SD	3.84±0.06	18.9±0.26	254±3.97	0.11±0.009
		CV	1.65	1.39	1.56	8.4
T1	3	Min-Max	4.52-4.70	32.2-33.5	513-524	0.19-0.21
		Mean±SD	4.63±0.06	32.3±0.46	518±4.00	0.20±0.009
		CV	1.38	1.4	0.77	4.28
T2	3	Min-Max	4.63-4.86	43.4-45.1	500-516	0.15-0.20
		Mean±SD	4.75±0.07	44.1±0.58	509±4.18	0.18±0.018
		CV	1.51	1.31	0.82	9.98
P1	3	Min-Max	3.86-4.01	39.6-41.0	577-591	0.16-0.20
		Mean±SD	3.95±0.06	40.6±0.52	584±4.94	0.18±0.015
		CV	1.45	1.28	0.84	7.97
P2	3	Min-Max	4.32-4.47	47.3-49.7	785-800	0.19-0.21
		Mean±SD	4.40±0.05	48.6±0.86	794±4.89	0.20±0.009
		CV	1.13	1.77	0.62	4.52

n-no. samples; EC-electrical conductivity; SD-standard deviation; CV-coefficient of variation.

Similar studies on honey samples from Romania showed various content of phenolic compounds: in linden honey samples were found from 16 mg GAE/100 g to 38 mg GAE/100 g, in acacia honey samples were found from 2 mg GAE/100 g to 39 mg GAE/100 g (Mărghitaș et al., 2009), in polyfloral honey samples were found 31 mg GAE/100 g and in linden honey samples were found 53 mg GAE/100 g (Dobre et al., 2010).

Flavonoids have antioxidant and anti-inflammatory properties. The total flavonoid content ranged from 0.97 mg Q/100 g to 2.59 mg Q/100 g, with the highest value for rapeseed honey (Table 4). The total flavonoids content of 4.7-6.9 mg Q/100 g for Romanian linden honey and of 0.9-2.4 mg Q/100 g on Romanian acacia honey were reported by Mărghitaș et al. (2009).

Several studies showed significant correlations between some characteristic parameters of honey. Pontis et al. (2014) found strong positive correlation between color intensity, flavonoid content and phenolic content; a positive correlation was observed by Ahmida et al. (2013), Sohaimy et al. (2015) between electrical conductivity and total ash content.

Table 4. Total phenols content and total flavonoids content of honey samples

Type	n	Descriptive statistics	TPC (mg GAE/100 g)	TFC (mg Q/100 g)	Type	n	Descriptive statistics	TPC (mg GAE/100 g)	TFC (mg Q/100 g)
R1	3	Min-Max	25.87-27.58	2.52-2.64	T1	3	Min-Max	29.43-30.63	2.00-2.17
		Mean±SD	26.80±0.56	2.59±0.05			Mean±SD	30.01±0.36	2.09±0.06
		CV	2.1	1.79			CV	1.19	2.77
R2	3	Min-Max	22.91-24.59	2.06-2.22	T2	3	Min-Max	22.24-23.59	1.48-1.55
		Mean±SD	23.91±0.60	2.12±0.05			Mean±SD	22.76±0.50	1.52±0.03
		CV	2.52	2.4			CV	2.18	1.67
S1	3	Min-Max	15.19-16.87	0.93-1.01	P1	3	Min-Max	30.90-31.98	2.24-2.34
		Mean±SD	16.10±0.55	0.97±0.03			Mean±SD	31.44±0.42	2.30±0.04
		CV	3.39	2.92			CV	1.32	1.53
S2	3	Min-Max	18.42-19.22	1.22-1.34	P2	3	Min-Max	29.34-30.85	1.85-1.99
		Mean±SD	18.90±0.28	1.28±0.04			Mean±SD	29.94±0.046	1.92±0.04
		CV	1.46	2.87			CV	1.54	2.2

n-no. samples; TPC-total polyphenols content: TFC-total flavonoids content; SD-standard deviation; CV-coefficient of variation

Khalil et al. (2012) observed strong correlation between phenolic and flavonoid contents, color intensity and flavonoid content.

Krishnasree and Ukkuru (2017) noticed positive correlations between pH and moisture, pH and acidity, acidity and ash.

Table 5 presents the Pearson correlation coefficients between parameters of the analyzed honey samples.

A strong positive correlation was found between color intensity, total phenols content and total flavonoids content, between refractive indices, total soluble solids and density, between acidity, electrical conductivity and ash content, between electrical conductivity and total phenols content and between total phenols content and total flavonoids content.

A strong negative correlation is observed between moisture, refractive index, total soluble solids and density.

The factor analysis based on chemical composition of honey samples are presented in Figure 2.

The refractive index, the total soluble solids and the moisture content were the main variables determining the ranking of honeys on factor 1 (38.15% of variance accounted for), whereas the second factor (32.07% of variance accounted for) would be explained mainly by the free acidity, electrical conductivity and ash and the third factor (22.69% of variance) by color, total phenols content and total flavonoids content.

Table 5. Correlation matrix of honey samples parameters (Pearson correlation coefficients)

	Color	RI	TSS	Moisture	Density	pH	Acidity	EC	Ash	TPC	TFC
Color	1.00										
RI	0.31	1.00									
TSS	0.31	1.00	1.00								
Moisture	-0.31	-1.00	-1.00	1.00							
Density	0.30	0.99	0.99	-0.99	1.00						
pH	0.05	-0.48	-0.48	0.48	-0.40	1.00					
Acidity	0.24	-0.22	-0.22	0.22	-0.17	0.61	1.00				
EC	0.11	-0.34	-0.34	0.34	-0.31	0.53	0.94	1.00			
Ash	0.12	-0.41	-0.41	0.41	-0.37	0.62	0.87	0.92	1.00		
TPC	0.66	-0.05	-0.05	0.05	-0.06	0.27	0.68	0.74	0.74	1.00	
TFC	0.94	0.16	0.16	-0.16	0.14	-0.01	0.31	0.27	0.31	0.82	1.00

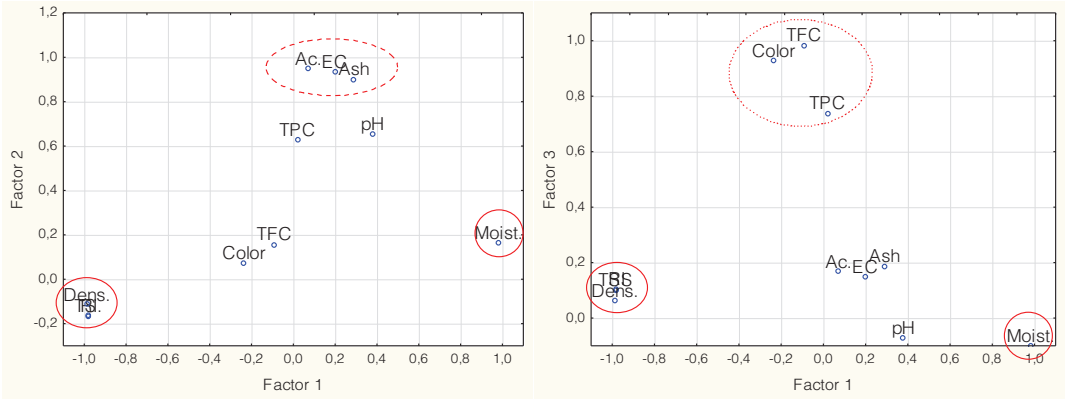

Figure 2. Discrimination of the most influential variables on the observed separations on the basis of factor analysis performed on data recorded on chemical composition of honey

FTIR spectroscopy is a rapid and non-destructive analytical method for quality control of honey through the spectrum obtained from 4000 cm^{-1} to 650 cm^{-1} spectral region. The spectrum shows peaks that correspond with the main classes of organic molecules (Anguebes et al., 2016; Nayik et al., 2015).

Moisture, carbohydrates and other minor compounds are mainly responsible for these variations in the spectral structural composition of honey samples (Figure 3).

There are some regions where the absorption bands were attributed to some bonds which belong to the structure of honey compounds.

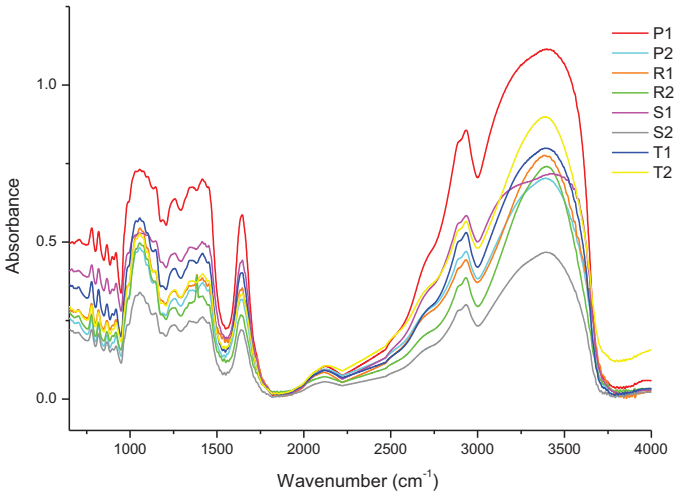

Figure 3. FTIR spectra of honey samples in the range 650-4000 cm^{-1}

The absorption bands between 3700 cm^{-1} and 3000 cm^{-1} are due to the stretching vibrations of the -OH functional group from carbohydrates, water and organic acids present in honey samples (Anguebes et al., 2016).

The band at 3000-2700 cm^{-1} corresponds to the stretching vibration of the C-H bonds which constitute the chemical structure of the carbohydrates, the stretching vibration of the O-H bonds of the carboxylic acids and NH_3 of free amino acids (Anguebes et al., 2016; Franca and Oliveira, 2011; Gok et al., 2015).

The band at 1700-1600 cm^{-1} was attributed to the deformation vibrations of O-H from the water and the stretching vibrations of the functional groups C=O (ketone) of fructose and

CH=O (aldehyde) of glucose (Anguebes et al., 2016; Gok et al., 2015).
The absorption band from 1600 cm^{-1} to 700 cm^{-1} is specific to the structure of carbohydrates of honey bee. There are many peaks of the stretching vibrations of bonds C-C, C-C, C-H and the bending vibrations of C-H which are present in chemical structure of carbohydrates (Anguebes et al., 2016; Gok et al., 2015).

CONCLUSIONS

The values of the main parameters required by legislation (moisture, free acidity, electrical conductivity) for all honey samples were found within the recommended limits.
Pearson coefficients showed strong correlations between some parameters: color with total phenols content and total flavonoids content and between electrical conductivity and ash content.
The total phenolic and flavonoid content depend mainly on type of honey.
FTIR analysis gives qualitative information on honey adulteration and confirms different levels of water and sugar in samples.

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AN EVALUATION OF GUELDER ROSE (*Viburnum opulus* L.) AND HAWTHORN (*Crataegus monogyna)* CONCENTRATES AS ALTERNATIVE ANTIOXIDANT SOURCES TO BHT AND NITRITE IN POULTRY MEAT MODEL SYSTEM

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Abstract

This study aimed to determine the effectiveness of hawthorn (HT) and guelderrose (GR) concentrates (65%) at different levels (1, 5, 10%) as an alternative antioxidant sources for nitrite (N) (25, 50, 100, 156 ppm) and butylatedhydroxytoluene (BHT) (0.01%) in cooked turkey ground meat stored under aerobic and anaerobic conditions at 4°C. Cooking loss (CL), pH, CIE L, a*, b*, texture profile analysis (TPA) and thiobarbituric acid reactive substances (TBARS) levels were determined. The use of 5% and 10% of the HT and GR concentrates increased CL compared to control and nitrite-containing groups (P<0.05). A significant differences were not found in terms of pH among groups stored under aerobic conditions. However, the highest pH values were determined in groups containing 10% HT or 100 ppm nitrite, whereas the lowest pH values were obtained in both BHT and control groups stored under anaerobic condition (P<0.05).TBARS increased during storage in both storage types (P<0.05). The lowest TBARS were determined in groups containing 156 ppm or 100 ppm nitrite, ora 10% HT in both storage conditions (P<0.05). The addition of GR and HT reduced the TBARS and this effect was further enhanced with increasing GR and HT levels (P<0.05). Furthermore, the groups containing 10% HT or GR were found to be have lower TBARS than the both control and BHT (P<0.05). It was determined that the all treatments did not have a significant effect on the L* values, the addition of nitrite and GR increased the a* values, and the addition of HT also increased the b* values (P<0.05). Addition of nitrite, BHT, HT or GR did not cause a significant changes chewiness, springiness, cohesiveness and adhesiveness. The lowest hardness and gumminess were determined in 10% HT or GR added samples compared with BHT or nitrite (156 ppm) containing groups (P<0.05). Study results suggested that the use of GR or HT (especially 10%) may be effective strategy in delaying the oxidative changes in poultry meat.*

Key words*: hawthorn, guelder rose, concentrate, poultry meat, antioxidant.*

INTRODUCTION

Poultry meat is more preferred than red meat due to low connective tissue and fat content, and high protein content (Ismail and Joo, 2017). However, it is highly susceptible to oxidation reaction due to its high content of polyunsaturated fatty acids (Arguelo et al., 2016).Oxidative deterioration is one of the most important chemical reactions limiting shelf-life and causing quality loss of meat products (Min and Ahn, 2005).During the oxidation, toxic compounds such as hydroperoxide, carbonyl compounds, aldehydes, acids, ketones, epoxides and carboxy acids are formed (Reitznerová et al., 2017). These compounds cause undesirable changes in the texture, color, taste and odor of meat products. Oxidation-related changes have a complex process and are influenced by many factors such as light, oxygen, storage temperature, metal ions and meat compositions (Sen and Mandal, 2017). The most commonly used method to prevent oxidation is the use of synthetic or natural antioxidants. Synthetic antioxidants are mainly used in meat industry to delay oxidation and prolong the storage period of meat products because of their strong antioxidant activity, and these additives are also cheaper than natural antioxidants (Karre et al.,

2013). However, many studies have shown that synthetic antioxidants such as sodium nitrite, butylatedhydroxytoluene (BHT), butylatedhydroxyanisole (BHA) and propyl gallate (PG) exhibit carcinogenic and teratogenic effects in living organisms. Therefore, many studies are focused on natural antioxidants that can be an alternative to synthetic antioxidants (Naveena et al., 2008).

The guelder rose *(Viburnum opulus* L.) is an edible and dark-red colorfruit which is known as "Gilaburu" in Anatolian region (Kalyoncu et al., 2013). It is known with several other names such as European cranberrybush (Akbulut et al., 2008), crampbark (Özrenk et al., 2011), whitten tree androse elder (Akbulut et al., 2008). GRis consumed as fruit juice, dried fruits, jam and pickles. GRfruits are especially used to treat of kidney problems. In addition, it is also reported to have antidiabetic and antispasmodic effects. Furthermore, GR fruits have antioxidant effects due to its high content of polyphenols such as chlorogenic acid, (+)-catechin, (–)-epicatechin, quercetin glycosides and proanthocyanidins (Levent et al., 2008; Özrenk et al., 2011; Moldovan et al., 2012; Kalyoncu et al., 2013; Ozola and Kampuse, 2018).Although there are studies showing the antioxidant activity of GRfruits (Levent et al., 2008; Şeker et al., 2016), there is no study on the use of this fruit on meat products.

Hawthorn used in the treatment of cardiovascular disease is a fruit having a high antioxidant activity. HT fruits contain high amount of flavonoids, phenolic acids (chlorogenic and caffeic acids) and oligomericprocyanidins. These compounds have lipid-lowering, antioxidant and anti-inflammatory properties (Liu et al., 2010; Shortle et al., 2014; Papuc et al., 2018). There are limited studies on the use of HT fruits on meat products. Ganhão et al. (2010a), Ganhão et al. (2010b), Shortle et al. (2014), Akcan et al. (2017) and Pabuc et al. (2018) investigated the efficiency of HT fruit extracts as inhibitors of oxidative reactions in cooked and raw pork patties, bovine muscle homogenates, ready-to-eat pork patties and minced pork, respectively. The goal of this study was to investigate the effectiveness of using HT and GR concentrates at different levels as a natural antioxidant sources in cooked turkey meat model system.

MATERIALS AND METHODS

Turkey breast meat (*Musculus superficiolis*) were purchased from a local slaughterhouse for each of two replications on separate production days. GR fruit concentrate (65%) was supplied by Kayseri Pazarı BioBitkisel Ürünler San.veTiç. A.Ş (Kayseri, Turkey). HT fruits were taken from a local market and concentrates were prepared according to the following procedure. HT fruit were dried in an air circulatory drier (FN 500, Nüve, Turkey) at 40°C for 48 h, and ground in an analytical mill to a grain diameter of less than 0.5 mm. The HT fruit powders were mixed with distilled water to be a concentration of 65%.

Sample Preparation: All experimental groups contained 2% sodium chloride and 10% distilled water over meat weight. Twelve experimental groups were formulated ascontrol (without additive) group and BHT (0,01%) ornitrite (25, 50, 100, 156 ppm) or HT (1%, 5%, 10%) or GR (1%, 5%, 10%; table 1) incorporated groups. The experimental samples formulated according to treatment groups (approximately 45 greach) were filled into 50 mL centrifuge tubes and cooked in a water bath until final internal temperature of 74^{o}C. Cooked samples were cooled to room temperature. Samples were stored under aerobic and anaerobic conditions at 4^{o}C for 30 days.

Physico-chemical analyses: The pH measurements were performed by using a portable pH meter (HI 9024, Hanna Instruments, Germany) with spear electrode (FC 200, Hanna Instruments, Germany). Color values of cooked treatments were measured with respect to CIE Lab Color System using a Minolta Colorimeter (Model CR-200, Minolta corp., Ramsey, Nj, USA). Thiobarbituric acid reactive substances (TBARS) analysis were applied according to the method stated by Kilic and Richards (2003). TPA tests were performed using a Brookfield CT3 Texture Analyzer (Brookfield Engineering Laboratories, Inc., USA) to determine hardness (N), adhesiveness (mJ), springiness, cohesiveness, gumminess (N), chewiness (N), and resilience. Conditions were: aluminium rectangular probe (9 mm x 35 mm x 0.05 mm), compression 70%, and 25 kg load cell.

Table 1. Coding for hawthorn (HT) and guelder rose (GR) concentrates, sodium nitrite (N) and butylatedhydroxytoluene (BHT) evaluated

Groups	HT, GR, and N treatments
Control	Without additive
BHT	% 0,01 BHT
N156	156 ppm sodium nitrite
N100	100 ppm sodium nitrite
N50	50 ppm sodium nitrite
N25	25 ppm sodium nitrite
HT1	1% Hawthorn concentrates
HT5	5% Hawthorn concentrates
HT10	10% Hawthorn concentrates
GR1	1% Guelder rose concentrates
GR5	5% Guelder rose concentrates
GR10	10% Guelder rose concentrates

Statistical Analysis: Statistical analysis was performed using the Minitab 17.3.1 program (Minitab Inc., UK).

The cooking loss data were implemented to one-way analysis of variance (one-way ANOVA).

The pH, color, texture profile analysis and TBARS data were implemented to two-way analysis of variance (two-way ANOVA).

The differences between means in all experimental groups were determined by using Tukey multiple range test P values < 0.05 were considered as significant.

RESULTS AND DISCUSSIONS

The CL results are shown in Table 2. The use of GR and HT concentrate at 5% or 10% increased the CL compared to the control and nitrite containing groups (P<0.05).

Ganhão et al. (2010a) reported that the addition of HT fruit extract (3%) in burger patties did not affect the moisture loss after cooking and chill storage.

In present study, an increasing added HT or GR concentrates in turkey meat formulation has been caused to an increase in cooking loss (P<0.05).

The highest CL value was determined in the sample containing 10% GR concentrate, whereas the lowest CL values were determined in the control samples and nitrite containing samples (P<0.05).

In addition, the cooking loss was increased with increasing GR concentrate levels (P<0.05). A similar effect were also present between HT5 and HT10 groups (P<0.05).

Table 2. The results of cooking loss in cooked turkey ground samples

Groups	Storage time (Day)
Control	9.17^{ef}±0.36
BHT	8.87^{ef}±0.29
N156	9.17^{ef}±0.08
N100	9.39^{de}±0.17
N50	8.51^{f}±0.35
N25	8.36^{f}±0.23
HT1	10.04^{cd}±0.25
HT5	10.44^{c}±0.20
HT10	11.96^{b}±0.45
GR1	10.21^{cd}±0.52
GR5	11.28^{b}±0.25
GR10	13.79^{a}±0.51

Means ± standard deviation (SD)

$^{a-f}$Within a column, values superscripted with different letters are significantly different (P<0.05)

The changes in pH values of the samples stored under aerobic and anaerobic conditions are presented in table 3 and table 4, respectively. There was no significant difference in the pH values of all treatment groups stored under aerobic conditions on processing day. No significant changes in pH were also observed during aerobic storage. At the end of 30 d storage period, the higher pH value was obtained in the GR1 group which was similar to HT5 (P<0.05). In the samples stored under anaerobic conditions, the lowest pH was determined in the group containing 25 ppm nitrite (N25), whereas the highest pH were determined in the groups of GR5 and GR10at the beginning of storage (P<0.05). There are no generally significant changes in pH values during storage in anaerobic conditions. At the end of the storage, the highest pH value was determined in group of HT10, whereas the lowest pH value was determined in group with

BHT (P<0.05). In the groups stored under anaerobic condition, the pH values of the samples containing HT concentrates were higher than the pH values of the samples containing GR concentrates (P<0.05). pH values were increased at increasing HT concentrate ratios in anaerobic storage conditions (P<0.05). Tengilimoglu-Metin et al. (2017) noted that the addition of the HT extract in beef and chicken breast meat had caused significant increase on pH values.

Table 3. Results of pH values of treatments stored under aerobic condition

	Storage time (Day)				
Groups	0	5	10	15	30
Control	5.75^{bcd}±0.01	5.76^{bcd}±0.02	5.78^{bcd}±0.01	5.70^{bcd}±0.03	5.61^{cd}±0.04
BHT	5.72^{bcd}±0.02	5.87^{abc}±0.01	5.82^{a-d}±0.02	5.81^{a-d}±0.01	5.59^{cd}±0.01
N156	5.70^{bcd}±0.01	5.76^{bcd}±0.01	5.74^{bcd}±0.03	5.83^{a-d}±0.01	5.71^{bcd}±0.01
N100	5.73^{bcd}±0.01	5.79^{a-d}±0.01	5.79^{a-d}±0.01	5.80^{a-d}±0.01	5.75^{bcd}±0.01
N50	5.71^{bcd}±0.01	5.81^{a-d}±0.02	5.78^{bcd}±0.01	5.83^{a-d}±0.01	5.76^{bcd}±0.01
N25	5.75^{bcd}±0.01	5.82^{a-d}±0.01	5.77^{bcd}±0.02	5.80^{a-d}±0.01	5.72^{bcd}±0.01
HT1	5.77^{bcd}±0.03	5.84^{a-d}±0.02	5.76^{bcd}±0.01	5.72^{bcd}±0.01	5.60^{cd}±0.01
HT5	5.67^{bcd}±0.01	5.75^{bcd}±0.01	5.75^{bcd}±0.01	5.83^{a-d}±0.01	5.81^{a-d}±0.01
HT10	5.87^{abc}±0.02	5.94^{ab}±0.02	5.73^{bcd}±0.02	5.72^{bcd}±0.03	5.71^{bcd}±0.02
GR1	5.82^{a-d}±0.05	5.79^{a-d}±0.01	5.73^{bcd}±0.02	5.74^{bcd}±0.02	5.98^{a}±0.05
GR5	5.88^{abc}±0.03	5.77^{bcd}±0.01	5.72^{bcd}±0.01	5.68^{bcd}±0.01	5.61^{cd}±0.02
GR10	5.72^{bcd}±0.04	5.89^{abc}±0.02	5.81^{a-d}±0.01	5.70^{bcd}±0.02	5.54^{d}±0.01

Means ± standard deviation (SD)
$^{a-d}$Within a table, values superscripted with different letters are significantly different (P<0.05)

Table 4. Results of pH values of treatments stored under anaerobic condition

	Storage time (Day)				
Groups	0	5	10	15	30
Control	5.70^{stu}±0.01	5.77^{l-s}±0.01	5.72^{q-u}±0.01	5.83^{e-l}±0.01	5.72^{q-u}±0.01
BHT	5.72^{q-u}±0.02	5.77^{k-r}±0.01	5.75^{n-t}±0.01	5.78^{j-r}±0.01	5.68^{tu}±0.01
N156	5.77^{k-r}±0.01	5.76^{l-s}±0.02	5.76^{m-s}±0.01	5.81^{g-n}±0.01	5.84^{e-k}±0.01
N100	5.74^{n-t}±0.01	5.81^{g-n}±0.01	5.98^{ab}±0.01	5.99^{a}±0.04	5.75^{m-s}±0.01
N50	5.73^{p-u}±0.01	5.86^{d-h}±0.01	5.89^{cde}±0.01	5.86^{d-h}±0.01	5.74^{n-t}±0.01
N25	5.67^{u}±0.02	5.80^{h-o}±0.02	5.86^{d-i}±0.01	5.81^{g-n}±0.01	5.75^{m-s}±0.02
HT1	5.76^{l-s}±0.01	5.83^{e-l}±0.01	5.81^{g-n}±0.01	5.76^{l-s}±0.02	5.71^{r-u}±0.02
HT5	5.81^{g-n}±0.01	5.87^{c-g}±0.01	5.83^{e-l}±0.02	5.87^{c-h}±0.01	5.80^{h-o}±0.01
HT10	5.72^{q-u}±0.03	5.92^{bcd}±0.01	5.84^{e-k}±0.01	5.89^{cde}±0.01	5.93^{abc}±0.01
GR1	5.78^{j-q}±0.03	5.79^{i-p}±0.02	5.75^{n-t}±0.06	5.74^{o-t}±0.01	5.75^{n-t}±0.01
GR5	5.88^{c-f}±0.01	5.83^{e-l}±0.01	5.82^{f-m}±0.01	5.70^{stu}±0.02	5.80^{h-o}±0.01
GR10	5.87^{c-g}±0.01	5.84^{e-t}±0.01	5.74^{n-t}±0.06	5.78^{j-q}±0.01	5.77^{k-r}±0.01

Means ± standard deviation (SD)
$^{a-u}$Within a table, values superscripted with different letters are significantly different (P<0.05)

The changes in TBARS values of treatments stored under aerobic and anaerobic conditions are shown in Table 5 and Table 6, respectively. There was no significant difference between the TBARS values of all treatment groups in both storage conditions at the beginning of storage. There was a gradual increase in TBARS values in all treatment groups stored under aerobic and anaerobic conditions during storage (P<0.05). In the samples stored under aerobic conditions, the higher (P<0.05) TBARS levels were determined in control, HT1 and GR1 groups compared to other treatment groups during first 15 days of storage. Similar results were reported by Akcan et al. (2017). Researchers pointed out that the highest TBARS values were obtained in the control group during storage period. On the 10thand 15thdays of storage, the lowest TBARS values were determined in N156, N100, HT10 and GR10 groups (P<0.05). The highest TBARS values were obtained in the control and GR1 groups on the last day of storage under aerobic conditions (P<0.05). The lowest TBARS values were also determined in HT10 and N156 groups (P<0.05). TBARS values obtained from

both HT10 and GR10 groups were lower than TBARS values of BHT group in the samples stored under aerobic conditions at the end of the storage (P<0.05).

Table 5. Results of TBARS values of treatment groups stored under aerobic condition

	Storage time (Day)				
Groups	0	5	10	15	30
Control	1.12^{vw}±0.07	6.13^{m-s}±0.52	13.55^{efg}±1.07	21.36^{b}±1.98	28.66^{a}±0.74
BHT	1.05^{vw}±0.03	2.59^{t-w}±0.48	7.18^{k-p}±0.68	9.98^{h-l}±0.70	16.26^{cde}±0.88
N156	1.15^{vw}±0.16	2.23^{t-w}±0.16	3.20^{s-w}±0.10	5.64^{n-t}±0.38	6.40^{m-s}±0.14
N100	0.98^{w}±0.21	3.01^{s-w}±0.50	3.94^{o-w}±0.09	5.65^{n-t}±0.11	7.24^{k-p}±0.54
N50	0.78^{w}±0.21	4.29^{o-w}±0.69	4.53^{o-v}±0.71	7.22^{k-p}±0.14	8.70^{j-n}±0.03
N25	1.44^{vw}±0.32	4.11^{o-w}±0.33	5.56^{n-t}±0.59	9.24^{j-m}±0.72	9.34^{i-m}±0.34
HT1	1.12^{vw}±0.21	6.97^{k-q}±0.56	9.63^{i-m}±0.16	17.99^{bcd}±1.55	15.03^{def}±1.86
HT5	1.28^{vw}±0.17	4.21^{o-w}±0.09	6.46^{l-s}±0.53	11.84^{f-j}±0.05	12.83^{e-i}±0.33
HT10	1.37^{vw}±0.45	1.40^{vw}±0.26	2.25^{t-w}±0.24	3.71^{p-w}±0.54	5.12^{o-u}±0.85
GR1	1.64^{uvw}±0.39	6.74^{k-r}±0.73	13.23^{e-h}±1.02	19.72^{bc}±1.18	27.00^{a}±0.45
GR5	1.18^{vw}±0.02	3.42^{r-w}±0.26	8.80^{j-n}±0.88	11.49^{g-j}±1.25	19.35^{bc}±0.52
GR10	0.88^{w}±0.14	1.56^{vw}±0.19	3.55^{q-w}±0.72	7.33^{k-o}±0.61	9.99^{h-k}±0.25

Means ± standard deviation (SD)
[a-w]Within a table. values superscripted with different letters are significantly different (P<0.05)

Table 6. Results of TBARS values of treatment groups stored under anaerobic condition

	Storage time (Day)				
Groups	0	5	10	15	30
Control	1.54^{p-x}±0.31	3.06^{j-u}±0.64	6.42^{c-f}±0.90	8.92^{b}±1.02	12.38^{a}±0.73
BHT	1.01^{t-x}±0.22	2.01^{m-x}±0.02	3.49^{j-r}±0.10	3.99^{g-n}±0.49	5.73^{d-i}±0.30
N156	1.12^{s-x}±0.35	0.73^{wx}±0.07	0.90^{u-x}±0.10	1.55^{p-x}±0.02	2.32^{l-x}±0.44
N100	0.83^{v-x}±0.21	0.85^{v-x}±0.11	1.04^{t-x}±0.36	2.15^{l-x}±0.35	2.86^{k-w}±0.27
N50	0.99^{t-x}±0.16	1.89^{n-x}±0.43	1.88^{n-x}±0.45	2.63^{k-w}±0.13	3.58^{i-q}±0.28
N25	1.15^{s-x}±0.03	1.73^{o-x}±0.14	2.94^{j-v}±0.42	3.67^{h-p}±0.06	3.83^{h-o}±0.33
HT1	0.88^{u-x}±0.00	2.09^{l-x}±0.26	5.08^{e-j}±0.63	7.48^{bcd}±0.44	8.61^{bc}±0.58
HT5	1.44^{q-x}±0.00	1.53^{p-x}±0.23	4.14^{g-m}±0.26	3.69^{h-p}±0.21	5.86^{d-h}±0.69
HT10	0.71^{wx}±0.17	1.60^{p-x}±0.02	3.05^{j-u}±0.47	3.10^{j-t}±0.43	3.15^{j-t}±0.04
GR1	1.42^{q-x}±0.00	2.83^{k-w}±0.14	4.60^{f-k}±0.29	6.95^{b-e}±0.86	8.41^{bc}±0.34
GR5	1.23^{s-x}±0.19	2.00^{m-x}±0.32	3.31^{j-s}±0.33	4.27^{e-l}±0.36	6.04^{d-g}±1.00
GR10	0.42^{x}±0.11	1.37^{r-x}±0.28	2.96^{j-v}±0.24	4.24^{f-l}±0.35	4.17^{g-m}±0.01

Means ± standard deviation (SD)
[a-x]Within a table values superscripted with different letters are significantly different (P<0.05)

Similarly, Pabuc et al. (2018) indicated the addition of HT berry ethanolic extract into minced pork meat was more effective than BHA in reducing lipid oxidation. Additionally, Keser et al. (2012) pointed out that the water and ethanolic extracts of HT showed powerful total antioxidant activities when compared to BHA and α-tocopherol. Levent et al. (2008) reported that GR extracts showed better antioxidant effect than BHT. In another study, it was reported that procyanidins obtained from HT fruit showed antioxidant activity at the similar level as trolox and BHT (Soko´ł-Łe¸towska et al., 2007). In present study, the addition of GR and HT concentrate (except for HT1 group) reduced the TBARS levels and this effect was further enhanced with increasing GR and HT concentrate levels (P<0.05). Akcan et al. (2017) indicated that the adding HT extract into the pork burger patties decreased the TBARS values. Additionally, researchers pointed out that increasing the amount of HT extract added was further reduced the TBARS values. Şeker et al. (2016) stated that the radical-scavenging activity levels of cake samples increased proportionally with the ratio of GR pomace incorporation. Additionally, HT10 group showed similar TBARS values

with N156 and N100 groups, and lower TBARS values than N50 and N25 groups (P<0.05). In the samples stored under anaerobic condition during the storage period the highest (P<0.05) TBARS values were determined in the control. In the first 15 days period of storage the lowest (P<0.05) TBARS values were determined in the group containing 156 ppm nitrite. According to the TBARS measurements performed at the end of the storage. the lowest (P<0.05) TBARS values were determined in N156, N100 and HT10 groups. Whereas there was no significant difference between TBARS values of HT and GR groups in the first 10 days of storage, it was determined that TBARS values decreased at increasing levels of HT and GR concentrates on the 15^{th} and 30^{th} days of storage stored under anaerobic condition (P<0.05). At the end of the storage, HT10 group had lower TBARS values than BHT containing group (P<0.05). Similar results were reported by Ganhão et al. (2010b) for the raw pork burger patties. Researchers noted that HT fruits exhibited strong antioxidant activity against lipid oxidation. In addition, Shortle et al. (2014) indicated the HT extracts significantly decreased the level of lipid oxidation in bovine muscle homogenates. The TBARS values of samples stored in aerobic conditions were higher than those of stored in anaerobic conditions (P<0.05). It is reported that the moleculer oxygen is a pro-oxidative factor which accelerates the oxidation of lipids in many studies (Min and Ahn. 2005; Kang et al., 2014; Ahmed et al., 2016).

The CIE color results (data is not presented) demonstrated the highest L* values were determined in GR1 (76.74±0.64) and GR5 (76.88±0.64) groups in aerobic storage conditions (P<0.05). The lowest L* values (P<0.05) in the samples stored under the same conditions were obtained in N100 (72.52±0.64) groups. No significant differences were found between the L* values of HT and GR groups in both storage conditions. Ganhão et al. (2010a) reported that the addition of HT extracts in cooked pork burger patties had no effect on L* values. In present study, a significant decrease and increase (P<0.05)in L* values was determined on the 5^{th}(72.42±0.41) and 10^{th}(75.60±0.41)day of aerobic storage, respectively. No significant changes were determined after the 10^{th} day of storage. In the groups stored under anaerobic conditions, the highest (P<0.05) L* values were determined in HT1 (74.77±0.79) and HT5 (74.83±0.79) groups. The lowest L* values (P<0.05) were determined in BHT (70.77±0.79) containing groups. Whereas a significant decrease in L* values were observed on the 5^{th} day of anaerobic storage, no significant changes were observed during anaerobic storage after the 5^{th} day of storage. In the samples stored under aerobic condition, whereas the highest a* value was determined in GR10 (7.96±0.17) group, and the lowest a* values were determined in control (1.74±0.17) and HT1 (2.01±0.17) groups (P<0.05). The a* values of the N156 (7.08±0.17) and N100 (6.45±0.17) groups were lower than those obtained from the GR10 (7.96±0.17) group but they had higher a* values than all the other experimental groups (P<0.05).The addition of GR increased a* values more than the addition of HT in both storage conditions (P<0.05). The addition of GR increased a* values and this effect was further increased at increasing GR ratios in both storage conditions (P<0.05). Similar effect was not determined in groups with HT. GR fruit flesh and skin have a dark-red color, therefore it caused to increase the redness values of cooked turkey meat (Levent et al., 2008; Özrenk et al., 2011). No significant differences were found between the a* values of N50 (4.91±0.17), N25 (4.50±0.17) and GR5 (4.93±0.17) groups in aerobic storage condition. In addition, a* values of HT10 (3.08±0.17) and HT5 (2.50±0.17) groups were determined to be higher (P<0.05) than the control (1.71±0.17). Similarly, Ganhão et al. (2010b) reported that significantly higher a* values in raw pork patties containing HT berry extracts compared to control group, on day 12 of aerobic storage. Additionally, researchers claimed that the protecting the colour characteristics of HT berry extracts in raw pork meat were as a result of the inhibition of lipid oxidation (Ganhão et al.. 2010b). In general, it was determined that there was a decrease (P<0.05) in a* values with storage in both storage conditions. Similarly, it has been reported to decrease in a* values during storage (Ganhão et al. 2010b). In the groups stored under anaerobic condition, whereas the highest

a* value was determined in GR10 (9.50±0.17) group, the lowest a* values were determined in control (2.27±0.17) and BHT (1.69±0.17) groups (P<0.05). The highest a* value among the groups containing nitrite was determined in the N156 (7.69±0.17) group, whereas the lowest a* value was determined in the N25 (5.48±0.17) group in anaerobic storage condition (P<0.05). The GR10 group was higher a* values than all nitrite containing groups(P<0.05).It was found that GR5 (5.96±0.17) group had a* values similar to all nitrite containing groups except N156 (7.69±0.17) group. In both storage conditions the highest (P<0.05) b* values were determined in the HT10 group, whereas the lowest(P<0.05) b* values were also determined in all nitrite containing groups. The b* values obtained from HT10 and HT5 groups were higher (P<0.05) than the groups containing GR or nitrite. The use of HT in cooked turkey meat increased b* values and this increase was also increased with increasing HT concentrate levels in both storage conditions (P<0.05). In the samples with GR. a similar relationship was found between the samples containing only 1% and 10% GR concentrates (P<0.05). No significant differences were found between b* values of nitrite containing groups in both storage conditions. b* values of treatments stored under aerobic conditions increased (P<0.05) at 5th days of storage, whereas no significant changes were observed in the b* values of the treatments stored under anaerobic conditions during storage. In addition, L* and b* values of treatments stored in aerobic conditions were higher, whereas a* values were lower than compared to the treatments stored under anaerobic conditions (P<0.05).

The results of texture profile analysis of treatment groups stored under aerobic and anaerobic conditions are presented in Table 7 and Table 8, respectively. The results of TPA demonstrated the use of nitrite, BHT, HT and GR in cooked turkey meat did not cause a significant changes in the values of chewiness, springiness and adhesiveness in the samples stored under aerobic storage conditions. In addition, no significant differences were found between resilience, cohesiveness, springiness, gumminess, chewiness and adhesiveness values of all treatment groups stored under anaerobic storage conditions. There was no significant difference between the groups stored under anaerobic conditions in terms of hardness values at the beginning of storage. There were no changes in hardness values of all treatment groups stored under anaerobic conditions during storage. At the end of the anaerobic storage, hardness value of N156 group was found to be higher than the value of HT5 group (P<0.05). In the samples stored under aerobic condition, whereas the highest resilience value was determined in GR5 group, the lowest resilience value was determined in N100 group at the beginning of storage (P<0.05). There were no significant changes in resilience and cohesiveness (except for control and GR5 groups) and hardness (except forGR5 group) values in all treatment groups during storage in aerobic storage conditions. Ganhão et al. (2010a) stated that the hardness and chewiness values increased in burger patties containing HT extracts during the 12 days of storage. In present study, on the 15th day of storage, there was a significant decrease in resilience and cohesiveness values of control and GR5 groups, and significant increase in hardness value of GR5 group (P<0.05). Ganhão et al. (2010a) reported the addition of HT extracts significantly increased the hardness of cooked burger patties. The highest hardness value was determined in BHT group, whereas the lowest hardness value was determined in GR5 group at the beginning of aerobic storage (P<0.05). At the end of the aerobic storage, the N156 group was higher hardness value than those of HT10 group (P<0.05). Furthermore, at the beginning of aerobic storage, the highest gumminess value was determined in HT5 group, whereas the lowest gumminess values were determined in GR5 and GR10 groups (P<0.05). There were no significant differences between the groups in terms of both gumminess and cohesiveness values at the end of the aerobic storage.

Table 7. Results of Texture Profile Analysis of treatment groups stored under aerobic condition at 30 days storage

	Hardness (N)			Adhesiveness (mJ)			Resilience			Cohesiveness		
Groups	0	15	30	0	15	30	0	15	30	0	15	30
Control	2.38^{de} ±0.19	4.02^{a-d} ±0.00	3.65^{a-e} ±0.69	1.31^{a} ±0.07	0.75^{a} ±0.21	1.10^{a} ±0.21	0.23^{ab} ±0.02	0.06^{d} ±0.01	0.08^{cd} ±0.02	0.81^{a} ±0.12	0.47^{cde} ±0.01	0.45^{cde} ±0.08
BHT	4.54^{abc} ±0.01	3.88^{a-e} ±0.81	4.22^{a-d} ±0.86	0.45^{a} ±0.07	1.00^{a} ±0.14	1.10^{a} ±0.17	0.11^{cd} ±0.01	0.06^{d} ±0.01	0.08^{cd} ±0.03	0.54^{b-e} ±0.08	0.43^{de} ±0.03	0.49^{b-e} ±0.07
N156	3.87^{a-e} ±0.16	3.49^{a-e} ±0.32	4.92^{a} ±0.32	0.65^{a} ±0.11	1.15^{a} ±0.21	1.25^{a} ±0.21	0.09^{cd} ±0.02	0.07^{d} ±0.01	0.07^{cd} ±0.00	0.49^{b-e} ±0.01	0.47^{cde} ±0.02	0.47^{cde} ±0.01
N100	4.05^{a-d} ±0.21	3.80^{a-e} ±0.37	4.62^{abc} ±0.35	0.95^{a} ±0.07	0.80^{a} ±0.00	1.35^{a} ±0.24	0.05^{d} ±0.00	0.07^{d} ±0.01	0.10^{cd} ±0.01	0.41^{e} ±0.03	0.42^{de} ±0.00	0.49^{b-e} ±0.01
N50	3.00^{a-e} ±0.25	4.33^{a-d} ±0.49	4.19^{a-d} ±0.10	0.40^{a} ±0.12	1.15^{a} ±0.07	1.10^{a} ±0.00	0.16^{bc} ±0.03	0.08^{cd} ±0.02	0.09^{cd} ±0.01	0.64^{a-d} ±0.04	0.46^{c-e} ±0.08	0.46^{c-e} ±0.05
N25	3.03^{a-e} ±0.01	3.61^{a-e} ±0.00	4.17^{a-d} ±0.62	0.15^{a} ±0.07	0.40^{a} ±0.00	1.05^{a} ±0.07	0.13^{cd} ±0.04	0.09^{cd} ±0.00	0.09^{cd} ±0.01	0.55^{b-e} ±0.10	0.56^{b-e} ±0.00	0.51^{b-e} ±0.05
HT1	3.72^{a-e} ±0.62	4.32^{a-d} ±0.22	4.56^{abc} ±0.01	1.30^{a} ±0.11	1.00^{a} ±0.00	0.95^{a} ±0.07	0.11^{cd} ±0.03	0.07^{d} ±0.02	0.10^{cd} ±0.01	0.53^{b-e} ±0.02	0.48^{b-e} ±0.01	0.47^{cde} ±0.03
HT5	4.45^{abc} ±0.56	3.77^{a-e} ±0.64	4.13^{a-d} ±0.04	0.35^{a} ±0.01	1.90^{a} ±0.14	1.40^{a} ±0.14	0.12^{cd} ±0.01	0.06^{d} ±0.01	0.07^{cd} ±0.03	0.60^{a-e} ±0.01	0.43^{de} ±0.08	0.45^{cde} ±0.06
HT10	3.45^{a-e} ±0.37	4.21^{a-d} ±0.18	2.87^{b-e} ±0.19	0.45^{a} ±0.09	1.20^{a} ±0.14	0.90^{a} ±0.18	0.12^{cd} ±0.02	0.08^{cd} ±0.03	0.06^{d} ±0.01	0.49^{b-e} ±0.08	0.43^{de} ±0.02	0.44^{cde} ±0.01
GR1	3.94^{a-d} ±0.78	4.77^{ab} ±0.33	4.51^{abc} ±0.37	0.75^{a} ±0.21	1.30^{a} ±0.14	1.10^{a} ±0.28	0.08^{cd} ±0.01	0.08^{cd} ±0.00	0.06^{d} ±0.01	0.66^{abc} ±0.08	0.48^{b-e} ±0.02	0.46^{c-e} ±0.01
GR5	1.93^{e} ±0.25	4.16^{a-d} ±0.20	4.14^{a-d} ±0.03	0.20^{a} ±0.04	1.45^{a} ±0.21	1.30^{a} ±0.14	0.27^{a} ±0.00	0.08^{cd} ±0.03	0.07^{d} ±0.02	0.70^{ab} ±0.01	0.55^{b-e} ±0.06	0.42^{de} ±0.05
GR10	2.79^{cde} ±0.16	3.08^{a-e} ±0.58	3.13^{a-e} ±0.04	0.25^{a} ±0.01	0.85^{a} ±0.14	0.85^{a} ±0.04	0.09^{cd} ±0.01	0.09^{cd} ±0.03	0.09^{cd} ±0.01	0.46^{cde} ±0.00	0.50^{b-e} ±0.11	0.47^{cde} ±0.04

	Springiness			Gumminess (N)			Chewiness (N)		
	0	15	30	0	15	30	0	15	30
Control	0.90^{a} ±0.11	0.91^{a} ±0.07	1.49^{a} ±0.21	1.92^{abc} ±0.44	1.90^{abc} ±0.05	1.61^{abc} ±0.03	1.74^{a} ±0.39	1.73^{a} ±0.18	2.41^{a} ±0.34
BHT	1.00^{a} ±0.20	0.91^{a} ±0.00	0.92^{a} ±0.02	2.45^{ab} ±0.41	1.66^{abc} ±0.25	2.02^{abc} ±0.11	2.49^{a} ±0.51	1.52^{a} ±0.23	1.85^{a} ±0.06
N156	0.87^{a} ±0.04	0.88^{a} ±0.04	0.93^{a} ±0.01	1.88^{abc} ±0.11	1.61^{abc} ±0.07	2.32^{abc} ±0.08	1.63^{a} ±0.17	1.42^{a} ±0.13	2.16^{a} ±0.05
N100	0.87^{a} ±0.01	1.65^{a} ±0.05	0.91^{a} ±0.02	1.66^{abc} ±0.04	1.59^{abc} ±0.14	2.27^{abc} ±0.09	1.44^{a} 0.01	2.70^{a} ±0.91	2.06^{a} ±0.13
N50	0.84^{a} ±0.01	1.68^{a} ±0.11	0.87^{a} ±0.01	1.91^{abc} ±0.26	1.96^{abc} ±0.09	1.91^{abc} ±0.25	1.60^{a} ±0.18	3.22^{a} ±0.71	1.66^{a} ±0.18
N25	0.78^{a} ±0.04	0.94^{a} ±0.00	0.88^{a} ±0.04	1.67^{abc} ±0.30	2.02^{abc} ±0.00	2.13^{abc} ±0.50	1.29^{a} ±0.18	1.90^{a} ±0.00	1.88^{a} ±0.22
HT1	0.85^{a} ±0.01	0.93^{a} ±0.01	0.89^{a} ±0.04	1.96^{abc} ±0.25	2.05^{abc} ±0.16	2.15^{abc} ±0.14	1.66^{a} ±0.23	1.91^{a} ±0.12	1.91^{a} ±0.03
HT5	0.90^{a} ±0.06	0.87^{a} ±0.06	0.92^{a} ±0.01	2.69^{a} ±0.48	1.60^{abc} ±0.03	1.87^{abc} ±0.25	2.44^{a} ±0.15	1.39^{a} ±0.13	1.72^{a} ±0.21
HT10	0.86^{a} ±0.01	0.91^{a} ±0.02	0.90^{a} ±0.01	1.70^{abc} ±0.47	1.79^{abc} ±0.14	1.25^{c} ±0.11	1.46^{a} ±0.39	1.62^{a} ±0.09	1.12^{a} ±0.11
GR1	0.96^{a} ±0.10	0.92^{a} ±0.04	0.91^{a} ±0.01	2.55^{ab} ±0.18	2.27^{abc} ±0.06	2.05^{abc} ±0.14	2.44^{a} ±0.08	2.08^{a} ±0.02	1.87^{a} ±0.11
GR5	0.79^{a} ±0.01	0.96^{a} ±0.04	0.90^{a} ±0.01	1.34^{c} ±0.20	2.28^{abc} ±0.12	1.72^{abc} ±0.17	1.06^{a} ±0.13	2.18^{a} ±0.19	1.54^{a} ±0.14
GR10	0.80^{a} ±0.01	0.88^{a} ±0.08	0.87^{a} ±0.01	1.28^{c} ±0.06	1.51^{bc} ±0.06	1.47^{bc} ±0.12	1.02^{a} ±0.06	1.32^{a} ±0.07	1.27^{a} ±0.11

Means ± standard deviation (SD)

[a-h]Values superscripted with different letters for each textural property are significantly different ($P<0.05$)

Table 8. Results of Texture Profile Analysis of treatment groups stored under anaerobic condition at 30 days storage

	Hardness (N)			Adhesiveness (mJ)			Resilience			Cohesiveness		
Groups	0	15	30	0	15	30	0	15	30	0	15	30
Control	2.54^{h} ±0.37	4.61^{a-h} ±0.39	5.20^{a-g} ±0.08	0.15^{b} ±0.01	1.55^{ab} ±0.21	1.30^{ab} ±0.25	0.15^{a} ±0.01	0.06^{a} ±0.00	0.09^{a} ±0.02	0.76^{a} ±0.04	0.49^{a} ±0.01	0.52^{a} ±0.04
BHT	4.19^{a-h} ±0.34	5.45^{a-f} ±0.17	5.25^{a-f} ±0.04	0.65^{ab} ±0.07	0.95^{ab} ±0.29	1.45^{ab} ±0.28	0.13^{a} ±0.01	0.12^{a} ±0.05	0.08^{a} ±0.04	0.59^{a} ±0.05	0.56^{a} ±0.12	0.47^{a} ±0.08
N156	4.40^{a-h} ±0.28	5.76^{a-d} ±0.49	6.22^{a} ±0.22	0.55^{ab} ±0.21	1.90^{ab} ±0.14	2.65^{a} ±0.35	0.11^{a} ±0.01	0.07^{a} ±0.02	0.06^{a} ±0.00	0.50^{a} ±0.02	0.54^{a} ±0.05	0.43^{a} ±0.01
N100	4.19^{a-h} ±0.29	5.68^{a-d} ±0.12	6.01^{ab} ±0.01	0.95^{ab} ±0.07	1.05^{ab} ±0.28	0.95^{ab} ±0.35	0.08^{a} ±0.01	0.11^{a} ±0.06	0.10^{a} ±0.04	0.50^{a} ±0.07	0.51^{a} ±0.08	0.51^{a} ±0.08
N50	4.48^{a-h} ±0.54	3.99^{a-h} ±0.01	4.42^{a-h} ±0.25	0.60^{ab} ±0.14	1.25^{ab} ±0.35	1.10^{ab} ±0.14	0.13^{a} ±0.01	0.07^{a} ±0.01	0.07^{a} ±0.03	0.56^{a} ±0.07	0.49^{a} ±0.07	0.45^{a} ±0.08
N25	4.14^{a-h} ±0.03	5.80^{abc} ±0.30	5.55^{a-e} ±0.03	0.55^{ab} ±0.11	2.65^{a} ±0.07	1.10^{ab} ±0.25	0.13^{a} ±0.03	0.09^{a} ±0.01	0.09^{a} ±0.05	0.62^{a} ±0.08	0.55^{a} ±0.01	0.49^{a} ±0.05
HT1	4.01^{a-h} ±0.29	3.98^{a-h} ±0.08	4.51^{a-h} ±0.47	1.05^{ab} ±0.24	1.70^{ab} ±0.28	1.60^{ab} ±0.37	0.13^{a} ±0.02	0.06^{a} ±0.01	0.06^{a} ±0.02	0.46^{a} ±0.03	0.79^{a} ±0.12	0.41^{a} ±0.04
HT5	3.51^{c-h} ±0.41	3.26^{e-h} ±0.13	3.79^{b-h} ±0.30	0.45^{ab} ±0.07	0.85^{ab} ±0.07	1.25^{ab} ±0.07	0.11^{a} ±0.02	0.08^{a} ±0.01	0.06^{a} ±0.02	0.53^{a} ±0.04	0.50^{a} ±0.05	0.46^{a} ±0.01
HT10	3.44^{d-h} ±0.01	3.14^{fgh} ±0.22	3.83^{b-h} ±0.16	0.45^{ab} ±0.11	0.90^{ab} ±0.17	1.75^{ab} ±0.49	0.09^{a} ±0.01	0.11^{a} ±0.01	0.07^{a} ±0.02	0.54^{a} ±0.01	0.59^{a} ±0.08	0.56^{a} ±0.16
GR1	5.32^{a-f} ±0.54	5.17^{a-g} ±0.74	5.83^{abc} ±0.47	1.50^{ab} ±0.28	1.60^{ab} ±0.42	1.40^{ab} ±0.14	0.06^{a} ±0.01	0.08^{a} ±0.01	0.08^{a} ±0.01	0.52^{a} ±0.08	0.48^{a} ±0.01	0.49^{a} ±0.06
GR5	3.16^{fgh} ±0.50	5.13^{a-g} ±0.45	4.81^{a-h} ±0.70	0.10^{b} ±0.14	1.25^{ab} ±0.07	1.30^{ab} ±0.42	0.14^{a} ±0.04	0.10^{a} ±0.00	0.09^{a} ±0.02	0.55^{a} ±0.04	0.51^{a} ±0.01	0.50^{a} ±0.04
GR10	2.89^{gh} ±0.50	5.05^{a-g} ±0.68	4.08^{a-h} ±0.11	0.50^{ab} ±0.14	1.95^{ab} ±0.33	1.35^{ab} ±0.07	0.10^{a} ±0.01	0.11^{a} ±0.02	0.06^{a} ±0.01	0.50^{a} ±0.00	0.59^{a} ±0.13	0.47^{a} ±0.14

	Springiness			Gumminess (N)			Chewiness (N)		
	0	15	30	0	15	30	0	15	30
Control	0.85^{a} ±0.01	0.90^{a} ±0.06	0.92^{a} ±0.00	1.92^{a} ±0.19	2.24^{a} ±0.25	2.67^{a} ±0.21	1.62^{a} ±0.14	2.01^{a} ±0.10	2.46^{a} ±0.19
BHT	0.90^{a} ±0.06	0.91^{a} ±0.06	0.90^{a} ±0.01	2.45^{a} ±0.11	3.03^{a} ±0.32	2.46^{a} ±0.43	2.19^{a} ±0.06	2.72^{a} ±0.46	2.21^{a} ±0.36
N156	0.89^{a} ±0.01	0.94^{a} ±0.03	0.94^{a} ±0.02	2.18^{a} ±0.23	3.08^{a} ±0.04	2.69^{a} ±0.21	1.92^{a} ±0.18	2.90^{a} ±0.12	2.52^{a} ±0.25
N100	1.50^{a} ±0.22	0.90^{a} ±0.01	1.29^{a} ±0.40	2.12^{a} ±0.43	2.92^{a} ±0.05	3.04^{a} ±0.50	3.35^{a} ±0.38	2.62^{a} ±0.91	4.03^{a} ±0.17
N50	0.84^{a} ±0.04	0.94^{a} ±0.04	0.87^{a} ±0.01	2.49^{a} ±0.02	1.95^{a} ±0.28	2.00^{a} ±0.47	2.09^{a} ±0.09	1.83^{a} ±0.33	1.75^{a} ±0.43
N25	0.87^{a} ±0.02	0.97^{a} ±0.01	0.91^{a} ±0.02	2.57^{a} ±0.36	3.06^{a} ±0.06	2.70^{a} ±0.28	2.22^{a} ±0.37	3.01^{a} ±0.04	2.44^{a} ±0.20
HT1	0.86^{a} ±0.01	1.00^{a} ±0.10	0.85^{a} ±0.06	1.85^{a} ±0.57	3.10^{a} ±0.58	1.81^{a} ±0.02	1.59^{a} ±0.46	3.18^{a} ±0.89	1.54^{a} ±0.12
HT5	0.89^{a} ±0.03	0.90^{a} ±0.01	1.18^{a} ±0.33	1.87^{a} ±0.48	1.60^{a} ±0.23	1.75^{a} ±0.08	1.67^{a} ±0.37	1.44^{a} ±0.18	2.07^{a} ±0.26
HT10	0.83^{a} ±0.05	0.90^{a} ±0.02	0.92^{a} ±0.04	1.84^{a} ±0.03	1.79^{a} ±0.44	2.13^{a} ±0.51	1.52^{a} ±0.11	1.60^{a} ±0.35	1.96^{a} ±0.32
GR1	0.96^{a} ±0.00	0.96^{a} ±0.04	0.94^{a} ±0.04	2.71^{a} ±0.02	2.45a ±0.29	2.86^{a} ±0.08	2.60^{a} ±0.03	2.34^{a} ±0.19	2.68^{a} ±0.18
GR5	0.79^{a} ±0.04	0.90^{a} ±0.05	0.91^{a} ±0.01	1.72^{a} ±0.13	2.60^{a} ±0.28	2.39^{a} ±0.52	1.36^{a} ±0.18	2.33^{a} ±0.38	2.17^{a} ±0.45
GR10	0.87^{a} ±0.01	0.92^{a} ±0.06	0.90^{a} ±0.02	1.45^{a} ±0.25	2.86^{a} ±0.33	1.92^{a} ±0.51	1.25^{a} ±0.23	2.61^{a} ±0.12	1.72^{a} ±0.42

Means ± standard deviation (SD)

[a-h]Values superscripted with different letters for each textural property are significantly different ($P<0.05$)

CONCLUSIONS

Results of the present study indicated that the addition of the GR or HT concentrates to ground turkey meat effectively delayed the reactions of lipid oxidation. The pH, color and textural properties of cooked ground turkey meat were not negatively affected by the use of GR or HT concentrates.

Overall results suggested that the use of GR or HT concentrates (especially 10%) to reduce lipid oxidation and the amount of added nitrite in poultry meat products can be an effective strategy for improving the color properties and shelf-life of poultry meat.

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THE TRACEABILITY OF FOOD PRODUCTS IN RELATION WITH FOOD INTEGRITY – A REVIEW

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Abstract

Traceability expresses the ability to detect and track raw materials, food products of animal or plant origin, a food-producing animal, or a substance intended to be embedded or expected to be incorporated into a food product, throughout all stages of production, processing and distribution. A traceability system in practice involves systematically and continuously completing and keeping records that can be uniquely identified for each batch unit and the information required at each stage of the food chain (up to consumption). For agri-food products, traceability makes a link between raw materials, their origin, processing, distribution and location after marketing. Food traceability must in principle aim at two objectives: the first one is to provide information to product use and the second one, to contribute to the safety of the food, allowing, as appropriate, withdrawal of non-conforming batches and recall of the product. The way the food goes through "from farm to fork" is called the food chain. The food chain has several links: farmers producing raw material, food processors, distributors and consumers. One of the benefits of traceability is the implementation of food contamination monitoring programs. Traceability facilitates the identification of key products in a particular food chain where sampling of products is required, to monitor the concentration of chemical, microbiological and biological contaminants.

Key words*: food traceability, food integrity, food chain, food consumer.*

INTRODUCTION

The paper presents the analysis of the literature on the concept of traceability, due to the fact that in recent years traceability issues have become recognized as an essential tool for guaranteeing food safety and food quality.

Traceability issues have become recognized as an essential tool for ensuring food safety and quality. Food integrity is another big challenge of nowadays. More and more consumers are interested in how their food is grown, processed and brought to their tables.

Traceability is the procedure that allows competent authorities to ensure and verify that a particular product is in compliance with legal provisions and regulations on food safety as well as quality requirements that are explicitly stated on the label.

A high level of public health protection is one of the fundamental objectives of the food law established by Regulation (EC) No 178/2002 of the European Parliament and of the Council of 23 February 2002 defining traceability as a traceability of food, feed, food - livestock or animal production are substance intended to be, or expected to be, incorporated into a food or feed for animals during all stages of production, processing and distribution. A traceability system implies, in practice, the completion and keeping of systematic and continuous recordings which can be uniquely identified for each tracking unit ("batch"), as well as the information required at each stage along the food chain, up to consumption.

GENERAL PRINCIPLES GOVERNING FOOD SAFETY

- Approach from farm to fork are the protection of health and life of humans starts to protect the power sources animals, plants and the environment.
- The consumer's right to safe food from the point of view of health and correct information as to the origin of the food and technological processes, which has been produced or processed.

This regulation not only sets out the principles of food safety but introduces the concept of "traceability". In other words, you have to make sure that all foods, feeds and ingredients can be traced through feed, from farm to fork.
However, you need to make sure that all foods, feeds and fertilizers can be obtained, whether produced, processed or imported, along the food chain. Each operator must be able to clearly identify suppliers for each raw material or material supplied by them but also to those who supply their products.
Important principles of food law are the following:

- Safety;
- Fairness,
- Responsibility;
- Transparency;
- Traceability;
- Withdrawal;
- Collaboration;
- Precautions;
- Flexibility;
- Objectivity;
- Privacy Policy.

The accuracy of the system will directly depend on the type of product and the characteristics of the production system, but also the objectives related to the traceability are important.
For example, in the field of food processing industry, traceability has become a mandatory requirement imposed by the European Union. Without the existence of solutions capable to store all the information relating to the raw materials entering into the composition of certain foodstuffs, in situations where problems arise manufacturer will have to withdraw the entire production on the market (European Union, 2002).

Figure 1. The way the food goes through to reach the final consumer's (http://www.agriculturae.ro)

Figure 1 shows the way the food goes through to reach the final consumer's tables.
Thus, only one negligence can lead to their contamination or alteration and may endanger the health of consumers. In order to be in good condition, manufacturers and distributors must always keep good manufacturing, storage, transport and distribution practices.

OBJECTIVES OF THE TRACEABILITY

The main objectives:
a) to support the objectives of safety and/or quality of food;
b) to meet the specification/specifications of the contracting authority;
c) to establish the history or origin of the product;
d) to facilitate the withdrawal and/or the recall of products;
e) to identify the organizations responsible along the food chaines;
f) to facilitate the verification of specific information concerning the product;
g) to communicate relevant information to stakeholders;
h) to fulfill any rules or local, regional, national or international policies, as the case may be;
i) to improve the efficiency, productivity, and profitability of the organization.
There is the basis for traceability both in the past and in the future, which together constitute an integrated traceability system for the agri-food chain.
The traceability of a product determines the physical location of that product, at any level along the food chain, in order to facilitate the management of the logistic, recalling the product and the dissemination of information to the consumer or other interested parties.
The food chain has several steps:
- farmers producing the raw material;
- the food producing factories (processors) that produce the end product;
- distributors which sells them on the market;
- consumers that buy the end products (Customer property, 2018).

Usually, the way we choose agri-food products is quite complicated, thus, negligence can lead to food contamination or modification, endangering consumer health.
In order to have good quality agri-food products, manufacturers and distributors must always keep good practice for production, storage, transport and distribution according to the legislation in force (Wilson and Clarke,

1998; Opara, 2003; Souza-Monteiro and Caswell, 2004).
To protect consumers, it is very important to continuously monitor how you go through each stage of the production or processing of agri-food products, a process that is called traceability (Matzembacher, 2018).

TYPES OF TRACEABILITY WITHIN A CHAIN OF PRODUCTION

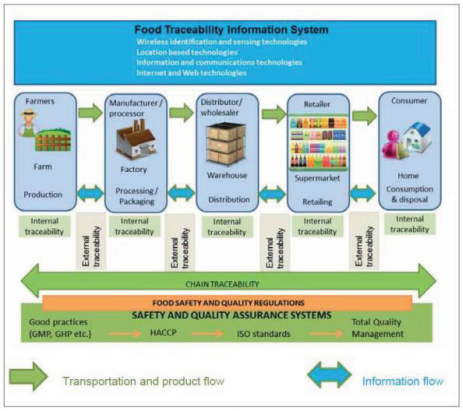
Internal traceability - is information that allows tracking of the product within an enterprise, and one or more raw materials and consumables that are subject to internal processing are received. Internal processing involves displacement, processing, storage, destruction;

External Traceability - is the information the company receives or provides to the other members of the food chain in respect of a particular product;
The traceability of the agri-food chain goes to the stages of the chain that accompanies the products from an agri-food point to another point so that its traceability can be done at all stages of production, processing and distribution.

STRUCTURE OF THE TRACEABILITY SYSTEM

Traceability must allow detection of the raw material or final product, identifying it along the production chain and providing information regardless of time and place on the technological flow.
Food security is enhanced by traceability in several ways:
- the exclusion from slaughter of diseased or suspected contagious animals;
- fraud control.
Traceability along with periodic audits can prevent fraud with regard to the origin of the products, the species of organisms used, the production method or the raw materials used.
- Promotion of marks, leading to consumer confidence and loyalty toward the good/service provided by the manufacturer, guaranteeing the originality of goods and/or services for which the mark has been created.
- Carrying out monitoring programs of the contaminants supply: traceability facilitates the identification of the key products from a particular food chain, where sampling is necessary in order to monitor the concentration of contaminants, such as chemical, microbiological and biological agents. (ANSVSA, Accesed in 2018).

THE TRACEABILITY IN PRACTICE



Source: Traceability in a food supply chain: Safety and quality perspectives Myo Min Aung, Yoon Seok Chang
Figure 2. Internal and external traceability

The traceability system is a system of type "guardian", whose activity is represented by the collection/storage of data, records, the monitoring of inputs and outputs etc.
Traceability will be essential only in the event of a crisis (when any program of self-regulation fails) or when there are non-compliant products on the market. During the crisis, the following aspects should be identified:
• Which product is involved?
• Quantities?
• The location of the product?
• How many consumers may be affected?

TRACEABILITY ON THE FOOD CHAIN

Traceability is a concept developed to be used in the production of food, being a key element of transparency. Associated with a flow of information, traceability represents a physical process, which consists in the pursuit of the food production in space and time (Iorga, 2016).
Traceability allows identification of a product along its path from raw material to consumers plate through identification and tracking with

supporting documents (Buhr, 2003; Gibbons, 2005; Skilton and Robinson, 2009).

For the consumer, traceability offers information about:

• which is the origin of the food (e.g. where an animal has been raisedor where vegetables and fruit shave been cultivated);

• when the raw material (meat, milk, fruits, vegetables) has been processed;

• what organizations have been involved in the processing and distribution of food.

For wholesale distributor, traceability offers information about:

• when new batches of products should be expected and the maximum capacity of distribution;

• for transport, storage requirements.

The traceability of food products should cover in principle two objectives:

• to provide information to users of the product;

• to help ensure the safety of the food product, enabling, as the case may be, the withdrawal of non-compliant batches and the recall of the product.

The structure of the traceability system depends on the characteristics of the technological process and is characterized by:

- width - describes the amount of information collected;
- depth - it refers to the distance of the cover system in terms of food safety depending on the time / stage of the food where the risk of contamination may occur;
- accuracy - reflects the degree to which the system of traceability can highlight a specific point of the trajectory of the culinary preparation, and its features.

Thus, the traceability means the ability of documentary evidence of all elements relevant to the product safety and quality - handling, process, control.

In conclusion, traceability is the main element in the responsibility of producers, farmers, operators and those directly involved in risk assessment and management across the food chain.

For the food industry, traceability is very important, because some recordings are essential from an ethical and legal framework point of view for both producers and consumers (Saak, 2016).

CONCLUSIONS

Traceability is advantageous from the following points of view:

Animal health protection - the task of the protection of animal health rests mainly at the farmer who has got all the interest to keep the animals in a very good health status for economic losses.

The control of the diseases of animals and birds by the fact that allows you to find the immediate traceability of the source from where come on the one hand, and on the other hand there is a check on all the processing chain links, which makes to exclude animal diseases to man.

The protection of the safety of man, is enhanced by the traceability system traceability multiple reasons: exclusion from the cut for public consumption of animals that are ill or suspected of contagious diseases haemopoletic-and the placing on the market of meat products and by-products obtained.

Control of fraud, the traceability together with the periodic audits of the records can prevent fraud with regard to the origin of the products, the species of organisms used to produce a product and veracity statements concerning the method of production, raw materials or products.

The facility allows the withdrawal, traceability and control measures for the prevention or reduction of the hazard identified on the basis of traceability both backwards and forwards in the situation in which the incident which put in danger the safety of consumers.

Promotion of marks, leading to the formation of consumer confidence in the loyalty toward the good/service provided by the manufacturer, guaranteeing the originality of goods and/or services for which the mark has been created.

Carrying out monitoring programs of the contaminants supply: traceability facilitates the identification of the products key from a particular food chain which is necessary for the sampling of the products in order to monitor the concentration of contaminated chemical, microbiological and biological agents.

The assessment of the risks arising from exposure to food: can easily be demonstrated by the correlation of information from the records carried out within the framework of the system of traceability.

Let's not forget:
The value of our money must be in the quality of the food we buy. Especially that our health is in the middle.

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*** ANSVSA (www.ansvsa.ro) Accesed in 2018

***) 7.5.4 Customer property (http://www.open-mind-solutions.com/Implementation/ISO_9001_CustomerProp.)

***) Regulation (EC) No. 178/2002 of the European Parliament and of the Council, Accesed in 2019 (https://eur-lex.europa.eu)

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IDENTIFICATION OF AMINOACID PROFILE AND PROXIMATE COMPOSITION OF THREE TROUT BREEDS REARED IN THE NORTH EASTERN REGION OF ROMANIA

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Abstract

Trout meat has sensory features and high nutritional value. The aim of the present study was to identify differences in the proximate composition, amino acid composition of three breeds (brown, brook and rainbow trout) reared under the same conditions in north eastern region of Romania. Chemical samples composition were determined using Association of Official Analytical Chemists (AOAC) methods. The moisture content recorded oscillated between 72.98 and 76.14 g, protein content of trout meat ranged between 17.37 and 19.31 g, the determined lipid content was between 4.41 and 5.82 g, and measured ash was between 1.11 and 1.21gfrom trout's flesh. The amount of essential aminoacids ranged between 26.49 and 28.08%. Among amino acids, the glutamic acid, aspartic acid, arginine, leucine and lysine were predominant. The measured essential to nonessential (E/NS) ratio for the trout flesh ranged between 71.73 and 75.87%. The present study demonstrate that trout meat is a highly source of protein and contains essential amino acids for promoting good health, prevention and healing of diseases in humans.

Key words*: proximate composition, amino acid, meat, trout.*

INTRODUCTION

Fish meat has outstanding sensory features and high nutritional value. In addition, fish meat also has a strong quantitative valence and plays an important role in ensuring daily protein requirements (Pariser and Wallerstein, 1980; Novikov et al., 1997; Tocher et al., 2003; Segal, 2002; Osibona et al., 2009; Sabetian et al., 2012).

The quality of fish protein depends on their digestibility and the content of essential amino acids such as lysine, methionine, leucine (Tuan et al., 1999; Oliva-Teles, 2000; Usydus et al., 2009; Robbins et al., 2010; Sabetian et al., 2012; Sarma et al., 2015).

Certain amino acids such as aspartic acid, glutamic acid and glycine are known for the important role they play in the process of wound healing. Amino acids, not only have high nutritional value, they also offer many benefits to human health such as reducing blood cholesterol and coronary heart disease (Gibbs et al., 2004).

Saito et al. (2003) finds that certain amino acids, such as tyrosine, methionine, histidine, lysine and tryptophan, have antioxidant action in the human body. Additionally, Kim et al. (1999) mentions that aspartic acid, glutamine, proline, glycine and leucine have strong cytotoxic activity against cancer cells, while Jones et al. (1999) notes the particular importance of glutamine in the proper functioning of many systems in the human body.

According to the literature, chemical composition of trout meat and the amino acids level is influenced by a number of factors such as breed, age, nutrition, fishing season, environment, salinity and water temperature (Shirai et al., 2002; Solvik and Rustad, 2005; Ionescu et al., 2006, Toppe et al., 2007; Erdem et al., 2009, Sabetian et al., 2012, Kaya et al., 2014).

Especially regarding brook trout, data from local and foreign literature consulted are less conclusive in terms of physical-chemical composition and amino acid profile, so our research represents a novelty for Romanian

literature and aims to bring new information to enrich it.
The aim of the present study was to identify differences in the proximate composition, amino acid composition of three breeds (brown, brook and rainbow trout) reared under the same conditions in north eastern region of Romania.

MATERIALS AND METHODS

Biological material was represented by 30 individuals of brook, rainbow and brown trout of both sexes, weighing about 250 grams, reared under the same condition in a trout farm from Suceava County. To achieve the proposed goals, from the biological material which was studied during 2017-2018, were made up three experimental batches, each of 10 individuals per batch, for the three studied breeds.
The fish samples were kept on ice in isothermal box, until they arrived to the laboratory and immediately frozen and stored at -21ºC until analyses.
To determine the physical-chemical composition of trout meat were gathered samples from side musculature of fishes from all three batches. All the analyses were conducted in duplicate replicate.
Determination of water and dry matter was realized through the method of drying in oven, which is the most used indirect method and suppose the drying of sample in oven at +105°C, till reaching a constant weight, in according to SR ISO 1442:2010. For this purpose, were used the Sartorius analytical balance (Gottingen, Germany) and the ECOCELL Blueline Comfort drying oven (Neuremberg, Germany).
Protein determination consists in decomposing the analyzed sample by heating with sulfuric acid in the presence of catalysts to reduce organic nitrogen to ammonium ions which can be determined by distillation / titration.The equipment used is Kjeltec Auto 2300 - Tecator, Sweden, which is a semi-automatic version of the crude protein Kjeldahl determination.
Determination of lipids content was realized using Soxhlet method, which consists in fat extraction from the analyzed sample using petrol ether using Velp Scientifica - SER 148 device (method specified by the manufacturer, AOAC Official methods o analysis 2005 and compatible with SR ISO 1443:2008.
Ash was determined by calcinations at 550ºC in calcinations oven according to SR ISO 936:2009.
Quantitative determination of amino acids was carried out in two stages in agreement with SR EN ISO 13903/2005 and AOAC Official Methods of Analysis (2005). The first stage was the hydrolysis of the peptide bonds existing between the constituent amino acids of the sample protein. In the case of determination of sulphur amino acids, the hydrolysis step is preceded by oxidation. The second stage was the determination (dosing) of the amino acids released following hydrolysis of the peptide bonds. In turn, this stage requires several steps: the individual separation of the amino acids from the existing mixture in the protein hydrolyzed, their detection and quantification.
Derivatization of pre-column sample was performed with OPA (ortho phthaldehyde), AMP (mercaptopropionic acid), FMOC (9-fluorenyl methyl chloroformate).
Amino acids were separated by high performance liquid chromatography using the HPLC Surveyor Plus Thermo Electron chromatographic system on a reversed-phase Hypersil BDS C18 (Thermo Electron) column, the reading being in the ultra-violet (338 nm). A Hypersil BDS (Base Deactivated Silica) C18 column with silica gel, with dimensions of 4.6 mm and a particle size of 5 μm. A gradient elution method is used, the chromatographic conditions being the following flow rate: 1 mL/min; injected volume: 25 μL; wavelength to be read: 336 nm; column temperature: 25°C.
Cysteine is determined as cystic acid in the hydrolysis of oxidized samples, but is calculated as cysteine using its molar mass. Also, methionine is determined as methionine sulfone from the oxidized and hydrolyzed samples, but is converted to methionine by the use of methionine moles.
The software used for statistical analysis was SPSS. We calculated the average, standard deviation, coefficient of variation and statistical significance of differences between samples.

RESULTS AND DISCUSSIONS

The chemical composition of trout meat, in addition to the genetic factors, is also influenced by environmental factors such as water quality, its pH and temperature, oxygen

content, technological factors, feeding, type of food used, season of the year, age and size of the fish (Fauconneau et al., 1993, 1995; Buchtova et al., 2007; Menoyo et al., 2007; Fallah et al., 2011; Vranić et al., 2011; Sabetian et al.; Kaya et al., 2014; Sirakov, 2015; Wang and Hun, 2017).

Water content of fillet (side muscles) obtained from the studied trout breeds had close values (Table 1) ranking between 72.98 g for brook trout samples and 76.14 g for brown trout samples, values which fall within the limits from literature (Fauconneau et al., 1993b; Corser et al., 1999; Plavša et al., 2000; Savić et al., 2004; García-Macíaset al., 2004; Bud and Mireşan, 2008; Celik et al., 2008; Alçiçek et al., 2010; Dinović et al., 2011; Fallah et al., 2011; Mocanuet al., 2012; Vranićet al., 2010, 2011; Sabetian et al., 2012; Kaya et al., 2014; Sirakov, 2015; Wang and Hun, 2017).

Table 1. Proximate composition of brook, rainbow and brown trout meat (g/100 g)

Specification		*Brook trout*	*Rainbow trout*	*Brown trout*
Water	$\bar{X} \pm s_{\bar{x}}$	72.98±0.84^{bc}	74.25±0.43^{ac}	76.14±0.27^{ab}
	V%	2.08	1.74	1.44
Dry matter	$\bar{X} \pm s_{\bar{x}}$	27.02±0.84^{bc}	25.75±0.43^{ac}	23.86±0.27^{ab}
	V%	5.61	4.52	3.36
Proteins	$\bar{X} \pm s_{\bar{x}}$	19.31±0.43^{bc}	17.87±0.61^{a}	17.37±0.24^{a}
	V%	5.69	2.68	4.35
Fats	$\bar{X} \pm s_{\bar{x}}$	5.82±0.23^{bc}	5.02±0.22^{ac}	4.41±0.21^{ab}
	V%	8.80	4.92	6.16
Ash	$\bar{X} \pm s_{\bar{x}}$	1.19±0.01^{bc}	1.11±0.01^{ac}	1.21±0.07^{a}
	V%	3.81	4.20	2.81

Mean values in rows marked with different letters differ significantly at $p<0.05$

As it can be observed from these data the highest dry matter content is registered at brook trout specimens 27.02 g, while in the case of brow brown trout samples were registered the lowest values of 23.86 g.

Proteins are the basic substances that offer products their nutritional value. Therefore, the quality of food is assessed primarily by their content of protein.

Within chemical composition of muscle tissue after water, proteins are the major constituents of animal bodies; proteins perform extremely varied functions and growth, maintenance and repair off all cells are dependent upon them, reflecting a high degree of structural organization and specialization (Sabetian et al., 2012; Wang and Hun, 2017; Simeanu et al., 2017).

Protein content of trout breeds fillet from the experimental batches ranged between 17.27 g for brown trout, and 19.31 g for brook trout, values similar to those mentioned in the specialty literature (Fauconneau et al., 1993b; Corser et al., 1999; Plavša et al., 2000; Savić et al. 2004; García-Macías et al., 2004; Bud and Mireşan, 2008; Celik et al., 2008; Alçiçek et al., 2010; Fallah et al., 2011; Dinović et al., 2011; Mocanu et al.,2012; Vranić et al., 2010, 2011; Sabetian et al., 2012; Kaya et al., 2014; Sirakov, 2015; Wang and Hun, 2017).

Lipids are among the most important biochemical constituents of fish (Aras et al., 2003), are found in sarcoplasm (in the form of fine droplets) under the skin, in the muscular tissue (Haliloglu and Aras, 2002) and blood plasma (Booth et al., 1999), but also in various organs such as the liver, spleen or gonads (Hatano et al., 1989; Hederson, 1996; Tocher, 2003; Segal, 2006), muscle fiber structures (mitochondria, microsomes, nuclei).

Lipids from trout meat vary within wide limits, ranging between 1.7 gand 9 gas it was found in the specialty literature (Fauconneau et al., 1993b; Corser et al., 1999; Plavša et al., 2000; Savić et al., 2004; García-Macías et al., 2004; Bud and Mireşan, 2008; Celik et al., 2008; Alçiçek et al., 2010; Fallah et al., 2011; Dinović et al., 2011; Mocanu et al., 2012; Vranić et al., 2010, 2011; Sabetian et al., 2012; Kaya et al., 2014; Sirakov, 2015; Wang and Hun, 2017).

Fish meat presents a good palatability when the lipid content ranges from 3.5% - 4.5% (Liu, 2002).

The fat content of the analyzed trout's fillet ranged between 4.41 g, in case of brown trout and 5.82 g for brook trout, values that place them in the category of fish medium lipid content (4–8%). And this time data obtained

were within the limits mentioned in the consulted specialty literature (Fauconneau et al., 1993b; Corser et al., 1999; Plavša et al., 2000; Savić et al., 2004; García-Macías et al., 2004; Bud and Mireşan, 2008; Celik et al., 2008; Alçiçek et al., 2010; Fallah et al., 2011; Dinović et al., 2011; Mocanuet al., 2012; Vranić et al., 2010, 2011; Sabetian et al., 2012; Kaya et al., 2014; Sirakov, 2015; Wang and Hun, 2017).

According to the literature, the ash content varies from 1 to 2 g (Plavśa et al., 2000; García-Macías et al., 2004; Bud and Mireşan, 2008; Fallah et al., 2011; Vranić et al., 2011; Mocanu et al., 2012; Sabetian et al., 2012; Kaya et al., 2014; Sirakov, 2015; Wang and Hun, 2017).

The ash content registered values ranging from 1.11 g for rainbow trout meat to 1.21 g for brown trout, values similar to those mentioned in literature. For all the constituents of chemical composition, there are, significant statistical differences between breeds ($p<0.05$).

Amino acid composition of the three trout meat is given in Table 2.

The protein compositions of the examined trout breeds contained the highest levels of glutamic acid (8.75% - 9.16%), followed by aspartic acid, leucine and lysine observations supported by Iwasaki and Harada (1985), Farmanfarmaian and Sun (1999), Beklevik et al. (2005), Sabetian et al. (2012), Kaya et al., (2014), Sirakov (2015), Wang and Hun (2017), tryptophan was not determined.

Wesselinova (2000) reported that the amounts and types of amino acids in fish muscle is affected by catching time and location, and Green et al. (2002) mentioned that the ratio of essential to non-essential amino acids (EAA/NEAA ratio) in dietary protein has important effects on protein utilization by fish.

The lowest levels in decreasing amounts were registered in the case of tyrosine (2.80–2.88%) and histidine (1.56%-1.63%), observations in according with those reported by Sabetian et al. (2012) (only in the histidine level), Kaya et al. (2014), Wang and Hun (2017), but in contradiction with the results mentioned by Sirakov (2015) which mention methionine and serine as limiting amino acids.

As the main amino acid constituent from the trout's meat composition, glutamine is essential for cell proliferation, as a nitrogen donor during purine and pyrimidine synthesis.

The human organism has the biological ability to produce a limited number of amino acids, which is why many of these must be obtained from sources of animal or vegetable protein by daily diets, trout meat being a rich source in essential amino acids needed for growth, and development.

The role of these essential amino acids is very important because, valine is an essential amino acid, needed to maintain the nitrogen balance, it performs its activity in synergy with leucine and isoleucine, participating in synthetic proteins, having anabolic role in muscle cells, also assuring the coordination of movements; leucine is required by ketogenic function, deficiency in leucine prevents normal growth, leading to body weight loss and a negative nitrogen balance; isoleucine participates in the synthesis of hemoglobin, regulates blood glucose levels, speeds recovery of the body after surgery or wounds and has anabolic effects; threonine is a lipotropic agent that prevents the accumulation of fat in the liver, and through degradation substances it participates in the synthesis of porphyrin; lysine is involved in the process of growth of the body, as well as in the formation of red blood cells, being a precursor of carnitine and is the limiting amino acid in cereal based diet; phenylalanine is a tyrosine precursor, with an important role in the synthesis protein, and is involved in mediating the transmission of nerve impulses as a dopamine precursor; methionone interferes with lipid metabolism, preventing fat accumulation in the liver and provides cysteine and S-adenosyl methionine biosynthesis, contributes as a methyl group donorand in the same time is essential in the diet for producing taurine, which exhibits clear antihypertensive effects (Wiley et al., 1986; Simeanu et al., 2015; Wang and Hun, 2017; Simeanu et al., 2017).

However, the contents and proportions of muscle amino acids were basically the same for all the trout breeds displaying a conservative pattern, which was in accord with the observations of Kizak (2013) on tench and Wang and Hun (2017) on rainbow trout.

The differences in the amino acid profiles for the three breeds can be related to different

aspects, such as feed utilization and its composition, the retention of amino acids and the amino acid profile of the fish body as well as environmental conditions, breeding system, size of the fish, catching season (Rodehutscord et al., 1997; Wesselinova, 2000; Green et al., 2002; Shirai et al., 2002; Solvik and Rustad, 2005; Toppe et al., 2007; Erdem et al., 2009; Kaya et al., 2014; Sirakov, 2015).

In this study, the total amino acid (TAA), total essential amino acid (EAA), total non-essential amino acid (NEAA) and total delicious amino acid (DAA) contents did not differ significantly ($P > 0.05$) between all the three trout breeds.

The ratio between EAA and TAA had the highest value in the case of brown trout samples of 0.73 and the lowest in the case of brook trout samples of 0.7 while the ratio between EAA and NEAA had higher value forthe rainbow trout samples of 0.75, the lowest value again at brook trout samples.

These results showed that the EAA to TAA and EAA to NEAA ratios for all of breeds were comparable to the reference values of nearly 40% and above 60%, respectively, recommended by the FAO/WHO, which indicates that all trout breeds may be considered high-quality protein food sources.

Each amino acid contributes, in different degrees, to the aroma of foods. Several amino acids taste sweet (glycine and alanine) or delicious (umami) to humans. Aspartic acid and glutamic acid have a sour taste, but they are responsible for the umami taste in the presence of sodium salt (Gunlu and Gunlu, 2014).

Table 2. Amino acid composition of trout meat (% from dry sample)

Amino acid	Brook trout	Rainbow trout	Brown trout
Aspartic acid (Asp)*	6.45±0.81^{bc}	7.03±0.46^{ac}	6.84±0.62^{ab}
Glutamic acid (Glu)*	9.16±0.49^{bc}	8.75±0.60^{ac}	9.81±0.66^{ab}
Serine (Ser)	3.18±0.43^{bc}	2.97±0.20^{ac}	3.02±0.13^{ab}
Glycine (Gly)*	4.28±0.16	4.44±0.35	4.41±0.14
Threonine (Thr)^{E}	3.17±0.57	3.79±0.35	3.72±0.42
Arginine (Arg)	5.38±0.31	5.68±0.50	5.74±0.55
Histidine (His)	1.56±0.06	1.65±0.25	1.63±0.08
Alanine (Ala)*	4.54±0.32	4.72±0.26	4.69±0.36
Tyrosine (Tyr)	2.88±0.21	2.88±0.12	2.80±0.08
Valine (Val)^{E}	3.47±0.38^{bc}	3.81±0.18^{a}	3.66±0.38^{b}
Phenylalanine (Phe)^{E}	3.37±0.23	3.31±0.20	3.33±0.15
Isoleucine (Iso)^{E}	3.56±0.28	3.63±0.16	3.60±0.19
Leucine (Leu)^{E}	6.38±0.58	6.85±0.47	6.46±0.76
Lysine (Lys)^{E}	5.82±0.34	5.82±0.32	5.79±0.14
Cysteine (Cys)	3.66±0.01^{c}	3.80±0.13	3.76±0.05^{a}
Methionine (Met)^{E}	3.68±0.04^{c}	3.85±0.12	3.84±0.05^{a}
Essential amino acids $\sum_{EAA}$	29.49±1.47	31.80±1.66	30.41±1.63
Delicious amino acids $\sum_{DAA}$	24.43±0.32	24.94±0.42	25.75±0.57
Non essential amino acids $\sum_{NEAA}$	41.11±1.52	41.91±1.60	41.66±1.21
Total amino acids $\sum_{TAA}$	70.60±1.98	73.02±3.41	73.13±2.96
$\sum_{EAA}/\sum_{TAA}$	41.77±1.72	43.54±2.53	41.58±2.29
$\sum_{EAA}/\sum_{NEAA}$	71.73±1.49	75.87±1.63	72.99±1.42
$\sum_{DAA}/\sum_{TAA}$	34.60±1.11	34.15±1.91	35.21±1.76

EAA – essential amino acids; NEAA – non-essential amino acids; TAA – total amino acids; Tryptophan was not determined;*denotes delicious amino acids (DAA);
Mean values in rows marked with different letters differ significantly at $p<0.05$

The level of total delicious amino acid (DAA) was higher in the case of brown trout samples than in rainbow and brook trout samples (Table 2), been suggested by Ruiz-Capillas and Moral (2004), that these free delicious amino acids are related to the characteristic fish flavour and that different contents of these amino acids may cause variations in fish flavour.

The ratio of DAA to TAA ranged from 34.15% in the case of rainbow trout to 35.21% for brown trout samples (Table 2). These values were higher to those reported for *Silurus asotus*

L. (31.83-32.33%) by Jiang (2012) but lower than those reported for Masu salmon (37.31-37.46%) by Wang et al. (2015), and for rainbow trout (36.61% to 36.92%) in the diploid fish and (36.01% to 37.68%) in the triploid fish by Wang and Hun (2017).

The results obtained from this study showed that the tested trout breeds have a well-balanced and high-quality protein source in the respect of the EAA/TAA ratio and the obtained data are in confirmation with those mentioned for rainbow trout by Sabetian et al. (2012) and Wang and Hun (2017), but lower for brown trout Kaya et al. (2014) and Sirakov (2015).

According to the obtained data, the sum of the sulphur-containing amino acids (methionine and cysteine) presented the highest score among rainbow trout proteins (Table 2), indicating that rainbow trout is rich in methionine and cysteine, while the lowest values were registered at brook trout proteins, while the sum of the aromatic amino acids (phenylalanine and tyrosine) presented the lowest score among brown trout proteins.

CONCLUSIONS

The values obtained after chemical determinations enlightened the fact that all trout breeds from the experimental batches fall into the limits cited in the literature, highlighted that all trout breeds have a good nutritional value.

After evaluation of chemical composition of trout meat, was observed that brook trout individuals had a higher content in dry matter and protein.

The protein compositions of the examined trout breeds contained the highest levels of glutamic acid, followed by aspartic acid, leucine and lysine.

The ratio between EAA and TAA had the highest value in the case of brown trout samples of 0.73 and the lowest in the case of brook trout samples of 0.7 while the ratio between EAA and NEAA had higher value forthe rainbow trout samples of 0.75, the lowest value again at brook trout samples.

The present study demonstrate that trout meat is a highly source of protein and contains essential amino acids for promoting good health, prevention and healing of diseases in humans.

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STUDY REFERRING TO THE APPEARANCE OF CONTAMINATION WITH DEOXYNIVALENOL IN GRAINS, GRAIN FLOUR AND BAKERY PRODUCTS ON THE ROMANIAN MARKET

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Abstract

The increase of the agricultural surfaces cultivated with cereals that came to represent ~ 50% of the total areas destined to the agricultural activity, as well as the last years' climatic changes mainly associated with the global heating which caused the increase of the temperature and humidity during the period of cereal harvest (in particular of wheat) required that monitoring of deoxynivalenol contamination would be a permanent concern in order to ensure the health of both, the human and animal population. In this study, the quantitative determination of deoxynivalenol contamination was pursued on a number of 584 samples, represented by unprocessed cereals, cereal flour, bread and bakery products, breakfast cereals and pasta. In the analysed samples, by the immuno-enzymatic technique and / or high performance chromatographic liquid, there were no registered values higher than the maximum level allowed for this parameter provided in Reg. EC no. 1881/2006 with the subsequent modifications and completions, but it was visible (even a doubling) of the deoxynivalenol contamination of the samples analysed in the year under test during which there were more abundant precipitation and increased temperatures in the wheat harvesting period. Following the study, it was found that the analysed samples correspond to the legislative requirements for the deoxynivalenol contaminant, and this parameter is permanently monitored in order to comply with the incident legislation.

Key words: *deoxynivalenol, food safety, contamination.*

INTRODUCTION

Cereals, cereal flour and bakery products are a staple food and represent about 45% of the world's energy source, which is why about half of the planet's arable area is cultivated with cereals. By their nature, cereals and products derived from them (cereal flour, breakfast cereals, flours, breads, bakery products) have a high risk of contamination with mycotoxins (deoxynivalenol, aflatoxin, ochratoxin A, etc.). The present study will address the evolution of deoxynivalenol contamination in the same geographical areas from our country but during different time periods (two consecutive years).

Contaminants are represented by any substance that is not intentionally added to food, which are present in them as a result of their production, manufacture, processing, preparation, treatment, packaging, packaging, transport or handling or as a result of environmental contamination. Foreign matters, such as insect fragments, animal hair, etc. are not included in this definition. (Codex STAN 193/2005).

The Codex Alimentarius definition of a contaminant implicitly includes natural toxins, including toxic metabolites of certain fungi that are not intentionally added to food and feed, respectively mycotoxins (Codex STAN 193/2005).

Mycotoxins

Mycotoxins are metabolism products of several mold species, the most commonly involved species being those of the genus *Aspergillus, Penicillium* and *Fusarium* which can develop on different nutritional substrates and under varied climatic conditions.

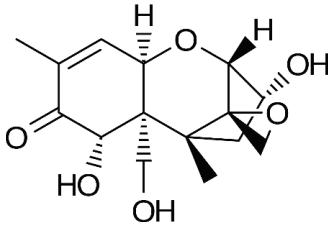
Mycotoxins may develop during cultivation, transport, storage or at other times during production. The end result is that they are found in many foods (especially those based on cereals). (Goran & Crivineanu, 2016).

The appearance of mycotoxins is influenced by numerous biological, environmental and harvesting factors. The most important biological factors are even represented by the plans susceptible to contamination with different mold species. Environmental factors need to be looked at considering two aspects, as well in the field, when we are talking about temperature, humidity, different biological vectors, as storage, when we talk especially about maintaining the products in optimum temperature and humidity conditions that prevent the occurrence of mycotoxin contamination. Harvesting factors are the represented by the harvesting method and they are very important for the harvesting period. As it is known, mycotoxin contamination varies from year to year, depending on the climate and other environmental factors. For example, contamination with deoxynivalenol is associated with rainy years and high temperature during the harvesting period which is subsequently reflected in increased contamination not only of cereals, but also of products obtained from them (flour, breakfast cereals, pasta, bread and bakery products).

Deoxynivalenol

Deoxynivalenol, also known as vomitoxin due to its effect on the body, is a type B trichothecene and it occurs mainly in cereal grains represented by wheat, barley, oats, rye and maize and rarely in rice, sorghum and triticale, being the most common contaminant of cereals and cereal products (Goran & Crivineanu, 2016).

The chemical formula of deoxynivalenol $C_{15}H_{20}O6$ is shown below:



Deoxynivalenol is a metabolism product, especially of the following species of molds: *Fusarium graminearum* and *Fusarium culmorum.*

Deoxynivalenol has been implicated in mycotoxicosis incidents in both, humans and farm animals, with the following clinical signs being most commonly described: decreased appetite, vomiting, intestinal transit disorders associated with immunodeficiency.

The regulations regarding the maximum allowed level of contaminants are represented at national level by observing the European legislation in force, respectively Reg. EC no. 1881/2006 with the subsequent modifications and completions (Table 1) and globally by the Codex Alimentarius regulations, respectively Codex STAN 193-1995.

Table 1. Maximum allowed level of Deoxynivalenol according to Reg. EC No. 1881/2006

Product type	Maximum allowed level µg/kg
Unprocessed cereals other than durum wheat, oats and corn	1250
Raw wheat and unprocessed oats	1750
Raw maize	1750
Cereals intended for direct human consumption, cereal flour, pasta	750
Bread (including bakery products), breakfast cereals	500
Dishes made from processed cereals and baby foods for infants and young children	200

Codex STAN 193-1995 has not yet set the maximum permitted level for deoxynivalenol contamination.

MATERIALS AND METHODS

In this study, it has been observed the quantitative determination of contamination with deoxynivalenol in cereals and different products derived from cereals for samples taken and analysed in an accredited laboratory.

During the period subjected to the study, number of 584 samples represented by the matrices defined in Reg. Ec 1881/2006.

At the reception of the samples the conformity of the sample, from the point of view of the quantity, with the batch from which it was taken, according to Reg. EC no. 401/2006.

The obtained results were compared with the maximum level allowed by Reg. EC no. 1881/2006 and expressed corrected for recovery and after that, reported depending on the extended uncertainty obtained after the validation of the analysis method.

The methods of analysis that were used were the immuno-enzymatic method and the liquid chromatographic method with DAD detector.

The minimum requirements that must be done for the analysis methods are the following:

- to allow the determination of mycotoxins at a level lower than the legal limit;
- the methods must be at least validated within the laboratory;
- the current trend is that all working methods have to be evaluated for accreditation.

The Immuno-enzymatic method (ELISA Enzyme linked Immunosorbent Assay)

It represents the method of detecting mycotoxin using an antigen-antibody complex. conjugated to an enzyme.

Benefits

It is a method of screening with high sensitivity that allows you to obtain, in a relatively short time, results for many samples and it does not involve a difficult stage of sample processing.

Disadvantages

The samples can be easily contaminated, false positive or false negative results can occur. Any possible result that does not comply requires a re-examination of the sample by a confirmation method (HPLC).

High performance liquid chromatography (HPLC)

High performance liquid chromatography has as a general principle the separation of the mixture of compounds by passing it through a stationary phase represented by a chromate-graphic column dedicated to the compound of interest, passing through the detector and issuing a characteristic signal transposed by a specific signal called 'peak chromatography'.

In order to use this technique to quantify deoxynivalenol contamination, the sample preparation stage is required by using the immunoaffinity columns which involve the passage of the extract by an immunoaffinity column coated with specific anti-deoxyniva-lenol antibodies, the stage in which antibody antigen complexes take place.

Benefits

This method has a high selectivity, being used as a confirmation method.

Disadvantages

Compared to the immune-enzymatic technique, it presents higher costs and specialized personal.

RESULTS AND DISCUSSIONS

The cereals and derived products are monitored from the point of view of contamination with deoxynivalenol at national level by applying the provisions of the Order of the President of ANSVSA no. 35/2016 with subsequent modifications and completions.

During the period under study, a number of 584 samples analysed during two years (2016 and 2017) were analysed, the samples came from the counties of Argeș, Botoșani, Brașov, Brăila, Bucharest, Călărași, Constanța, Dâmbovița, Dolj, Galați, Giurgiu, Gorj, Ialomița, Ilfov, Mureș, Olt, Prahova, Teleorman and Timiș. Because of the fact that Braila and Mureș counties are not found with samples analysed during two consecutive years, they will be eliminated from the statistical calculation (Table 2).

Table 2. Total number of samples analysed in the study

Year	No. of samples analysed	No. of samples analysed by ELISA	No. of samples analysed by HPLC
Year 1 - 2016	264	243	21
Year 2 - 2017	320	201	119

From the samples analysed by the two techniques it is noted that most of the samples were analysed by the immune-enzymatic technique, which has a higher sensitivity but cannot be used as confirmatory methods, therefore the results obtained by the HPLC method are generally expressed as undetectable because the limit of quantification of the method is much higher (157µg / kg by HPLC compared to 26.6 µg / kg by ELISA).

The centralized data, as a way of expressing ELISA vs HPLC results, are expressed in Tables 3 and 4.

Table 3. Expression of HPLC results Not detectable vs. Numerical values

Year	No. of samples analysed by HPLC	Results expressed as undetectable	Results expressed <limit of quantification of the method	Results with values
Year 1	21	21	-	-
Year 2	119	111	5 results <157µg/kg	3

Table 4. Expression of ELISA results Not detectable vs. Numerical values

Year	No. of samples analysed by ELISA	Results expressed as undetectable	Results expressed <limit of quantification of the method	Results with values
Year 1	243	163	4 results <20.6 µg/kg	76
Year 2	201	51	1 result <20.6 µg/kg	195

Following the statistical calculation, it is observed that in the second year (2017), the number of samples contaminated with deoxynivalenol increased, so that the number of samples at which values were recorded, but without exceeding the admitted level, doubled. In this way, in the first year (2016) values were registered at a percentage of 28.78% of the analysed samples, and in the following year, at a percentage of 61.87%, correlated with the meteorological conditions from year 2 of study.

In the first year of study (2016), a number of 112 unprocessed cereal samples were analysed, in 51 samples there were recorded values ranging from 21.52 µg / kg to 721.88 µg / kg in a barley sample, with an average contamination of 92.73 µg / kg.

In the second year of study (2017) a number of 152 cereal samples were analysed, a number of 85 samples were recorded values that ranged from 21.05 µg/kg to a maximum of 868 µg/kg in one sample wheat, with an average deoxynivalenol contamination of 226.24 µg/kg (Figure 1).

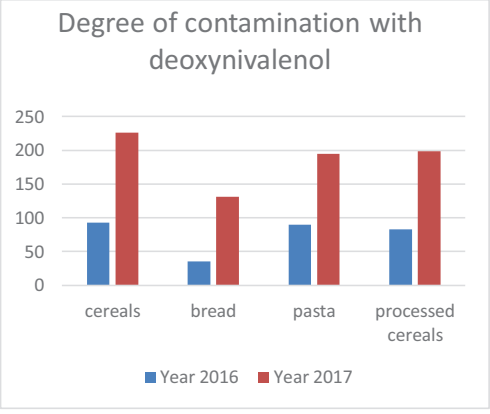


Figure 1. Graphic representation: contamination with deoxynivalenol of the different samples analysed

In 2016, a number of 86 bread samples, bakery products including biscuits and different products were analysed, in 12 samples there were values ranging from 23.31 µg / kg to 57.87 µg / kg in a bread sample, with an average contamination of 35.11 µg / kg. In the year 2017, a number of 97 bread samples were analysed, bakery products including biscuits and different products for a total of 36 samples were recorded values that ranged from 24.16 µg / kg to a maximum of 354.41 µg / kg in a bread sample, with a mean deoxynivalenol contamination of 131.49 µg / kg (Figure 1).

In the first year of study, there were analysed a number of 24 samples of processed products, especially pasta, in 4 samples there were values ranging from 28.23 µg / kg to 173.55 µg / kg in a sample of pasta, with an average contamination of 89.63 µg / kg.

In the second year of study, there were analysed a number of 32 samples processed products, especially pasta, at a number of 14 samples were recorded values ranging from 44 µg / kg to a maximum of 372.78 µg / kg at a sample of pasta, with an average deoxynivalenol contamination of 194.53 µg/kg (Figure 1).

In 2016, a number of 42 processed cereal samples were analysed – especially flour and breakfast cereals, at a number of 10 samples were recorded values ranging from 31.56 µg / kg up to a maximum of 172.37 µg / kg in a flour sample, with an average deoxynivalenol contamination of 82.84 µg / kg (Figure 1).

In 2017, a number of 39 samples represented by processed cereals, especially flour and breakfast cereals were analysed, at a number of 22 samples were recorded values that ranged from 59.79 µg/kg to a maximum of 453.64 µg/kg in a breakfast cereal sample, with an average deoxynivalenol contamination of 198.79 µg/kg (Figure 1).

CONCLUSIONS

From the point of view of deoxynivalenol contamination, all samples corresponded to the analysed parameter reported in Reg. What no. 1881/2006 with subsequent modifications and completions.

In 2017, there was not only a 100% increase in the number of samples analysed, but also an

increase in the level of deoxynivalenol contamination, as well as a doubling of the average level of contamination, mainly due to the weather conditions of the respective period.
There was noticed a correlation between the level of contamination in unprocessed cereals and the level quantified in the derived products, finding a decrease in the level of contamination following the different processing stages (milling, heat treatment, etc.) being directly proportional to the level of contamination.
Monitoring the level of contamination with deoxynivalenol should be continued taking into account the levels identified by deoxynivalenol, which in some cases are close to the maximum regulated level for taking the necessary legal measures.

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*** Commission *Regulation* (*EC*) No *1881/2006* of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs

*** COMMISSION REGULATION (EC) No *401/2006 of 23 February 2006* laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs

*** Codex STAN 193-1995 *Codex General Standard For Contaminants And Toxins In Food And Feed.*

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STUDY ON NUTRITIONAL QUALITY OF SMOKED AND MARINATED PRODUCTS FROM HERRING *(CLUPEA HARENGUS)*

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Abstract

The study aimed a comparative analysis of the nutritional characteristics of smoked and marinated products from herring (Clupea harengus). Thirty products were analyzed, fifteen samples of smoked and fifteen of marinated herring, five samples for each type of study product (manufacturers codification: A, B, C, D, E and F). The catch areas of analyzed herring products from al manufacturers were FAO 27, North-East Atlantic. The proteins, lipids, collagen and water content was determined using the automated analyzer Food Check (infrared spectrophotometer); mineral substances were determined by calcination at 550 ^{0}C and the carbohydrates content and energy value were determined by calculation, using conventional relations. The most important differences between the marinated herring products analyzed, have targeted the content of lipids, with difference of 3% (13.10% lipids for product C compared with 16.11% lipids for product A). There are very significant differences ($p<0.001$), between the two product categories analyzed, marinated herring having over 50% more fat for product A than for the smoked one (5.54% for E and 7.45% for F product).

Key words*: marinated, smoked herring, proteins, lipids, salt.*

INTRODUCTION

The consumption of fish as well as fish products has significantly increased during the last two decades (FAO, 2016). The popularity of fish is mainly due to the overall high quality and the positive effects on human health. Fish and seafood products, have a high nutritional value regarding beneficial amounts of protein, lipids as well as essential micronutrients and have a high content of omega 3 long chain polyunsaturated fatty acids (n-3 LC PUFA) compared to land living animals (Khalili Tilami and Sampels, 2017; Tacon and Metian, 2013). Strong links between fish and seafood consumption and positive health effects, especially with the decreased risk of coronary heart and cardiovascular diseases, decreased inflammatory disease as arthritis and prevention of cancer have been shown by many researchers (Calder, 2004; Rudkowska et al.; 2010; Lund, 2013). The main effects of fish consumption have been attributed to the high content of n-3 LC PUFA; also other nutrients from fish have positive effects on human health.

Fish and other seafood have also a well-balanced amino acid composition; contain high proportions of taurine and choline, the vitamins D_3 and B_{12} and the minerals: calcium, phosphorus, iodine, selenium and also might provide significant proportions of vitamin A, iron and zinc (Lund, 2013). However, one of the problems of fish consumption is represented by the fact that this meat produces allergies, by the content of biogenic amines such as histamine, that play essential roles in the normal development, metabolism and physiological functions of humans (Smith, 1980, Bardocz, 1995; Halasz, et al., 1994; Ladero, et al., 2010). Along with this issue, apart from a few pathogenic species, most infections of fish meat are considered to be relatively innocuous (Feist, et al., 2004; Lazar et al., 2012).

Salting, marinating and smoking are popular methods of processing the fish. Different types of marinades and salads based on marinated fish have had an important share in fish consumption in Europe for many years. Because of seasonal availability of fish and distance from the fishing ground, the majority of marinades are produced based on frozen fish (Szymczak et al., 2012, 2013 and 2018). During ripening, proteolysis of herring meat occurs under the influence of endogenous enzymes. It results in the release of high amounts of free amino acids and peptides that impart the typical taste and aroma of the

mature meat (Szymczak et al., 2015a, 2016a, 2016b). Hence, the degree of hydrolysis determines the sensory quality, nutritive value, and a high content of biologically-active compounds of a food product (Szymczak et al., 2015b; Felisiak, 2019).

The ripening of marinade meat proceeds as a result of multiple physical, biochemical, and microbiological transformations. Most of these transformations are associated with the hydrolysis of proteins and lipids and with their interactions. In marinades with low pH values, active are acidic aspartyl proteases (cathepsin D and E), as well as cysteine proteases (cathepsin B and L) and pepsin (Szymczak and Lepczynski, 2016; Szymczak and Kolakowsi, 2016; Szymczak, 2017).

Food smoking is one of the oldest preservation methods and is still widely used (Ledesma et al., 2015; Lingbeck et al., 2014; Stołyhwo and Sikorski, 2005). However, smoking is nowadays mainly used to obtain the desired colour, flavour, aroma, and appearance in the smoked food rather than for preservation purposes (Theobald, et al., 2012; Fasano et al., 2016, Hokkane, et al., 2018). The study aimed a comparative analysis of the nutritional characteristics of some smoked products from herring fillets and marinated herring medallions marketed in Iasi, Romania in spring of 2019.

MATERIALS AND METHODS

Thirty products were analyzed, fifteen samples of smoked and fifteen of marinated herring, five samples for each type of study product/ manufacturers (codification: A, B, C, D, E and F). The catch areas of analyzed herring products from al manufacturers were FAO 27, North-East Atlantic (Norway, Iceland, Faroe Islands, Scotland, Ireland and Netherlands).

The samples were chopped and homogenized with an electric shredder.

The water, proteins and lipids content were determined using the Food Check Near Infrared Spectrophotometer (NIRS technology); the energy value was determined by calculation using conventional formulas and crude ash content was assessed after AOAC, 1990, by calcinations (at 550°C for 16 h after a preliminary carbonization).

The energy conversion factors were: 4.27 for proteins, 9.02 for lipids and 3.87 for carbohydrates (according to FAO relations, 2003).

The achieved results were statistically processed through the main descriptors computation (arithmetic mean, $\bar{x}$, standard deviation, s, and coefficient of variation, V%, and by analysis of variance test (ANOVA multiple comparisons), using the GraphPad Prism 8.1 software.

RESULTS AND DISCUSSIONS

The most important differences between the marinated herring products analysed (Tables 1, 2 and 3) have targeted the content of lipids, with difference of 3% (13.10% lipids for product C compared with 16.11% lipids for product A).

Table 1. Chemical composition and energy value of marinated herring (A product)

Chemical components	$\bar{x} \pm s_{\bar{x}}$	s	V%	Min.	Max.
Lipids%	16.11± 0.84	1.68	10.44	14.30	17.75
Proteins%	17.95± 0.09	0.17	0.99	17.30	18.90
Collagen%	2.52± 0.03	0.06	2.07	2.66	2.80
Water%	61.15± 0.27	0.54	0.91	59.60	63.90
Ash%	3.78± 0.11	0.22	5.87	3.50	4.00
Salt%	3.21± 0.04	0.07	2.23	3.11	3.28
Dry matter%	38.13± 0.27	0.54	1.37	34.10	40.40
Organic matter%	34.35± 0.37	0.75	2.08	32.10	36.90
Carbohydrates %	0.81± 1.23	2.46	7.42	0.10	2.30
GE kcal/100g	222.84± 3.31	6.62	1.88	223.37	237.69
GE kJ /100g	932.37± 13.84	27.68	3.18	934.57	1094.51

GE = Gross Energy; s= standard deviation; V%=coefficient of variation.

On the same note, there are very significant differences between the two product categories analysed (Fig. 1, Tables 1-6), marinated herring having over 50% more fat for product A than for the smoked one (5.54% for E product, and 7.45% for F product). These differences can be attributed to the fact that the marinated herring is presented in the form of medallions, thus

including abdominal muscles that are richer in fat compared to the fish fillet and eventually losses of fat from smoking process; at the same time, the season when the fish was cached it can`t be determined, knowing that the proportion of fat varies greatly with the season and the breeding cycle (Stroud, 2001; Nielsen et al., 2005). However, these mean values of the lipid content were lower than those determined by Szymczak et al. (2018), that found 18.70±0.30% in size 4-8 herring (149 g/fillet, and whole fish weight above 350-400 g).

Table 2. Chemical composition and energy value of marinated herring (B product)

Chemical components	$\bar{x} \pm s_{\bar{x}}$	s	V%	Min.	Max.
Lipids%	14.86±0.28	0.62	4.16	10.20	15.70
Proteins%	18.24±0.04	0.10	0.52	17.10	18.90
Collagen%	2.98±0.01	0.03	0.98	2.93	3.20
Water%	62.62±0.18	0.40	0.65	61.20	63.90
Ash%	3.54±0.01	0.01	0.36	3.53	3.56
Salt%	3.10±0.07	0.15	4.29	2.30	3.60
Dry matter%	37.38±0.18	0.40	1.08	37.10	37.80
Organic matter%	33.84±0.18	0.41	1.21	33.54	34.27
Carbohydrates %	0.74±0.31	0.70	94.70	0.44	1.87
GE kcal/100g	214.80±1.66	3.71	3.29	213.03	220.69
GE kJ /100g	898.72± 6.94	15.51	1.73	891.30	923.37

GE = Gross Energy; s= standard deviation; V%=coefficient of variation.

After some authors (Stroud, 2001; Nielsen et al., 2005; Wianecki, 2007; Tacon and Metian, 2013; Adeyemi et al., 2015), unlike most white fish, the chemical composition of herring varies considerably with the season and the breeding cycle; the fat content of herring may be less than 1% immediately after spawning, and more than 20% as spawning time approaches again.

Table 3. Chemical composition and energy value of marinated herring (C product)

Chemical components	$\bar{x} \pm s_{\bar{x}}$	s	V%	Min.	Max.
Lipids%	13.10±0.72	1.25	9.56	11.80	14.30
Proteins%	18.17±0.32	0.55	3.03	17.60	18.70
Collagen%	3.04±0.06	0.10	3.33	2.94	3.14
Water%	64.17±0.03	0.06	0.09	64.10	64.20
Ash%	3.60±0.04	0.11	0.18	2.89	3.98
Salt%	2.62±0.02	0.03	1.12	2.59	2.65
Dry matter%	35.83±0.03	0.06	0.16	33.80	37.90
Organic matter%	32.23±0.05	0.09	0.18	29.20	34.30
Carbohydrates %	0.97±0.38	0.67	68.88	0.40	1.70
GE kcal/100g	199.47±3.71	6.42	3.22	192.86	285.69
GE kJ /100g	834.60±15.51	26.86	1.37	806.94	978.59

GE = Gross Energy; s= standard deviation; V%=coefficient of variation.

The water content decreases as the fat content increases. In addition, the protein content varies with water content; as the water content increases, the protein content raises a little (Stroud, 2001).

Table 4. Chemical composition and energy value of smoked herring (D product)

Chemical components	$\bar{x} \pm s_{\bar{x}}$	s	V%	Min.	Max.
Lipids%	9.35±0.31	0.61	6.56	8.60	10.10
Proteins%	17.73±0.25	0.50	2.82	17.20	18.20
Collagen%	3.28±0.04	0.08	2.42	3.18	3.37
Water%	69.78±0.22	0.45	0.64	69.20	70.30
Ash%	2.90±0.03	0.06	2.16	2.81	2.96
Salt%	2.30±0.09	0.18	7.94	2.10	2.50
Dry matter%	30.23±0.22	0.45	1.49	29.70	30.80
Organic matter%	27.32±0.22	0.43	1.58	26.79	27.84
Carbohydrates %	0.25±0.31	0.62	247.85	0.06	0.79
GE kcal/100g	160.99±2.48	4.96	7.12	154.93	197.01
GE kJ /100g	673.57±10.38	20.77	3.08	648.24	798.78

GE = Gross Energy; s= standard deviation; V%=coefficient of variation.

Table 5. Chemical composition and energy value of smoked herring (E product)

Chemical components	$\bar{x} \pm s_{\bar{x}}$	s	V%	Min.	Max.
Lipids%	5.54±0.11	0.25	4.51	5.30	5.90
Proteins%	19.03±0.25	0.55	2.90	17.77	19.10
Collagen%	3.70±0.05	0.12	3.32	3.43	3.72
Water%	70.01±0.37	0.82	1.17	66.10	71.80
Ash%	4.94±0.08	0.15	3.09	4.83	5.15
Salt%	4.42±0.06	0.14	3.20	4.13	4.73
Dry matter%	29.51±0.37	0.82	2.79	29.20	31.00
Organic matter%	25.56±0.38	0.85	3.31	24.24	26.17
Carbohydrates %	0.48±0.23	0.51	51.49	0.34	0.67
GE kcal/100g	135.06±1.60	3.57	2.64	128.54	137.26
GE kJ/100g	565.08±6.68	14.93	2.64	537.81	574.29

GE = Gross Energy; s= standard deviation; V%=coefficient of variation.

The water content (Fig. 2) was higher for smoked herring (70.49% for E product, 69.78% for D product and 68.96% for F product) and smaller for marinated herring (61.88% for A product), this having the smallest average value of proteins (17.45%), and the highest value of lipids content (16.10%).

Table 6. Chemical composition and energy value of smoked herring (F product)

Chemical components	$\bar{x} \pm s_{\bar{x}}$	s	V%	Min.	Max.
Lipids%	7.32±0.10	0.23	3.20	6.05	8.46
Proteins%	18.80±0.29	0.50	2.64	17.60	20.20
Collagen%	3.94±0.06	0.14	3.52	3.66	4.11
Water%	68.96±0.64	2.56	3.72	64.50	72.80
Ash%	4.44±0.58	1.17	26.33	2.69	5.06
Salt%	3.48±0.38	0.99	28.50	2.10	4.50
Dry matter%	31.04±0.64	1.18	3.81	27.20	34.10
Organic matter%	29.07±1.05	1.56	5.35	25.16	33.00
Carbohydrates %	0.59±1.97	2.28	12.24	0.30	1.90
GE kcal/100g	145.61±5.55	5.77	3.96	112.90	168.90
GE kJ/100g	609.21±23.24	24.15	3.96	472.39	706.66

GE = Gross Energy; s= standard deviation; V%=coefficient of variation.

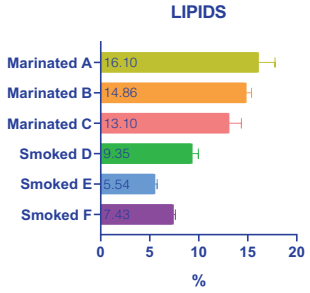

Figure 1. The lipids content of marinated and smoked herring

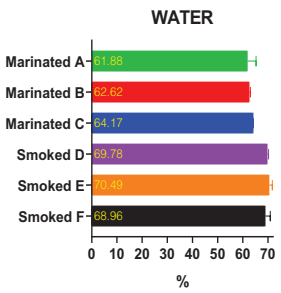

Figure 2. The water content of marinated and smoked herring

After Nielsen et al. (2005), the lipids content in herring meat was lower in February (in average 4.3%) and higher in July (17.6%) and September (17.3%).

The same authors found minim values of 1.3% lipids in February and 25.7% in July, these determinations showing how much it varies the chemical composition of this meat.

The proteins content (Fig. 3) was higher for smoked herring: E product (19.03%) and F product (18.69%); the lowest value was observed for marinated herring, product A (17.45%) this having the highest average values of lipids content (16.10%).

The collagen content (Fig. 4) was higher for smoked herring (3.94% for F product and 3.70% for E product) probably because the total proteins content was higher and, in the same time, the lipids content was lower (tables 5 and 6).

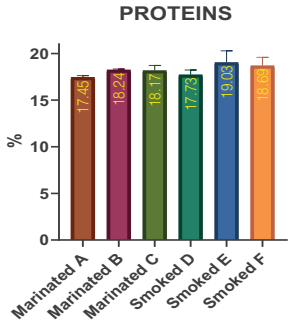

Figure 3. The proteins content of marinated and smoked herring

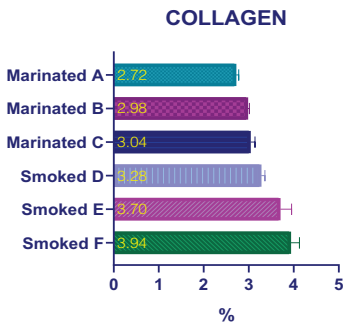

Figure 4. The collagen content of marinated and smoked herring

The ash content (Fig. 5) was the highest for product E (4.94%), this being and the saltiest product (4.42% salt), and the lowest value of ash (2.90%) and salt (2.30%), was observed for product D (fig. 6), both being smoked product (Tables 4 and 5).

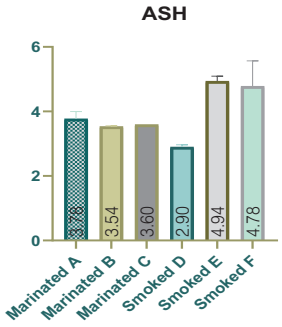

Figure 5. The ash content of marinated and smoked herring

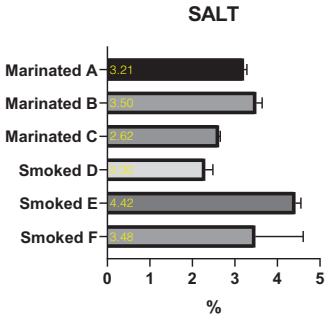

Figure 6. The salt content of marinated and smoked herring

The highest dry matter (DM), and in the same time the highest organic matter (OS) content of herring products analysed, was observed for the marinated one (39.85% for A product, 37.38% for B and 35.83% for C), the differences compared with the smoked one (D, E and F products) being on the base of lipid content that were found highest in this products (Fig. 7 and Fig. 8).

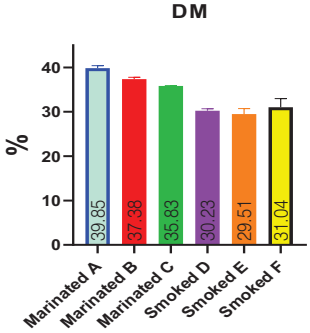

Figure 7. The dray matter content of marinated and smoked herring

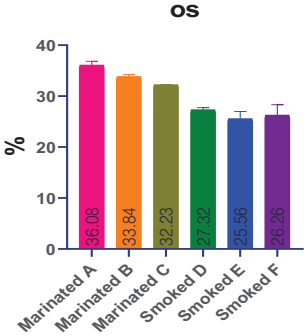

Figure 8. The organic matter content of marinated and smoked herring

The energy value (Fig. 9), was higher for marinated herring products (222.84 kcal/100g /898.37 kJ/100g for A manufacturer, 214.8 kcal/100g/ 898.2 kJ/100g for B manufacturer and 199.47 kcal/100g /834.60 kJ/100g for C manufacturer) compared with smoked herring, this having the highest lipids content.

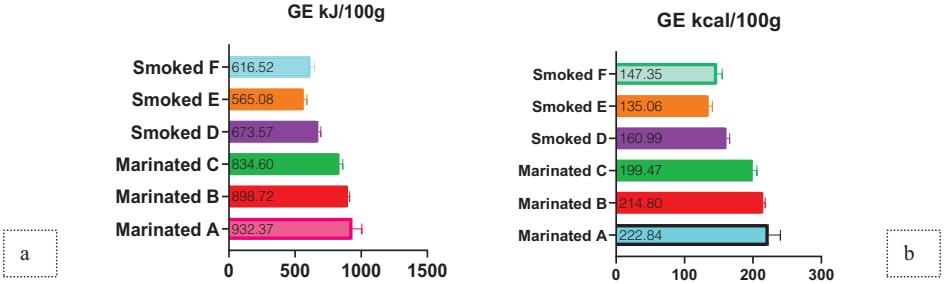

Figure 9. The gross energy content of marinated and smoked herring: a) kJ/100 g; b) kcal/100g

The statistical differences on chemical composition and energy value of marinated and smoked herring (Table 7) were preponderant significant, distinct significant and highly significant ($p<0.001$), with the exception of proteins and salt content where was found mostly not significant differences.

Table 7. The statistical significance of the differences on chemical composition and energy value of marinated and smoked herring (P value)

ANOVA	Lipids	Proteins	Collagen	Water	Ash	Salt	D.M.	O.S.	GE kcal/100g
A-B	<0.0001	0.0046	0.1751	0.9863	0.9738	0.9851	0.0696	0.1803	0.7156
A-C	0.0045	0.3785	0.1217	0.5206	0.9963	0.8484	0.0039	0.0127	0.0152
A-D	<0.0001	0.9246	0.0006	<0.0001	0.1367	0.3966	<0.0001	<0.0001	<0.0001
A-E	<0.0001	0.0864	<0.0001	<0.0001	0.0217	0.1041	<0.0001	<0.0001	<0.0001
A-F	<0.0001	0.3064	<0.0001	<0.0001	0.0191	0.9829	<0.0001	<0.0001	<0.0001
B-C	0.0025	0.9988	0.9950	0.8191	>0.9999	0.4714	0.5519	0.5946	0.1686
B-D	<0.0001	0.5481	0.0935	<0.0001	0.3732	0.1089	<0.0001	<0.0001	<0.0001
B-E	<0.0001	0.6889	<0.0001	<0.0001	0.0024	0.2709	<0.0001	<0.0001	<0.0001
B-F	<0.0001	0.8529	<0.0001	<0.0001	0.0012	>0.9999	<0.0001	<0.0001	<0.0001
C-D	<0.0001	0.9529	0.3942	0.0033	0.4122	0.9864	<0.0001	0.0010	<0.0001
C-E	<0.0001	0.5762	<0.0001	0.0005	0.0130	0.0117	<0.0001	<0.0001	<0.0001
C-F	<0.0001	0.9739	<0.0001	0.0042	0.0119	0.4010	<0.0001	<0.0001	<0.0001
D-E	0.1991	0.3554	0.0068	0.9887	<0.0001	0.0009	0.9552	0.4074	0.0015
D-F	0.0004	0.3062	<0.0001	0.9665	<0.0001	0.0642	0.8817	0.7831	0.1154

Moisture content is one of the determining factors for storage. Low moisture content is a good indicator against spoilage.

Protein and lipids contents of the smoked herring obtained by Atanda et al. 2015, were lower compared to the unsmoked fish. This loss may arise from the denaturing and exudation of protein occasioned by the loss of essential amino acid as lysine, which is labile at heat during the smoking process. Carbonyls present in the smoke might have reacted with lysine which caused the reduction. This is consistent with previous studies (Atanda et al, 2015; Oluwaniyi et al. 2009; Karthikeyan et al., 2012). The loss of lipids in the smoked fish in contrast to the unsmoked fish could also be due to exudation which agrees with a previous report (Akineye et al, 2007). Thus, high lipids content might lead to increase rancidity in the course of storage (Atanda et al., 2015).

Wianecki, 2007, found lower proteins content in fresh herring (15.4%), similar content of lipids (8.2%) and higher content of water (74.4%) that in this study. The same author found in frozen herring small differences in proteins content 15.6%, lipids 8.4% and water 73.9% (these being closer of those determined in this study).

Only in the last decade, research has focused on the beneficial health effects of fish protein in human nutrition (Rudkowska et al., 2010; Pilon et al., 2011).

Studies related to inflammation, metabolic syndrome, osteoporosis, insulin resistance, obesity-related comorbidity and development of cancer have been executed and fish protein, peptides or hydrolysates have shown of

importance in nearly as many areas as fish lipids. For example, a sardine protein diet showed to lower insulin resistance, improved hyperglycemia and decreased adipose tissue oxidative stress in rats with induced metabolic syndrome (Madani et al., 2012).
The authors suggested fish protein as a possible prophylaxis against insulin resistance (Khalili Tilami and Sampels, 2017).

CONCLUSIONS

The most important differences between the marinated herring products analyzed, have targeted the content of lipids, with difference of 3% (13.10% lipids for product C compared with 16.11% lipids for product A). Between the two product categories analyzed were very significant differences, marinated herring having over 50% more fat for product A than for the smoked one (5.54% for E and 7.45% for F product); must be taken into account that the marinated herring was in the form of medallions, richer in fat, compared to the smoked one (fillet), eventually losses of fat from smoking process; and the season when the fish was captured.

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FISH MEAT CONTAMINATION WITH HEAVY METALS – A REAL CONCERN FOR THE FOOD CONSUMPTION

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Abstract

As a result of the increasingly concern of people regarding the food safety, different public media focused on the data collected from reference laboratories inducing a false perception regarding the threat of sea food consumption. Starting with the Minamata disaster more and more people became worried about the sea water pollution with heavy metals. Using the reference data of Commission Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs and the Codex Alimentarius, 21 fish species from sea water or freshwater were studied for Cd, Mg and Pb. The data were collected from specialized laboratories during the following years: 2016 and 2017. The obtained results were in the acceptable limits for European standards. For 67 of 110 samples the value of LOD/LOQ could not be determined. These data are correlated with the water tests made in the last years, which shows a general decrease of the heavy metal pollution in sea waters.

Key words*: food safety, pollution, water.*

INTRODUCTION

Metals such as Fe, Zn, Cu, Mn are chemical elements indispensable to aquatic organisms acting as essential catalysts in metabolic processes (Canli and Atli, 2003). On the other hand, non-essential metals such as Pb, Hg and Cd, which have no vital role for these organisms, in excessive levels, in ecotrophic systems can lead to serious health problems in humans.

Extractive activities, coal-fired power plants, deforestation, animal farming are sewage are sources of release of these metals into the environment (WHO, 1996).If heavy metals enter in the food chain, especially in the aquatic species, they can be harmful to human health (Goyer, 1997; Copat, 2012; Weiss, 2014). Most vulnerable are the young organisms: fetuses, babies, preschoolers (Giles, 1988; Waalkes, 2000; El-Moselhy, 2014).

The levels of heavy metal accumulation in fish depend on the growth rate, metabolism, feeding pattern and ecological requirements of a given fish species (Uluozlu, 2007; Tüzen, 2003). Taking into account the increasing demand for fish meat-imports in Romania have increased tenfold in the last fifteen years-large quantities of fish and seafood have been brought from the waters adjacent to Asian countries with urban and industrial explosion (Noor, 2014; Rohasliney, 2014).

These are the geographical areas most exposed to heavy metal contamination. As a consequence, the attention of consumers to food safety increases as the information media are presented on this topic.

MATERIALS AND METHODS

Muscle tissue of fish (dorsal muscle) was used in this study because it is the major target tissue for metal storage and is the most edible part of the fish. The study was followed in two years, 2016 and 2017, on 14 species of fish from sea waters or inland waters. For year 2016 the included species are listed in the table: *Acipenser gueldenstaedtii, Cyprinus carpio,*

Gadus macrocephalus, Hypophthalmichthys molitrix, Liza aurata, Merluccius merluccius, Pangasius pangasius, Salmo salar, Salmo trutta, Sander lucioperca, Sardina pilchardus, Scomber scomber, Sparus aurata. For year 2017 the included species are listed in the table: *Acipenser gueldenstaedtii, Alosa immaculata, Clupea harengus, Cyprinus carpio, Dicentrarchus labrax, Esox lucius, Gadus macrocephalus, Hypophthalmichthys molitrix, Hypophthalmichthys nobilis, Liza aurata, Salmo salar, Salmo trutta, Sander lucioperca, Sardina pilchardus, Scomber scomber, Sparus aurata, Thunnus albacares.*

A total of 72 fish were analized using the Analytical Methods for Atomic Absorption Spectrometry with graphite furnace for Pb and Cd (Kito, 1986; Endo, 1995) and Cold Vapor Atomic Absorption Spectroscopy or CVSAA for Mg. The Thin Layer Chromatography or TLC, which is an old method, was used for economical reasons, as it is known to be a money saving analyses.

The mercury content was also determined by SAACV technique.

The AAS spectrometer measures the absorbance of a component-specific (HCL, EDL) absorber that is directly proportional to the metal concentration in the sample and read on the calibration curve previously plotted. CVSAA - Atomic Absorption Spectrophotometry is based on the determination of the concentration of a chemical element in the sample to be analyzed (Perkin, 1996). The samples subjected to the analysis were properly processed (chopping, crushing, homogenization) so as to achieve a uniform and homogeneous mass according to SR EN 13804/2013. Any kind of impurification were excluded during processing. The sample thus processed was stored in tightly closed plastic bags. The second step was the mineralization of samples. A suitable amount of sample, between 0.2-1.0 grams was placed in the reaction vessel and added 6 ml concentrated nitric acid and 2 ml hydrogen peroxide. A mercury / or MRC test and a blank test of the reagents was performed. After the reaction was stabilized (about 20 minutes), the reaction vessel was closed well and placed in the digester (Wet Mineralization Equipment with Pressure and Controlled Temperature). At the end of the program, the reaction vessels were allowed to cool in the oven for 20 minutes. The extract was carefully filtered into graduated tubes, yielding an appropriate volume depending on the matrix.

The digestion vessels were washed, decontaminated with 10% v / v soil nitric acid and passed through a short digestion program for additional decontamination. From these sample solution thus obtained there were also determined the elements: Cd, Pb.

For determination of mercury with SAACV it was drawn the curve representing absorbance obtained by concentration.

The clean porcelain crucible dried and stored in the desiccator was tilted to the analytical balance, then a suitable amount of sample was placed in it and weighed again. All weightings were performed with an accuracy of 10 mg and noted in the work book. For high-water matrices, pre-drying is at the oven.

The product crucibles or capsules were placed on the asbestos screen or on the lonely triangle and burn to the small flame of the cooker to the charcoal stage. The resulting ash was uniform in color (white or gray) and no more black coal. In parallel, a blank test was prepared, with mineralization reagents and a sample enriched with analytes to be detected.

For determination of mercury with SAACG – SAA the technique with atomization in graphite furnace - SAA-CG was performed.

An optimal graph was established, taking into account, in particular, the temperature and time parameters decomposition, temperature and atomization time. Aspiration according to the working diagram of the blank sample solution, the MRC solution / fortified sample, the sample set from which a double sample and the MRC / fortified sample at the end of the sample set read, the concentration was recorded.

From the same solution of the sample, the other elements were determined under the same conditions, using the standard solutions, the specific cathode lamps and the parameters specific to each lamp (FAO– Faolex, 2003).

RESULTS AND DISCUSSIONS

Table 1. LOD and LOQ values of heavy metal

Year	Matrix denomination	Chemical element	Results	Val. LOD/ LOQ mg/kg
2016	Scomber scomber	Cd	0.018	
2016	Salmo salar	Pb	0.023	
2016	Scomber scomber	Cd	0.029	
2016	Salmo salar	Pb	0.036	
2016	Hypophthalmichthys molitrix	Pb	<LOD	0.002
2016	Merluccius merluccius	Pb	0.012	
2016	Merluccius merluccius	Cd	0.008	
2016	Merluccius merluccius	Hg	0.026	
2016	Scomber scomber	Pb	0.019	
2016	Scomber scomber	Cd	0.024	
2016	Scomber scomber	Hg	0.076	
2016	Merluccius merluccius	Pb	0.016	
2016	Merluccius merluccius	Cd	<LOD	0.001
2016	Merluccius merluccius	Hg	0.015	
2016	Salmo trutta	Pb Cd Hg	<LOD <LOD 0.012	0.002 0.001
2016	Sander lucioperca	Cd	<LOD	0.001
2016	Pangasiuspangasius	Cd	<LOD	0.003
2016	Sparus aurata	Cd	0.003	
2016	Liza aurata	Cd	0.007	
2016	Scomber scomber	Hg	0.026	
2016	Acipenser gueldenstaedtii	Hg	0.020	
2016	Hypophthalmichthys molitrix	Cd	<LOD	0.001
2016	Liza aurata	Cd	<LOD	0.001
2016	Sardina pilchardus	Cd	0.022	
2016	Gadus macrocephalus	Cd	0.018	
2016	Sparus aurata	Cd	0.006	
2016	Salmo trutta	Cd	<LOD	0.003
2016	Cyprinus carpio	Cd	<LOD	0.001
2016	Cyprinus carpio	Pb	<LOD	0.002
2016	Salmo trutta	Pb Cd Hg	<LOD <LOD 0.011	0.002 0.001
2016	Cyprinus carpio	Pb Cd Hg	<LOD <LOD 0.010	0.002 0.001
2017	Alosa immaculata	Hg	0.063	
2017	Salmo trutta	Pb Cd Hg	0.029 <LOD 0.010	0.002 0.003
2017	Sardinapilchardus	Cd	0.024	
2017	Salmo salar	Pb	0.037	
2017	Dicentrarchus labrax	Hg	0.031	
2017	Sparus aurata	Cd	0.006	
2017	Sparus aurata	Hg	0.023	
2017	Hypophthalmichthys nobilis	Pb	0.029	
2017	Salmo salar	Pb	0.03	
2017	Scomber scomber	Hg	0.04	
2017	Hypophthalmichthys nobilis	Cd	<LOD	0.001
2017	Sparus aurata	Cd	<LOD	0.001
2017	Scomber scomber	Cd	<LOD	0.001
2017	Liza aurata	Cd	<LOD	0.001
2017	Salmo trutta	Pb Cd Hg	0.03 0.017 <LOD	 0.010
2017	Cyprinus carpio	Pb Cd Hg	0.01 <LOD 0.01	 0.001
2017	Sander lucioperca	Cd	<LOD	0.003
2017	Thunnus albacares	Pb Cd	<LOD 0.012	0.002
2017	Acipenser gueldenstaedtii	Hg	0.028	
2017	Sparus aurata	Cd	0.02	
2017	Sardina pilchardus	Cd	0.018	
2017	Scomber scomber	Cd	0.011	
2017	Salmo salar	Pb	<LOD	0.002
2017	Dicentrarchus labrax	Hg	0.047	
2017	Salmo trutta	Pb Cd Hg	<LOD <LOD <LOD	0.002 0.001 0.010
2017	Sparus aurata	Cd	0.04	
2017	Sparus aurata	Hg	0.024	
2017	Clupea harengus	Hg	0.02	
2017	Scomber scomber	Hg	0.024	
2017	Salmo salar	Pb	<LOD	0.002
2017	Salmo trutta	Pb Cd Hg	<LOD <LOD <LOD	0.002 0.003 0.010
2017	Cyprinus carpio	Pb Cd Hg	0.007 <LOD <LOD	 0.003 0.010
2017	Esox lucius	Hg	0.1	
2017	Cyprinus carpio	Pb Cd Hg	<LOD <LOD <LOD	0.002 0.001 0.003
2017	Salmo trutta	Pb Cd Hg	0.02 <LOD <LOD	 0.001 0.010
2017	Gadus macrocephalus	Cd	<LOD	0.001
2017	Gadus macrocephalus	Cd	<LOD	0.003
2017	Hypophthalmichthys molitrix	Pb Cd Hg	0.03 <LOD <LOD	 0.001 0.010
2017	Cyprinus carpio	Cd	<LOD	0.001
2017	Sardina pilchardus	Cd	0.01	
2017	Cyprinus carpio	Cd	<LOD	0.001

To evaluate the correct values, any analysis needs to be calibrated. Due to the extremely low working values, the errors due to the devices are frequent, which is why blank samples are essential. Each time the standard deviation was established and the validation criteria were those specified in Regulation (EC) No. 333/2007 establishing the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo (a) pyrene in foodstuffs.

In the course of sampling, precautions shall be taken to avoid any changes which would affect the levels of contaminants, adversely affect the analytical determination or make the aggregate samples unrepresentative (Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed).

LOD is the smallest amount of substance that can be distinguished from the absence of that substance with a declared level of confidence (generally 99%).

LOQ is the minimum amount of substance that can be quantitatively determined with appropriate precision: the limit to which the difference between two distinct values can be reasonably discussed.

LOD, Limit of Detection, minimum detectable value, detection limit, CCβ (term used in the EU directives)

Determine the standard deviation (s) of ten independent measurements of a blank sample or of a sample with very low concentrations of the measuring. Limit of detection = LOD = s*3.3

LOQ, quantification limit, limit of quantitation, limit of determination, reporting limit, limit of reporting and application limit.

Determine the standard deviation (s) of ten independent measurements of a blank sample or of a sample with very low concentrations of the measure and Limit of quantitation = LOQ = s*10.

Consider the fitness of purpose of using the concentration at which imprecision (coefficient of variation) of the method is 5%.

Regulation (EU) 333/2007 specifies the LOD as 3 times the standard deviation of the mean of blank determinations and LOQ as six or 10 times the standard deviation of the mean of blank determinations (ISO (2000). ISO 11843-2). Interpretation of analysis results with LOD and LOQ can be noticed in the Figure 1.

The Table 1 shows the LOD and LOQ values for the three elements surveyed.

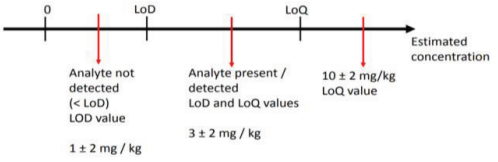

Figure 1. Analysis results with LOD and LOQ

The laboratory test must be homogeneous and representative, with no secondary contamination.

The LOD/LOQ report is interpreted according to the displayed result. If the absolute value displayed has the third decimal (0.00xx), then the result is not taken into account in the contamination of heavy metal fish. For Pb the maximum permissible level in food is 0.30 mg/kg, for Cd is 0.50 mg/kg, for Hg is 0.50 mg/kg.

CONCLUSIONS

Although the principle of biomagnification is demonstrated in many species in the animal world, substances resulting from human activities have different degrees of assimilation and cumulation. The heavy metal toxicity is the result of their binding to the important enzyme systems in the animal cell or certain cell membrane components.

Methylmercury is known to form in aquatic ecosystems via bacterial methylation of inorganic mercury. Methylmercury is excreted slowly over a period of several months, mostly as inorganic mercury in the faeces. It may take 45–70 days for the methylmercury concentrations to fall by a half in a person's blood, and 70–80 days in the entire body, but substantial variations in time-scale can occur (Nielsen and Grandjean, 2000). The need to research the concentration of heavy metals in food was obvious because the accumulation of heavy metals could impact health hazards to human. Following the study above, it can be observed that the concentration of mercury, lead, and cadmium of all analyzed fish types is lower than the maximal allowed concentration (MAC) by the legislation in force, which shows that the presence of heavy metals in the body of the fish (higher concentrations are recorded in the skin and their liver, and smaller in the white muscles) it is more a press topic than a reality.

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ON THE OCCURRENCE OF POTASSIUM SORBATE (E202) IN CERTAIN FOOD AND BEVERAGE PRODUCTS

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Abstract

Potassium sorbate (E202) is one of the food additives approved by the European Union to be used as preserving agent with antibacterial and antifungal actions in certain food groups, such as the ones investigated in this study: jams, jellies, marmalades (apricot jam) and aromatized alcoholic beverage (ciders). Six commercial products (three from each group) were investigated through spectrophotocolorimetric methods to identify and quantify the usage of the potassium sorbate. The inclusion level of E-202 in the first food category (apricot jam) was 28.4-47.2% lower than the maximum allowed inclusion level (MAIL) in product (100 mg potassium sorbate/100 g jam). The estimated daily intake through a portion of jam (100 g) represented 6.7-7.4% of the maximal allowed daily intake (MADI) in children and 3.1-4.0% in adults, respectively. The potassium sorbate concentration in Apple cider product was 51-52% below the MAIL (20 mg additive/100 ml), while the consumption of one portion of such beverage (660 ml, equivalent of two bottles) would lead to an intake of 3.93-4.84%, compared to MADI, in adults. Although the potassium sorbate has lower toxicity, compared to other commonly used antiseptic food additives (sodium nitrite and sodium benzoate), it still represents a risk factor in certain consumers known with immunity issues, because is a factor involved in the onset of certain allergic reactions localized in the upper respiratory and digestive tracts, as well as in certain contact dermatitis (cutaneous eruptions, itching etc.). Therefore, the cumulative consumption of the foods containing the E202-potassium sorbate should be avoided.

Key words*: potassium sorbate, apricot jam, apple cider, maximum allowed daily intake.*

INTRODUCTION

The spectacular development of food industry, in terms of manufacturing quantities, varieties of types of foods and length of distribution logistic chains rendered an increased role of food additives in the processing industry, because they provide safety in usage and long shelf life. The natural ingredients, used in the past both for spicing, coloring and preserving were replaced by synthetic substances, easier and cheaper to produce, store, use and also more stable at different treatments (Banu, 2010).

Food additives are known to preserve products quality and safety throughout a longer period of time, improve products taste, ensures the control of acidity and alkalinity of products, maintain the texture, flavor and color of food. It is not to be neglected that the usage of food additives plays a serious role in influencing consumers' choices and preferences, especially of those related to sensorial features. Among the additives most used to preserve food, benzoic acids and its salts, sorbic acid and its salts, as well as sodium and potassium nitrites and nitrates are the most used ones (Ciobanu, 2003).

Sorbic acid and the potassium sorbate are part of an additives group playing antiseptic role in food preserving, therefore protecting consumers against microbiological hazards that could be transmitted through food. However, according to certain studies (Cho et al., 2000) such additives could be incriminated in developing side effects in laboratory animals used as animal model in certain studies simulating the response of the human consumers to food preserved in such manner. The main undesired effects occur in upper respiratory ways due to nasal epithelia squamation, to microvascular inflammation, to pronounced edema.

Potassium sorbate generated genotoxic effects in some in-vitro studies on human lymphocytes: chromosomal aberrations and interchromatidic mutations, DNA disaggregation and decrease of cell division speed. Such effects could be involved in the carcinogenic processes, mostly when the additive exerts its

action onto the immune system cells (Mamur et al., 2010).
According to other authors (Luck and Jager, 1995) the chronic consumption of food containing sorbates and benzoates or sorbic acid and benzoic acid could induce the occurrence of generalized inflammatory status, visible through clinic signs such as dermatitis (itching), respiratory difficulties of asthma type and even anaphylactic shock in consumers with allergic issues to food additives. The consumption of foods belonging to dressings and sauces, very rich in antiseptic additives like sorbic acid and its salts with potassium and sodium induced peri-oral dermatitis – like effects (itching, edema, redness) in pre-scholar children (Maritim and Universitäts medzin, 2010). However, other authors underlined that an absolute correlation between the intake of foods preserved with sorbic acid and its salts and the side effects like local inflammatory responses could not be formulated. However, when systemic responses like toxicity syndromes occur, the sorbates act in synergy with other food preservatives (eg. nitrites) (Walker, 1990).
Heating and high temperature storage could induce genotoxicity and cell-transforming capacities of the food containing sorbic acid and its salts with sodium and potassium, especially when these additives are used in mixture with nitrites (Piper and Piper, 2017).
In accordance with the F.A.O. Codex Alimentarius (FAO, 2011) and the EU regulations (European Comission, 2011, 2012), the sorbic acid and its salts could be used in several food categories, with specified maximal inclusion limits. For instance, the potassium sorbate (E202, the subject of this study) could be used up to: 200 mg / liter in wines, cider, acidulated fermented beverages made of fruits, mead, beverages with less than 15% alcohol; 300 mg / liter in nonalcoholic aromatized beverages (dairy products excluded); 1000 mg / kg in fillings for ravioli and other similar products, dried fruits, sauces based on fruits and vegetables, jams, jellies and marmalades, olives and derivate, cheese and cheese with added food products, dehydrated egg products kept refrigerated or frozen; 2000 mg/kg in melted cheese, pre-packed sliced bread. Also, the Maximal Allowed Daily Intake of potassium sorbate (E202) was limited to 25 mg/kg body weight.

MATERIALS AND METHODS

The purpose of this study was to investigate two categories of food in which the usage of E-202 – Potassium sorbate is allowed: (a) jams, jellies, marmalades and (b) wines, cider and other fruits fermented acidulated beverages, mead.
Three commercial products were chosen from each category and coded jam A, jam B, jam C (apricot jam), respectively cider A, cider B, cider C (apple cider), because the purpose of the study was to test only the occurrence and quantify the concentration of E202, regardless the brand..
The used analytical method was in accordance with the AOAC 960.38 protocol (benzoic acid and its salt/preservatives assessments in Beverages and Beverage Materials/Beverages containing small amounts of alcohol, Beverages and Beverage Materials/Soft Drinks, Fruits and Fruit Products/Jelly, Vegetables/Catsup, Fruits and Fruit Products/Jam), applied on a UV-VIS VWR UV-6300PC double beam spectrophotometer. After the calibration of the curve, using an aqueous solution of 0.2% potassium sorbate, there were run 20 readings per investigated sample at the wavelength of 250 nm.
The acquired values were statistically processed to obtain the main statistical descriptors (mean, standard deviation, standard mean error, coefficient of variation). The data was afterwards compared with the maximal admitted inclusion limit (MAIL) for each food category and with the maximal allowed daily intake (MADI) for each consumer category (child of 30 kg body weight, adult woman of 55 kg, adult man of 65 kg). The size of the daily consumed portions was estimated accordingly to the consumption behaviors: apricot jam, 10 teaspoons = env. 100 g (consumed by both children and adults); apple cider: 2 bottles of 330 ml = 660 ml (consumed by adults only).

RESULTS AND DISCUSSIONS

The analytical results for the apricot jam product are presented in Table 1 and Figure 1.

It could be noticed that the limits of potassium sorbate inclusion were not exceeded, compared with the rate legally regulated (100 mg E-202/100 g product).
Thus, in the first analyzed product – Jam A, the analytical values varied between 54-56 mg potassium sorbate/100 g, resulting a mean of 55.40±0.18 mg potassium sorbate/100 g jam, thus 55.4% of the legal limit.

Table 1. Average values of potassium sorbate (E-202) content in the three foods analysed from the group jams, jellies, marmalades

Product	Analytical values			MAIL* (mg/100 g)	% of MAIL
	$\bar{X}$	$\pm s_{\bar{x}}$	CV%		
Jam A	55.40	0.18	1.48	100	55.40
Jam B	50.60	0.23	2.07	100	50.60
Jam C	54.20	0.17	1.42	100	54.20

* MAIL = maximal allowed inclusion level (mg/100 g product)

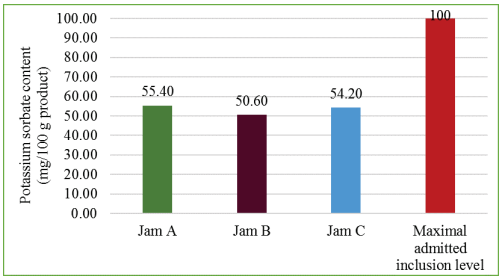

Figure 1. Potassium sorbate inclusion in the analysed samples from jams, jellies and marmalades category

The jams produced by other companies presented lower levels of potassium sorbate in product B (50.60±0.51 mg/100 g) or close ones, in product C (54.20±0.37 mg/100 g), hence the inclusion rates between 50.6-54.2%, comparing to the legal limit.
Starting from an estimated consumed portion of 100 g / day (equivalent of circa 10 teaspoons), the data related to the daily intake dose were obtained, referring to the three consumers' categories (children and both genders adults) (Table 2 and Figure 2).
In children weighing 30 kg, daily ingested dose is between 1.687 and 1.847 mg potassium sorbate per kg body weight, intake that represented 6.7-7.4% of the Maximal Admitted Daily Intake (25 mg E202/kg body weight). In adults, these doses were even lower, reaching 0.920-1.007 mg E-202/kg body weight in women (3.68-4.03% of the MADI), respectively 0.778-0.852 mg potassium sorbate/kg body weight in men (3.11-3.41% of the MADI regulated for the studied additive).

Table 2. Calculation of daily ingested dose of potassium sorbate (E-202) through the three food products from the category jams, jellies and marmalades

Daily ingested dose, related to consumer type	Product		
	Jam A	Jam B	Jam C
Maximal allowed daily intake (MADI) (mg potassium sorbate/kg body weight)	25	25	25
Child, 30 kg body weight(mg potassium sorbate/kg body weight)	1.847	1.687	1.807
% of MADI	7.4	6.7	7.2
Adult, woman, 55 kg body weight(mg potassium sorbate/kg body weight)	1.007	0.920	0.985
% of MADI	4.03	3.68	3.94
Adult, man, 65 kg body weight(mg potassium sorbate/kg body weight)	0.852	0.778	0.834
% of MADI	3.41	3.11	3.34

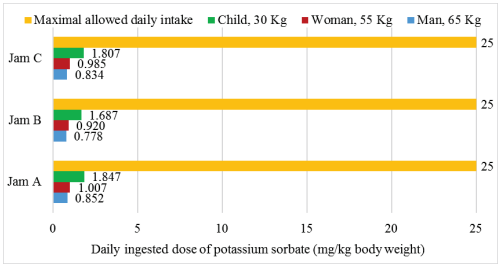

Figure 2. Daily ingestion of potassium sorbate through the consumption of 100 g portion of apricot jam

Although the intake through the apricot jam was quite low when compared to the MADI, the cumulative intake must be taken into account, especially in children alimentary patterns, knowing that E202 is also allowed to be included in several foods and beverages (flavored nonalcoholic beverages, pasta filling, sauces, olives, cheeses, egg products, sliced pre-packed bread etc.).
The second studied group of products is designed exclusively for adults, knowing the cider contains environ 4-5% alcohol. The results related to the usage of potassium sorbate in the three analyzed commercial products are presented in table 3 and figure 3.

Table 3. Average values of potassium sorbate (E-202) content in the three products analysed from the group wines, cider, acidulated fermented beverages made of fruits, mead

Product	Analytical values			MAIL* (mg/ 100 ml)	% of MAIL
	$\bar{X}$	$\pm s_{\bar{x}}$	CV%		
Cider A	9.80	0.17	7.83	20	49.00
Cider B	9.60	0.11	5.24	20	48.00
Cider C	9.80	0.09	4.19	20	49.00

* MAIL = maximal allowed inclusion level (mg/100 ml product)

In the Cider A product, the analytical values felt within the 9-11 mg/100 ml interval,

resulting an average potassium sorbate content of 9.80±0.37 mg/100 ml, reaching thus 49% of the Maximal Allowed Intake Level (20 mg potassium sorbate/100 ml). For the other studied cider brands, the potassium sorbate content measured in laboratory was found between 9.60±0.24 mg/100 ml and 9.80±0.20 mg/100 ml, that meant 48-49% of the regulated MAIL.

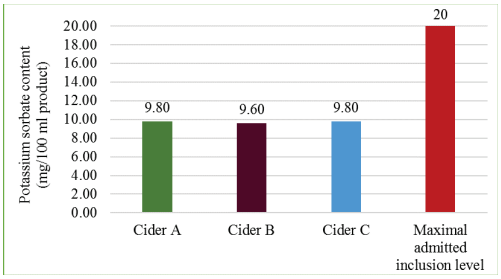

Figure 3. Potassium sorbate inclusion in the analysed samples from wines, cider, acidulated fermented beverages made of fruits, mead category

In order to estimate the daily intake dose of potassium sorbate (Table 4 and Figure 4), the consumption portion was considered to be 660 ml (2 bottles of 330 ml), knowing that apple cider is rather drunk as refresher beverage than like an alcoholic beverage itself.

Table 4. Calculation of daily ingested dose of potassium sorbate (E-202) through the three products from the category wines, cider, acidulated fermented beverages made of fruits, mead

Daily ingested dose, related to consumer type	Product		
	Cider A	Cider B	Cider C
Maximal allowed daily intake (MADI) (mg potassium sorbate/kg body weight)	25	25	25
Adult, woman, 55 kg body weight(mg potassium sorbate/kg body weight)	1.187	1.162	1.211
% of MADI	4.75	4.65	4.84
Adult, man, 65 kg body weight(mg potassium sorbate/kg body weight)	1.004	0.984	1.025
% of MADI	4.02	3.93	4.10

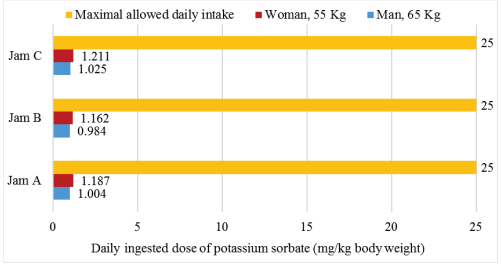

Figure 4. Daily ingestion of potassium sorbate through the consumption of 660 ml portion of apple cider

Thus, for the considered consumption rate (660 ml cider), the calculated values of the daily intake dose oscillated between 1.162 and 1.211 mg potassium sorbate/kg body weight in women, respectively between 0.984-1.025 mg potassium sorbate/kg body weight in men.
In comparison with the MADI regulated for the studied additive, the daily intake doses represented 4.65-4.84% in women and 3.93-4.10% in men consumers. Although the potassium sorbate has a lower systemic toxicity in humans, comparing with other antiseptic additives frequently used in food and beverages (sodium nitrite and sodium benzoate), it should be remembered that it is still a risk factor in triggering certain allergic reactions localized in the respiratory tracts as well as in certain contact dermatitis (skin rushes, itching etc.).

CONCLUSIONS

The potassium sorbate level of inclusion in the apricot jam was 45.8-49.4% lower than the maximal admitted level (100 mg E202/100g product).
The daily ingested dose through a portion of apricot jam (100 g) represented 6.7-7.4% comparing to the maximal admitted daily intake in children, respectively 3.1-4.0% in adults.
The measured concentration of potassium sorbate in apple ciders was 51-52% below the maximal admitted inclusion level (20 mg E202/100 ml).
Consuming a portion of apple cider (660 ml) resulted in reaching 3.93-4.84% of the maximal tolerated daily intake in adults.
All levels felt within the legal limits. However, it must be noticed that in certain products, the labeling was not honest, as all products contained the investigated additive in certain concentrations and only a few producers mentioned it as ingredient in their product.

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USAGE OF HISTOLOGICAL AND RHEOLOGICAL TECHNIQUES IN ASSESSMENT AND PREDICTION OF MEAT TEXTURAL PROPERTIES

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Abstract

The research carried on a comparative assessment of chicken meat texture using both histological and instrumental – rheological techniques, in order to find out certain correlations between the methods and the possibility to predict one in relation with another. Therefore, 50 samples of Pectoralis major and Pectoralis minor muscles, issued from 50 individuals of Cobb-500 chicken broilers were submitted to paraffin inclusion technique followed by microscopic measurements (Motic Image 3+ software after image acquiring at 400 x magnification), respectively to rheological analysis, using a common Adams consistometer and a Perten Instruments texturometer with Warner-Bratzler shear chamber. The histometric values in Pectoralis minor muscles oscillated between 25.18 and 44.37 µm thickness of myocites, a muscle density of 579-683 fibers/sqmm of muscle and a proportion of pure muscular tissue of 62.51% vs. 37.49% connective elements. In Pectoralis major muscles, the proportion of pure muscular tissue reached 59.83% while the connective compounds represented 40.17%, which corresponded to a density of 405-669 fibers/sqmm of muscle and to a myocites thickness within the 28.63-47.19 µm limits. These findings were consistent and highly positive correlated (0.89) with the Warner-Bratzler shear force values (47.10 Newtons in Pectoralis major and 42.63 Newtons in Pectoralis minor muscles) or moderate negatively correlated (-0.53) with the Adams consistency (1,8 Adams units in M. Pectoralis minor and 1,2 Adams units in M. Pectoralis major). Therefore, shear force instrumental assessments could be used as predictors for the histological properties of the meat, including for the tissual composition of muscles (pure muscular vs. connective tissue participation in meat structure).

Key words*: COBB-500, myocytes, thickness, density, shear force, texture.*

INTRODUCTION

Textural properties (tenderness, juiciness) were always considered key point quality and consumer acceptance indicators, because they influence directly the sensorial and technological traits of the meat (Schilling et al., 2003; Zhuang et al., 2007). It was reported that meat texture, especially in poultry, varies due to several influential factors, such as: genotype, due to higher firmness and lower tenderness in pure breeds and slow growing broilers (Wattanachant et al., 2004); selection against certain physical properties of the meat, such as the ultimate pH value (Alnahhas et al., 2015); age at slaughter (Coro et al., 2002); type of culling prior to slaughter and of post-mortem electrical stimulation (Froning and Uijttenboogaart, 1998); farming system, i.e. conventional vs. organic (Grashorn and Serini, 2006); type of applied heat and pressure treatments onto meat (Zamri et al., 2006; Del Omo et al., 2010; Kruk et al., 2011); certain abnormal meat rheological conditions (pale, soft exudative-PSE; dry, firm dark meat–DFD, wooden breast condition-WBC, white stripping-WS) (Barbut et al., 2005; Zhang and Barbut, 2005; Chatterjee et al., 2016; Sanchez et al., 2016); meat chemical composition, including collagen levels (Chumngoen and Tan, 2015). Also, meat texture is directly related to live development of animals and, subsequently, to carcass weight, because the growing process influences both the size of myocytes as well as the ratios between pure muscular and connective tissues in both domestic and wild animal species (Żochowska et al., 2005;Petracci and Cavani, 2012). Muscle

ultrastructure, assessed through conventional histometric techniques was longtime used as predictor of meat texture (Stanley and Swatland, 1976), along with sensorial descriptive panel testing (Lyon and Lyon, 2000) and with several objective instrumental methods, such as needle Instron puncture, Allo–Kramer, Warner–Bratzler and razor blade shears tests (Cavitt et al., 2005; Xiong et al., 2006; Luckett et al., 2014). Within this conjuncture, we aimed to test two methods (conventional cyto-histometry and Warner Bratzler shear test) in order to identify the correlations between the results and to find out if both tests could be used together to better predict poultry texture traits, as well as the tissual composition of muscles.

MATERIALS AND METHODS

Fifty samples of *Pectoralis major* and *Pectoralis minor* muscles, issued from 50 individuals of Cobb-500 chicken broilers (conventional farming system, slaughtered at 42 days) were submitted to shaping in 1.5 x 1.5 x 0.3 cm (Width x Length X Thickness) pieces and included in histological processing cassettes. The remnants of the samples were shaped, along the muscle fibres growth direction, in cubic blocks of 1.5 x 1.5 x 1.5 cm (Width x Length X Thickness) and kept in refrigerator throughout 24 hours, in order to allow maturation, then they were submitted to textural instrumental analysis, (Zhuang and Savage, 2009), using a common Adams consistometer to test deformation and a Perten Instruments TVT 7600 texture analyzer equipped with Warner-Bratzler accesories (Steffe, 1996).

The Adams consistometer was equipped with a dial with concentric circles, graded from 0 (origin) to 10, the gap between two consecutive circle measuring 1 cm, sub-graded in mm. Every cm on the scale represents one Adams unit. Each meat sample was placed in the origin spot then a weight of 500 g was added on top. In relation with the meat texture characteristics, the sample flattened and extended more or less on the dial, as well on the scale. The extension was measured on the consistometer scale and was expressed in Adams units with one decimal. Therefore, as the meat is more tender, the extension on the consistometer is wider. Two samples were tested for each muscle; therefore 100 repetitions were run in order to acquire Adams deformation data.

The Pertentexture analyser was equipped with a straight Warner Bratzler blade and with the appropriate rig - heavy duty stand - to test the cutting strength in a single cycle compression mode. Prior to repeated testing, the probe was accordingly calibrated for cutting parameters (starting distance from sample 5 mm; compression 25 mm; initial and testing speed 2 mm/s; trigger force 5 g, data rate 200 pps). The acquired force curves during tests were recorded through the dedicated software (TexCalc 4.0.2.) and the maximum peak force (Newtons) was considered as the shear force necessary to cut the meat sample. Two samples were tested for each muscle; therefore 100 repetitions were run in order to acquire cutting strength data.

The samples prepared for histology processing were immersed in fixation bath (formaldehyde 10%, at 4°C, throughout 30 days).Then, they were submitted to the paraffin infiltration technique using a spin tissue processor - histology line - THERMOSCIENTIFIC STP-120-2(Pappas, 1994) and following a 3 stages protocol: dehydration in 5 consecutive ethyl alcohol baths (70% ethanol, 1 hour; 95% ethanol, 1 hour; 1st absolute ethanol, 1 hour; 2nd absolute ethanol 1,5 hours; 3nd absolute ethanol, 1.5 hours; 4th absolute ethanol, 2 hours); immersion in clearing agent (1st Xylene bath, 1 hour; 2nd Xylene bath, 1 hour); paraffin impregnation (1st paraffin bath at 58°C 1 hour; 2nd paraffin bath at 58°C, 1 hour).

The resulted samples were transferred into stainless molds, accompanied by an appropriate amount of paraffin, at 58°C, then they were cooled down. The resulted solidified blocks were submitted to micrometric cutting using a rotary automatic microtome - histology line - THERMOSCIENTIFIC HM355S adjusted to 5µm step increment. The resulted slices were mounted in groups of 3 pieces on cleaned histological glass slides. These slides were introduced then in a thermos-regulated oven at 65°C, throughout 20 minutes in order to induce slices bonding on the glass.

Slices were then introduced into a trichromic Masson staining protocol, using an automatic

tissue stainer - histology line - Varistain Gemini AS – THERMOSCIENTIFIC. The procedure comprised 16 steps: deparaffinizing and rehydration using 3 successive baths of absolute, 95% and 70% ethanol; washing in distilled water; re-fix in Bouin's solution at 56°C, 1 hour; rinsing in tap water, 5-10 minutes; staining in Weigert's iron hematoxylin solution, for 10 minutes; rinsing in tap water, 10 minutes; washing in distilled water, 5 minutes; stain in Biebrich scarlet-acid fuchs in solution, 10-15 minutes; washing in distilled water, 5 minutes; immersing in phosphomolybdic-phosphotungstic acid solution, 10-15 minutes; transfer and staining without rinse into aniline blue solution, 5-10 minutes; rinsing briefly in distilled water; immersing in 1% acetic acid solution for 2-5 minutes; washing in distilled water, 2-5 minutes; dehydrating quickly through 95% ethyl alcohol, absolute ethyl alcohol clearing in xylene; mounting square microscopic slides over the stained sample and sealing with resinous reagent.

The resulting stained and fixed smears were analyzed by microscopic measurements (Motic M230 with camera, calibrated with the default objective micrometric scale for 10 x 10 and 10 x 40 ocular x objective associations) and computations (Motic Image 3+ software after image acquiring at 100X and 400X magnify-cation factors) to assess myocytes and 1st order muscular fascicles diameters (µm) and cross section areas (sqµm).

Two hundred and forty-five myocytes and eight 1st order muscular fascicles were analyzed in each muscle.

Myocytes density (number of muscle cells per sqmm of muscle) and proportion of main tissue categories (% pure muscular tissue and % connective tissue) were also calculated. Muscle cells density was obtained using the relation (Radu-Rusu et al., 2007):

$$\text{Myocytes density} = \frac{\text{myocytes amount in MFI x 1000000}}{\text{cross section area of MFI (sqµm)}},$$

where:
MFI = 1st order muscle fascicle
1000000 = multiplication factor (1 sqmm=1000000 µm)

The proportions of pure muscular and connec-tive tissues in muscle structure were calculated using the relation (Radu-Rusu et al., 2007):

$$P_{MT}(\%) = \frac{\Sigma \text{ myocytes cross section areas (sqµm) in MFI}}{\text{cross section area of MFI (sqµm)}}$$

$$P_{CT}(\%) = 100 - P_{MT}(\%)$$

The acquired data were statistically processed to obtain the main descriptors (mean, standard deviation, coefficient of variation - CV%) and running of comparisons between the used methods (one-way ANOVA), as well as to assess the correlation level between the results, using Graphpad Prism 8.0 for Windows software, in accordance with the appropriate methodology for animal science experiments (Kaps and Lamberson, 2014).

RESULTS AND DISCUSSIONS

Breast myocytesdimensional properties are presented in Table 1, as a comparative analysis between *Pectoralis major* and *Pectoralis minor* muscles.

In *Pectoralis major* muscle (superficial pectoral in breast mass), cells thickness varied between 28.36 µm and 47.19 µm, resulting an average diameter of 37.31±0.28 µm. Hence the coefficient of variation was calculated at 11.72%, the homogeneity of the analyzed trait could be considered low.

Table 1. Dimensional features of myocytes in breast meat

Trait	M	Mean	±SME	CV%	Min.	Max.
Thickness (µm)	PM	37.31	0.28	11.72	28.63	47.19
	Pm	36.22	0.25	10.90	25.18	44.37
ANOVA:	PM vs. Pm (distinct significant): 0.001 <*P*(0.003) < 0.01					
Cross section area (sqµm)	PM	1580.22	11.44	11.33	1157.55	1996.14
	Pm	1537.43	12.27	12.49	1065.11	1987.78
ANOVA:	PM vs. Pm (significant): 0.01 <*P*(0.011) < 0.05					

M = muscle, where PM = Pectoralis major, Pm=Pectoralis minor.
SME = Standard error of the mean
CV% = coefficient of variation

Myocytes in *Pectoralis minor* samples (profound pectoral in breast mass), had diameters comprised within the 25.18 µm and 44.37 µm, therefore an average thickness of 36.22±0.25 µm, while the variation was situated, as well, above the 10% homogeneity threshold. The data we found is comparable with the results reported by MacRae et al., 2007, (38.6 µm – 41.6 µm) in a study analyzing the muscle fibers characteristics in the pectorals of three genetic strains of broilers' genitors. The difference of 1.09 µm (3%) between the cells thickness in the two muscles composing the breast meat was distinct significant ($p < 0.01$).

Average thickness values led to a similar distribution of myocytes cross-section areas when the two muscles were compared. Thus, in *Pectoralis major,* the average cross-section area (1580.22±11.44 sqμm) was significantly 2.8% higher than the one measured in *Pectoralis minor* samples (1537.43±12.27 sqμm) ($p<0.05$). All the values measured within the 1157.55-1996.14 sqμm, respectively between 1065.11-1987.78 sqμm intervals presented low homogeneity (coefficient of variation above 10%).Our results revealed lower cross-section areas that the ones reported in other studies run on same muscles (Berri et al., 2007; Petracci et al., 2013; DalleZotte et al., 2017).

Muscle fibers density was higher in the *Pectoralis minor* samples (624.00±14.62 myocites/sqmm) than in the *Pectoralis major* ones (551.86±33.81 myocites/sqmm), an expected fact, hence the fibers are thicker in *Pectoralis major* (Table 2). The differences between means did not pass the α 0.05 significance threshold.

Table 2. Myocytes density and tissue proportions in breast meat

Trait	M	Mean	±SME	CV%	Min.	Max.
Density (myocites /sqmm)	PM	551.86	33.81	16.21	405.00	669.00
	Pm	624.00	14.62	6.20	579.00	683.00
ANOVA:	PM vs. Pm (not significant): 0.05 <*P* (0.073)					
Striate muscular tissue (%)	PM	59.83	1.15	5.10	56.00	64.20
	Pm	62.51	1.41	5.96	58.00	67.10
ANOVA:	PM vs. Pm (not significant): 0.05 <*P* (0.167)					
Connective tissue (%)	PM	40.17	1.15	7.60	35.80	44.00
	Pm	37.49	1.41	9.94	32.90	42.00
ANOVA:	PM vs. Pm (not significant): 0.05<*P*(0.167)					

M = muscle, where PM = Pectoralis major, Pm=Pectoralis minor.
SME = Standard error of the mean
CV% = coefficient of variation

These findings were correlated with the main tissual categories in muscles, knowing that the thinner and more densified are the muscle cells, the less room remains for connective tissue in that particular muscle (Petracci et al., 2013). Thus, pure muscle tissue participation reached 59.83±1.15% in *Pectoralis major* and 62.51±1.41% in *Pectoralis minor* while the connective tissue was higher in the former muscle (40.17±1.15%) than in the latter one (37.49±1.41%). Although these differences were not found as statistically significant ($p>0.05$), the histological findings suggest that the meat of *Pectoralis minor* muscles would have a better texture, with thinner cells and less connective tissue, therefore a better sensorial quality.

The textural instrumental analysis (table 3) on both muscles revealed, as indicated also by the histological findings, a 10.49% lower cutting strength in the *Pectoralis minor* samples (42.63±0.21 Newtons), compared with the *Pectoralis major ones* (47.10±0.33 Newtons). Significant differences were calculated between the two muscles ($p<0.05$). The findings were homogenous, if the lower values of coefficients of variation are considered (5.01-6.97%).

Table 3. Texture instrumental analysis of breast meat samples

Trait	M	Mean	±SME	CV%	Min.	Max.
Shear Force (Newtons)	PM	47.10	0.33	6.97	43.18	52.90
	Pm	42.63	0.21	5.01	38.82	44.70
ANOVA:	PM vs. Pm (significant): 0.01<*P* (0.028) < 0.05					
Adams consistometry (A.U.)	PM	1.2	0.01	6.27	1.1	1.3
	Pm	1.8	0.01	9.34	1.6	2.1
ANOVA:	PM vs. Pm (highly significant): *P* ($5.31x10^{-5}$) < 0.001					

M = muscle, where PM = Pectoralis major, Pm=Pectoralis minor.
SME = Standard error of the mean
CV% = coefficient of variation

Meat extension on the Adams consistometer revealed values higher in *Pectoralis minor* (1.8±0.01 Adams units) and lower in *Pectoralis major* samples (1.2±0.01 Adams units), suggesting a better tenderness in *Pectoralis minor* and a better firmness in *Pectoralis major* ($p<0.001$).

The acquired data related to cutting strength and firmness are consistent to those reported in other similar studies (Xiong et al., 2006; Zhuang and Savage, 2009; Chatterjee et al., 2016).

Certain histological traits were highly correlated with the textural ones: strong positive correlations between the myocytes thickness and shear force (cutting strength) (r=+0.89 in *Pectoralis major* and r=+0.95 in *Petoralis minor*) (Table 4).

Table 4. Correlations between hitsometrical and rheological traits

Muscle	Trait	Shear Force	Adams Consistometry
Pectoralis major	Myocites thickness	r=+0.89	r=-0.53
	Myocites density	r=-0.68	r=-0.29
	Muscular tissue	r=-0.88	r=+0.14
	Connective tissue	r=+0.88	r=-0.14
Pectoralis minor	Myocites thickness	r=+0.95	r=-0.11
	Myocites density	r=-0.86	r=-0.17
	Muscular tissue	r=-0.43	r=+0.05
	Connective tissue	r=+0.43	r=-0.05

Also, the shear force was intense positive (+0.88) or medium positive correlated with the connective tissue proportion. In fact, the higher the connective tissue proportion, the stronger the correlation with the shear force value, knowing that the connective tissue (either specific cells, such as adipocytes or tenocytes, either the extracellular matrix, particularly the collagen) negatively affects the meat textural characteristics, assessed both instrumentally and sensorial (Roy et al., 2006; Nishimura 2010).

On the contrary, shear force was negatively and intense correlated with myocytes density and with the muscular tissue proportion in the analyzed muscles, suggesting that higher the amount of muscle cells, lower will be the force necessary to cut this particular sample.

Adams consistometry revealed poor to medium negative correlations with most of the histological traits, except with the muscular tissue proportion. Therefore, the sample extensibility (deformation) is higher when less connective tissue is present in the sample or sample firmness is higher as this particular tissual category increases its participation in muscle formation.

CONCLUSIONS

Pectoralis minor muscles had thinner fibers, higher myocytes density and higher proportion of pure muscular tissue, compared to *Pectoralis major* muscles.

The instrumental texture analysis indicated lower shear force and better extensibility in *Pectoralis minor*, suggesting better tenderness, versus the *Pectoralis major* findings.

Acquired data confirm that certain textural descriptors of the chicken meat (especially shear force) are highly and positively correlated with some of the meat histological characteristics (especially myocytes dimensional features). Therefore, one trait, once measured, could predict the other one with which is highly correlated.

As follow-up, it is indicated to enlarge the panel of textural descriptors to be measured instrumentally and sensorial, as well, in order to correlate the human consumer perception for those descriptors with the textural instrumental or histological findings. In this particular situation, our study reveals better tenderness and masticability of *Pectoralis minor* muscles, but this statement remains to be confirmed by the sensorial analysis.

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PROTEINS PROFIL OF SAUSAGE LAYING CHICKEN MEAT WITH ANGKAK (RED RICE) USED AS NATURAL FOOD MATERIAL

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Abstract

This study examines the potential of Angkak as a binding compound, color improvement in the process of making sausage chicken layered rejects as a curing material. Testing protein profiles related to dissolved protein, texture, water binding capacity to form a compact and soft texture. The design was carried out in a completely randomized design (CRD) 4 x 4, as a treatment using the Angkak level, ie without Angkak (0%), Angkak 0.5%, Angkak 1% and Angkak 1.5% with replications 4 times followed by a test BNJ. The data obtained were analyzed by analysis of variance (Analysis of Variance) which included chemical, color, total protein, and protein profile determination using the SDS-PAGE method (Sodium Dodecyl Sulphate Polyacrilmide Gel Electrophoresis). The results of profil proteins with SDS-PAGE (sodium dodecyl sulphate polyacrilmide gel electrophoresis), where R0 is a sausage without the addition of Angkak, R1: sausages that use 0.5% Angkak concentration, R2: sausages that use a concentration of Angkak 1.0% and R3: sausage who use the Angkak concentration of 1.5%. The conclusion of the study was that the addition of Angkak gave a chemical change and protein profile in the sausages of rejected laying hens and the range of molecular weights found in R0, R1, R2 and R3 were the same, namely 12.44 - 47.89 kDa.

Key words*: sausage meat, laying chicken, Angkak, curing.*

INTRODUCTION

Reed laying chicken meat as well as other livestock products is livestock commodities that need to be developed and improved. In general, the Indonesian people have known laying chicken meat rejects as a source of food that is mainly expected to be eggs, but the obstacles that have existed during this time rejects laying chicken meat is not much in demand by the public, because laying chicken meat apart from clay meat that is not favored by consumers also variations in processing chicken rejected laying into processed products is very limited, so that a processing technology is pursued to utilize and increase the added value of rejected laying chicken meat. One processing technique that can be develop.

According to Tisnadjaja (2006) that the use of nitrite in the process of curing in meat is 125 ppm, but this use has not been strictly monitored on food products, while the use of Angkak as a substitute for saltpeter or nitrite can be reduced by up to 60% without any apparent changes in organoleptic properties. Angkak pigments are used as a partial substitute for nitrite in the processing of meat cured like ham and sausage beef, both in improving the red color of meat products and in inhibiting the growth of spore-forming bacteria such as *Bacillus cereus* and *Bacillus stearothermophillus*.

According to Astawan (2012), the use of Angkak can reduce the use of nitrite in food. Nitrite is often used as a component of saltpeter, a substance used to maintain the red color of meat, especially in making sausages, smoked meat and cornet. Through its antimicrobial properties, the use of Angkak in making sausages is not only a red giver, but also as a safe preservative for health. Another advantage of using Angkak in making sausages is improving the texture and flavor. Furthermore, according to Sheu et al. (2000), states that the pigments produced by *Monascus purpureus* are very stable and do not change the taste of nata de coco. The dosage used for animal food coloring ranges from 2000-4000 ppm Monascus extract while for soft drinks, the concentration used can be lighter which is 0.002% - 0.005% (2-5 ppm).

The curing solution formulation can increase the red color stability of the product during storage, in ham and beef sausage products, the use of nitrite in the curing solution can be reduced from 125 ppm to 80 ppm by adding 2.5 g / kg meat of Angkak pigment. (Fardiaz et al., 2008). Farisandi and Pangesthi (2013) examined the combination of administration of Angkak with sodium nitrite to the organoleptic properties of corned beef, the result was that giving 1% Angkak combined with 50 ppm sodium nitrate affected the color of corned beef products but not flavor and aroma. According to Pattanagu et al. (2007), the optimum use of Angkak in meat products is 1.6% (w / w).

Proteins are high molecular weight complex organic compounds which are polymers of amino acid monomers that are connected to each other by peptide bonds. Proteins generally have a high molecular weight, because of the large weight of protein molecules, so proteins tend to form colloids. Protein solubility depends more on its structure and function and not on its molecular weight. Soluble proteins are good buffers and are very important in maintaining equilibrium reactions. Proteins are formed by units of amino acids that make up polymers so they are long compounds. Protein quality depends on the amino acids it contains. The principle of determining protein profiles by electrophoresis is to separate protein molecules with different charges.

MATERIALS AND METHODS

The ingredients used for the manufacture of sausages are 20 - 24 month old reject chicken meat which has been skinned, washed, 2x2 cm in size taken from the chest and thighs, then separated into 4 parts.

Then the meat is ground and then seasoning is added with a formula from a combination of Bhattacharyya, Mita and Biswas (2005) and Pearson and Dutson (1988), namely: 2% salt, 1.67% sugar, 1.5% garlic, 0.5% pepper, ginger 0.75%, nutmeg 0.5%, which is given in powder form, oil 15%, tapioca flour 5.7%, skim milk 3.5%, ice cubes 16.7% and STPP 0.3% by weight meat.

Each part is given Angkak 0%, 0.5%, 1% and 1.5% in the mixture put in a sleeve with a length of 10 cm and a diameter of 2.5 cm. Then cooked by steaming at 85^0C for 30 minutes. Then cooled and analyzed.

The research design carried out was descriptive research. The independent variable in this study was the addition of Angkak to the sausage meat of layered laying hens with concentrations of 0%, 0.5%, 1.0% and 1.5%.

Tools used for protein profile analysis are microtube, beaker glass, Erlenmeyer, electrophoresis chamber, power supply, rotator, mortal cup and spectrophotometer. The method used is SDS-PAGE (Rantam, 2003): Supernatant sample: 20 ml PB (Posphate Buffered Saline) solution added 0.5 M NaCl at pH 7.2. A 10 gram sausage sample was pounded with mortar then add a 0.01 ml PBS buffer of 3 ml. Then centrifuged at 6000 rpm for 15 minutes at 4^0C.

The work procedure of SDS-PAGE is as follows:

Prepare samples:

The protein sample is supplemented by 1:1 Reducing Sample Buffer (RSB) in the Eppendorf tube. Then the sample is heated at 100^oC for 5 minutes. After being cold, if the sample is not directly used, the sample can be stored at -20^0C

Prepare separating and stacking gel for 2 plates: Gelling plate is arranged as a guide. 15% separating gel is made by: 10% SDS - 60 ml, 10% APS – 60 ml, TEMED 10 ml

Enter the sample in the gel well

A plate that already contains gel is inserted into the electrophoresis chamber. Running buffer is poured until the top and bottom of the gel are submerged. If air bubbles form on the base of the gel or between sample wells, they must be removed. A standard 10 µl marker is inserted in one of the wells (can be gargled at the edge or in the middle well).

Samples of 10-20 µ (with a minimum protein content of 0.1 µ and a maximum of 20-40 µ) are carefully inserted into the bottom of the gel well, using Hamilton syringe. Syringes are rinsed to 3x using water or by running buffer before being used to insert different samples in the next gel well.

Running sampel

To start running the electrophoresis device is connected to the power supply. Running is carried out at a constant current 20 mA for approximately 40-50 minutes or until tracking

dye reaches a distance of 0.5 cm from the bottom of the gel. After completion, the running buffer is poured and the gel is taken from the plate

Coloring of Gel:

For this stage, a staining solution is needed for coloring gel proteins, the coloring used is Comasie Brilliant Blue or Silver Stain depending on usability. Staining is carried out for 30 minutes. Destaining solution to remove color in the gel and clarify the protein bands formed.

RESULTS AND DISCUSSIONS

Water Content

Decreasing the water content of duck sausage is caused by the increased concentration of Angkak used. Angkak can experience oxidation when heated which causes a decrease in the water in the meat is lost, so that the water content drops (Fardiaz and Zakaria, 1996).

According to Zanardi et al. (2002), the addition of the Angkak concentration produced from the *Monascus purpureus* mushroom resulted in a positively charged meat protein and binding to the H + charge, consequently there is no H + that is free or which binds to O which produces free water molecules.

The sausage water content according to the Indonesian National Standard (1995) is a maximum of 67.0%, so the sausage water content from the research which ranges from 63.70 - 64.16% still meets SNI standards.

Fat

This decrease in fat levels is caused by lovastin compounds in Angkak which act as inhibitors of HMG-CoA reductase (an enzyme that plays a role in cholesterol biosynthesis), where lovastin is hydrophilic and lipophilic but tends to be lipophilic (Dalimartha, 2001).

The fat content of this study ranged from 8.38 - 9.43%, while fat content according to the Indonesian National Standard (1995) was a maximum of 25%, so the fat content of the results of this study still met the standards.

Table 1. The average value of the chemical properties of sausage meat in laying hens is rejected

Parameter	Consentration Angkak			
	0%	0,5%	1%	1,5%
Water Content	$64.16 \pm 0,168^{a}$	$63.26 \pm 0,078^{a}$	$63.33 \pm 0,213^{b}$	$63.70 \pm 0,082^{c}$
Fat	$9.43 \pm 0,056^{a}$	$9.21 \pm 0,055^{b}$	$8.98 \pm 0,057^{c}$	8.38 ± 0.089^{d}
Carbohidrate	$7,75 \pm 0,090^{a}$	$8.14 \pm 0,110^{b}$	$9.31 \pm 0,029^{c}$	$10.89 \pm 0,028^{d}$
Protein	$15,48 \pm 0,369^{a}$	$15,81 \pm 0,088^{a}$	$16,09 \pm 0,118^{c}$	$16,83 \pm 0,131^{d}$

Remarks: different notations show significant differences between treatments ($P<0.05$)

Carbohidrate

The results of the analysis of carbohydrate values indicated that the higher the concentration of Angkak added, the higher the carbohydrate level is. While the carbohydrate carbohydrate levels in this study were 10.89% so that the higher the Angkak added, the higher the carbohydrate content of sausage chicken meat sausages.

Protein

The results of the study for protein levels showed that the higher the concentration of Angkak added, the higher the level of sausage protein in the rejected laying chicken meat.

The addition of Angkak in this experiment was immediately given together with seasonings in the process of making sausages. Monascus mushrooms that produce enzymes such as α-amylase, β-amylase, glucoamylase, lipase, protease, glucosidase and ribonuclease are able to grow in materials containing starch, protein or lipids (Pattanagu et al., 2007), this condition allows an increase in levels of chicken sausage protein with increasing levels of addition of Angkak to treatments R1, R2, and R3.

Angkak proteins undergo decomposition (oxidative degradation) through a transamination reaction (the enzymatic transfer of amino acid groups from one amino acid to another amino acid) that can bind meat proteins, this is supported by Kramlich (1971) which states that meat protein plays a role in increasing destruction meat during cooking to

form a compact product structure. The role of other proteins is the formation of meat emulsions, which are proteins that function as fat emulsifiers.

Protein content of chicken sausages with Angkak colorant added to the results of this study ranged from 15.48 - 18.83% so that the sausage protein levels still meet SNI standards.

Proteins generally have a high molecular weight, because of the large weight of protein molecules, so proteins tend to form colloids. Protein solubility depends more on its structure and function and not on its molecular weight. Soluble proteins are good buffers and are very important in maintaining equilibrium reactions.

Profile protein

The results of SDS-PAGE sausages from rejected laying hens have 7 protein bands that appeared in sausages without the addition of Angkak and the addition of Angkak (0.5%, 1%, 1.5%) with protein molecular weight ranging from 12.44 - and 47.89 kDa (Table 2). Calculation of molecular weight (BM) of protein bands contained in the gel by comparing the molecular weight of the marker and Retardation Factor (Rf), then proceed with making a standard curve with the value of Rf as the x axis and the molecular weight logarithm value as the y axis.

Table 2. Weight of sausage protein molecules

Pita	Rf	Ro	R1 BM	R2	R3
2.1	0.33	47.89	47.89	47.89	47.89
2.3	0.36	42.36	42.36	42.36	42.36
2.5	0.39	37.48	37.48	37.48	37.48
2.1	0.45	29.33	29.33	29.33	29.33
3.9	0.48	25.95	25.95	25.95	25.95
3.6	0.56	19.10	19.10	19.10	19.10
4.3	0.67	12.44	12.44	12.44	12.44

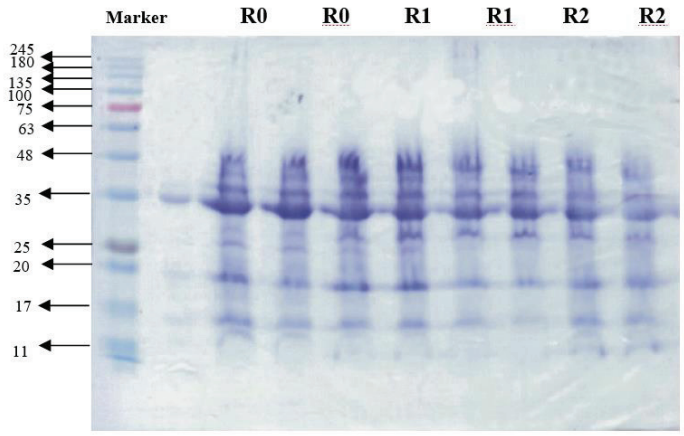

Figure 1. SDS-PAGE Electrophoresis Results
(R0: Sausage + Without Angkak,
R1: Sausage + Angkak 0.5%, R2: Sausage + Angkak 1%
and R3: Sausage + Angkak 1.5%, M: Marker)

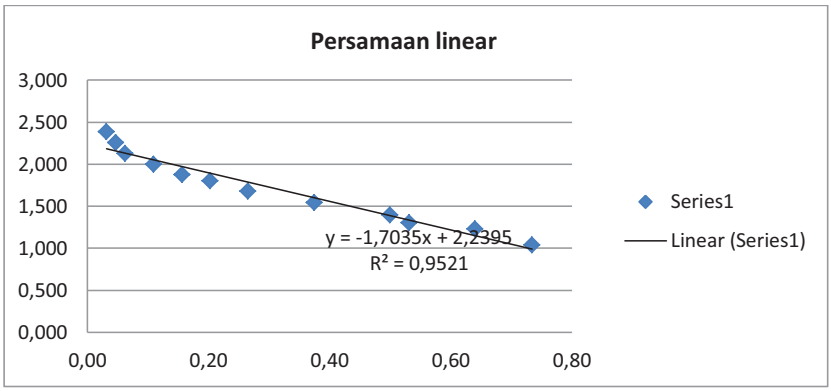

Figure 2. Molecular Weight Curve

According to Laemmli (1970), protein bands that are close together indicate that the protein has the same number of amino acids, whereas according to Soeparno (2011) that proteins are formed from amino acids which are bound together to form a series. A small difference in the formation of a series will produce a different type of protein. The types of protein bands detected in processed products are closely related to the functional level of protein damage. Amino acids are increasingly showing the low functional damage to proteins. This is evidenced by the type of protein bands found in meat sausages without the addition of Angkak and added ones which have molecular weights of 12.44 kDa, 19.10 kDa, 25.95 KDa, 29.33 kDa, 42.36 kDa, 37.48 kDa, and 47.89 kDa.

CONCLUSIONS

The addition of Angkak to laying hens sausages can provide changes in water, fat, carbohydrate and protein levels. The molecular weight of the treatment is 0%, 0.5%, 1% and 1.5% ranging from 12.44 kDa - 47. kDa.

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EFFECT OF YEAST AND LACTIC ACID BACTERIA IN CULLED LAYING HENS SALAMI AGAINST *ESCHERICHIA COLI, STAPHYLOCOCCUS AUREUS* AND *SALMONELLA SP*

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Abstract

The purpose of this study was to determine the effect of yeast and lactic acid bacteria in culled laying hens salami against Escherichia coli, Staphylococcus aureus and Salmonella sp. The study was conducted using Completely Randomized Design (RAL) with 5 treatments and 4 replicates. The treatments were: P1 = Salami with 2% lactic acid and 1% yeast, P2 = Salami with 2% lactic acid and yeast 2 %, and P3 = Salami with 2% lactic acid and 3% yeast, and P4 = Salami with 2% lactic acid and 4% yeast. Escherichia coli and Staphylococcus aureus were analyzed with quantitative and qualitative method, and Salmonella sp. were analyzed with qualitative method. The test for inhibitory bacteria Escherichia coli, Staphylococcus aureus, Salmonella sp. used disc diffusion method, that the salami product of this research meets the requirements of SNI 01-3820-1995. It can be concluded that the use of yeast and lactic acid bacteria could inhibit the growth of pathogenic bacteria in culled laying hens salami.

Key words: *lactic acid bacteria, pathogenic bacteria, salami, yeast.*

INTRODUCTION

Nowadays the number of inventions by experts reported that microorganisms play a lot in fermentation processes through various kinds of biochemical reactions. Utilization of microorganisms is used both to produce food products and to preserve existing food products. Utilization of yeast in traditional food and fermented product in Indonesia is still relatively small, mainly utilizing only a few species such as *Saccharomyces cerevisiae, Kluyveromyces lactis / Kluyveromyces kefyr and Zygosaccharomyces* spp., especially in producing bread, tape, brem, wine, soy sauce, salt vegetables, etc. Whereas, in some other countries, yeast has been used in producing fermented milk and other products. Common cultures used in salami processing include classes of lactic acid bacteria such as *Lactobacillus, Pediococcus, Micrococcus* and *Streptococcus.*

Yeasts have antimicrobial properties with specific proteolytic activity, such as *Candida,* known to have proteolytic ability. In addition, there are extracellular proteolytic capabilities such as *Candida, Cryptococcus, Rhodotorula, Phicia, Hansenulla* and *Metschnikowia.* Species *Saccharomycopsis fibuligera* R64 strains are known to have antimicrobial activity in some pathogenic bacteria such as *Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli. Kluyveromyces thermotolerans* and *Kloeckera apiculata* have long been known to inhibit the growth of *Lactobacillus plantarum*. A certain yeast culture belonging to *Debaryomyces* has been shown to help form and stabilize the color on the sausage surface. The presence of yeast in fresh meat is very different from that of processed meats: in fresh meat yeast growth is not expected because if the growth exceeds 10^5 - 10^6 cells / g it will be quickly damaged, therefore, in the sale of meat is expected to be at refrigerator temperature (4-5^{o}C) or packed in a vacuum. In carcasses only 5 - 10% of the total number of microflora and not significant cause damage (Samelis and Sofos, 2003). Unlike the case in processed meat products, the presence of yeast can add flavor and aroma for example in fermented sausage. *Debaryomyces hansenii* on Italian salami, gives a good flavor to the product; and affect the quality of sausages (Samelis and Sofos, 2003). *Yarrowia lipolytica*

is also often isolated from fresh beef and sausage.

Meat is a food contains nutrients needed by the body, but it also has drawbacks such as perishable foodstuffs, considering the meat contains protein and fat as a source of nutrition for utilized by the development of microorganisms. Meat damage can occur due to early contamination of microorganisms as it enters the bloodstream during livestock slaughter. Fresh meat can be contaminated by a large number of bacteria including pathogenic bacteria, so the food is damaged. The type of bacteria such as *Bacillus cereus concerned, Clostridium perfringens, C. jejeni, Escherichia coli, Listeria monocytogenes, Salmonella* sp. and *Staphylococcus aureus.* One of the requirements of quality poultry products are free of pathogens, such as *Salmonella* sp*., Staphylococcus aureus, Escherichia coli,* and *Campylobacter* sp. (Baumler et al., 2000). The microbial growth occurs in a short time and in appropriate conditions, such as the availability of nutrients, pH, temperature, and water content of food. The microbial group of spoil turns the fresh food into rotten and can even produce toxin (poison). Sometimes it shows no signs of change or physical damage (less really bad smell), so that food is still consumed (Djaafar and Siti, 2007). *Salmonella* and *Campylobacter* sp. is a major pathogen and is present in foods associated with poultry and often causes disease in humans (Mbata, 2005). However, other pathogens also exist such as *Clostridium perfringens*, *E. coli* O157 and *Listeria monocytogenes* and new pathogens, *Acetobacter* and *Helicobacter* sp. (Mbata, 2005). Therefore in this study will examine how the influence of yeast and lactic acid bacteria on quantitative analysis of *Escherichia coli, Staphylococcus aureus* and qualitative analysis of *Salmonella* and the inhibitory of each pathogenic bacterium against the culled laying hens salami.

MATERIALS AND METHODS

The material used was 30 culled laying hens strain Isa Brown 96 week old. Seasonings for the manufacture of salami such as garlic, ginger, pepper, nutmeg, sugar and salt, cornstarch, skim milk and fat. The starter used is yeast *Trichosporon beigelii* yeast identification using RapiD^{TM}Yeast Plus System and lactic acid bacteria *Lactobacillus acidophilus* and *Lactobacillus plantarum* isolation from culled laying hens. The equipment used in this research is: Food Processor Philips HR 7620 brand used for grinding meat and mixing salami, basin for laying chicken, plastic to store spices, gloves, ohaus scales with capacity 600 g Model: Scout Pro SPS 6000F, knife and cutting board, thermometer to measure the temperature of the room, Harnir-Shuma brand brush as a filler into the sausage casings, Salami casings (casing) brand "*NaloFaser*" with the size Caliber 45, Lange 60.0 comes from Germany. Strap casing (mattress yarn), stove and oven for curing salami.

Salami production consists of culled laying hens and fat with a ratio of 80 : 20 (g/g). meat and fat are simultaneously ground, then frozen for 24 hours; the ground meat is frozen and then milled again using a food processor while added spices such as salt, sugar, garlic, ginger, pepper, nutmeg, cornstarch, skim milk and 2% lactic acid bacteria culture as well as yeast species (*Trichosporon beigelii* T0, T1, T1 (1%), T2 (2%), T3 (3%) and 4% (T4) until well-mixed. Into salami dough added cornstarch, milk powder and chicken fat. After mixing, the dough is inserted into the 30 mm diameter casing, then tied to a distance of 10 cm and then hung on a rack and held for 24 hours at room temperature (Arief et al., 2008), then fermentation process at room temperature for 6 days and interspersed with fumigation for one hour per day. Temperatures during fumigation are maintained 27 - 30°C, when heat exceeds that temperature, a temperature decrease by adding ice to the cubic chamber when the temperature exceeds 30°C. The fuel used is dry coconut shell.

The experiment was conducted using Randomized Completely Randomized Design with 5 treatments and 4 replications, so that were 20 treatment combinations (Gaspersz, 1995) were obtained: T0 = Salami with 2% lactic acid and 0% yeast without adding spices, T1 = Salami with 2% lactic acid bacteria and 1% yeast, T2 = Salami with 2% lactic acid and 2% yeast, and T3 = Salami with 2% lactic acid and 3% yeast, and T4 = Salami with the use of

2% lactic acid bacteria and 4% yeast. Quantitative analysis methods of *Escherichia coli* (APHA, 1992), quantitative analysis of *Staphylococcus aureus* (Fardiaz, 1992) and qualitative analysis of *Salmonella* sp. (Andriani et al., 2005) were then tested for *Escherichia coli* bacterial inhibition, *Staphylococcus aureus, Salmonella* sp. using disc diffusion method (Kirby-Bauer test).

RESULTS AND DISCUSSIONS

Quantitative analysis of *Escherichia coli* (MPN/g), *Staphylococcus aureus* (CFU/g) and qualitative analysis of *Salmonella* (per 25 g)

Table 1. Quantitative analysis of *Escherichia coli* (MPN/g), *Staphylococcus aureus* (CFU/g) and qualitative *Salmonella* sp. (per 25 g) against Culled Laying Hens Salami

Sample/ Salami	Quantitative Analysis		qualitative analysis	Infor-mation
	Escherichia coli (MPN/g)	*Staphylococcus aureus* (CFU/g)	*Salmonella* (per 25 g)	
T0	< 3	< 10	Negative	Qualify
T1	< 3	< 10	Negative	Qualify
T2	< 3	< 10	Negative	Qualify
T3	< 3	< 10	Negative	Qualify
T4	< 3	< 10	Negative	Qualify

Notes: Qualify SNI (Kesmawet Laboratory DKI Jakarta, 2015)

Based on the results of laboratory tests (Table 1) showed that salami culled laying hens using yeast starter and lactic acid bacteria, *Escherichia coli* salami bacteria of laying hens were <3 MPN/g for each sample. These data suggest that salami products are safe with negative results for *Escherichia coli* bacteria. In accordance with SNI 01-3820-1995 the *Escherichia coli* in sausage should be <3 MPN/g. The main habitat of this bacterium exists in the digestive tract (especially in the intestines) of humans and can be found in soil, water, and other places that are the native habitat of this bacterium (Jay, 2000). The bacteria are easy to contaminate the water, therefore the contamination of these bacteria in food usually comes from the contamination of the water used. Foodstuffs that are often contaminated by *E. coli* include, chicken, beef, pork during slaughter, fish and other seafood products, eggs and other dairy products, vegetables, fruits, juices, and beverages milk and others. This bacterium is very sensitive to heat and can be activated at food pasteurization temperature or during cooking of food (Supardi and Sukamto, 1999).

Table 1 showed that the culled laying hens salami with starter yeast and lactic acid bacteria for all treatments against *Staphylococcus aureus* bacteria is <10 CFU/g; according to SNI 01-3820-1995 that the bacterium *Staphylococcus aureus* should be <10 CFU/g. The results of the study met the recommendations of National Standardization Agency (1995). The cells of *Staphylococcus aureus* are gram-shaped positive, generally arranged in groups like grapes. The bacteria are immobile, facultative anaerobic, growing on products containing up to 16% NaCl (Buckle et al., 2010). The presence of *S. aureus* in the diet comes from the skin, mouth, or nasal cavity of food processors, making it easy to contaminate food. Meat contaminated or containing enterotoxigenic *S. aureus* is very harmful to consumer health due to the absence of other competing microorganisms and can usually inhibit the growth and formation of *S. aureus* toxins (Djaafar and Siti, 2007).

In contrast to the qualitative test that laying chicken affection salami negative to *Salmonella*. The results are in accordance with the recommendation of the National Standardization Agency (1995), meaning that the resulting salami product is feasible and safe for consumption, because it is free from *Salmonella* bacteria.

Salmonella contamination in meat is most common, usually occurring during animal slaughtering processes (Hanes 2003; Goncagul et al., 2005; Stevens et al., 2006; Cortez et al., 2006). Cortez et al. (2006) and Nogrady et al. (2008) showed that the contamination of chicken meat in slaughterhouses occurs through feces, fur, soaking hot water before scalding water, evisceration water, chiller water, and carcass rinse water, as well as Humphrey (2006) stated that *Salmonella* contamination in carcass / poultry meat often occurs during the cutting process, especially during evisceration, and during dipping in soft scalding.

Salmonella is gram-negative pathogenic bacteria can be isolated from the soil, water, food, and digestive tracts of humans and animals (Anderson and Ziprin, 2001). Animals containing (infected) *Salmonella* often do not show clinical symptoms (subclinical), so the

bacteria tend to spread easily between flocks. In addition, animals become carriers of a persistent disease, so the prevalence of *Salmonella* incidence is not easily detected, except through routine sampling and examination (Namata et al., 2009). Another source of *Salmonella* infection in poultry is contaminated feed, rodensia, worms, and other wild animals (Humphrey, 2006).

Some pathogenic microbes such as *Escherichia coli*, *Salmonella* and Staphylococcus sp. often contaminate meat. The microbial content of meat comes from farms and unhygienic animal slaughterhouses (Mukartini et al., 1995). Meat processing long enough, allowing the occurrence of microbial contamination in its processed products. Processed meat products such as sausages must meet the provisions of quality requirements. Based on SNI 01-3820-1995, *Salmonella* contamination in meat sausage must be negative, and *Clostridium perfringens* negative, and *S. aureus* up to 10^2 colonies /g.

Inhibition of Escherichia coli (MPN / g), Staphylococcus aureus (CFU / g), Salmonella (per 25 g)

The average inhibition of the *Escherichia coli* inhibition on the culled laying hens salami showed in Table 2.

Table 2. Mean of inhibition against *Escherichia coli* (MPN / g), *Staphylococcus aureus* (CFU / g) and *Salmonella* (per 25 g) in culled laying hens salami with Starter Yeast and Lactic Acid Bacteria

Sample / Salami	Bacteria inhibition		
	Escherichia coli (MPN/g)	*Staphylococcus aureus* (CFU/g)	*Salmonella* (per 25 g)
T0	0.27	0.23	0.45
T1	0.20	1.60	0.25
T2	0.27	1.98	0.80
T3	0.20	1.65	0.40
T4	0.27	2.33	0.40

Table 2 showed that the higher percentage of yeast used in laying chicken salami caused the resulting *Escherichia coli* inhibition to be relatively stable, that means all of treatments showed the same and not significantly different (P>0.05). The research on antimicrobial activity of laying hens salami was done by disc diffusion methods (Kirby-Bauer method). Figure 1 showed that the inhibition zone activity of laying hens rejecting the growth of *Escherichia coli* bacteria in the presence of clear zones around each disc forming a drag zone with an average diameter of 0.275 mm; 0.2 mm; 0.275 mm; 0.2 mm and 0.275 mm, although not statistically significant (P>0.05). This means that lactic acid bacteria can produce lactic acid and other metabolites that are antibacterial, so the growth of pathogenic bacteria can be inhibited (Savadogo et al., 2004). In addition to producing organic compounds, several strains of lactic acid bacteria also produce bactericidal protein compounds against gram-positive and gram-negative bacteria called bacteriocin (Tahara et al., 1996), as well as yeasts capable of producing antimicrobial compounds in the form of organic acids hexanoate, octanoate and decanoate) and proteins.

Arief et al. (2008) reported that lactic acid bacteria of *Lactobacillus plantarum* sp. can produce antimicrobial compounds of hydrogen peroxide. Hydrogen peroxide serves to decrease the permeability of *E. coli* structure molecules through the mechanism of lactoperoxidase and thiocyanate, hydrogen peroxidase and can inhibit the growth of pathogenic bacteria *E. coli*, *Salmonella* and *Staphylococcus* (Jennie and Rini, 1995). The diameter of the growth zone of bacterial growth indicates bacterial sensitivity to anti-bacterial agents followed by the width of the diameter of the inhibition zone formed, so that the bacteria become more sensitive (Hastowo, 1992). *Escherciha coli* include harmless microorganisms, but also unfavorable under normal circumstances. These bacteria can be pathogenic with a moderate level of danger and rapid spread (Fardiaz, 1992).

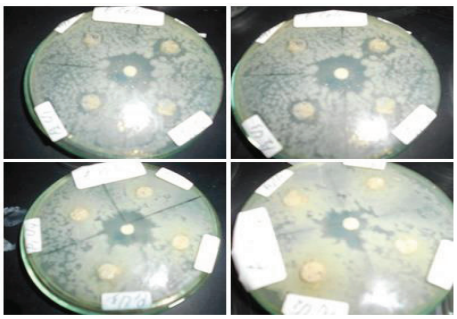

Figure 1. Inhibition of culled laying hens salami against *Escherichia coli* bacteria

Table 2 showed that the higher percentage of yeast with the addition of 2% lactic acid bacteria results in a significantly increased and significantly different inhibitory of *Staphylococcus aureus* ($P<0.05$). This means that the starter yeast 4% is the best treatment in inhibiting the growth of *Staphylococcus aureus* bacteria in culled laying hens salami. Clarified again with the observations shown in Figure 2 that the higher percentage of yeast starter shows clear zone as the percentage of yeast is T0 (0.23 mm), T1 (1.60 mm), T2 (1.98 mm), and T3 (1.6 mm) and T4 (2.33 mm). Means yeast in inhibiting the growth of different bacteria *Staphylococcus aureus* for each treatment so it can be concluded that yeast can inhibit the growth of *Staphylococcus aureus* bacteria, especially at 4% treatment (T4) obtained the highest is 2.33 mm. Differences zone resistor for each treatment due to the difference of yeast starter; this is because the higher starter yeast the higher the content of active substances in it. Thus the formation of a strong inhibitory zone can be due to the work of active substances as antimicrobials contained in culled laying hens salami.

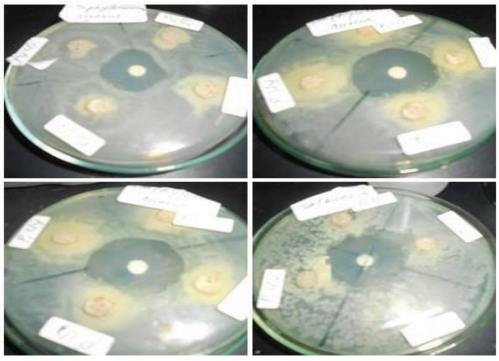

Figure 2. Inhibition of culled laying hens salami against *Staphylococcus aureus* bacteria

Based on the measurement of the inhibition zone diameter, the clear zone is visible. Means of salami with strarter yeast has an inhibitory effect on the growth of *Staphylococcus aureus* bacteria and occurs in all treatments.

The properties of yeast antimicrobials such as organic acids (hexanoate, octanoate, and decanoate) and proteins are known to inhibit the growth of bacteria and molds (Roostita, 2004). Bilinski et al. (1985) reported several types of yeast such as *Kluyveromyces thermotolerans* and *Kloeckera apiculata* showed activity in inhibiting the growth of *Lactobacillus plantarum*. The results of Arkoudelos et al. (1998) showed inhibition of pathogenic *S. aureus* bacteria in fermented meat products by starter *Lactobacillus plantarum*. Other antimicrobial compounds that can inhibit *S. aureus* growth are hydrogen peroxide as a result of the action of lactic acid bacteria (Leroy and Vuyst, 1999).

Similarly, with *Salmonella* spp bacteria (Table 2), the higher percentage of yeast in laying chicken salami was higher causing inhibitory effect on *Salmonella* bacteria growth and relatively stable although statistically not significantly different ($P>0.05$). T2 showed that the largest *Salmonella* bacterial inhibition (0.80 mm) increased from 0.25 mm for T1 treatment, then decreased again in treatment of T3 and T4 to 0.40 mm. The high drag in T2 treatment is caused by 2% yeast starter able to inhibit *Salmonella* bacteria growth optimally so that it has strong resistor power; whereas in the treatment of T3 and T4 the inhibitory power to the growth of *Salmonella* bacteria decreased or weakened. Optimum point of *Salmonella* bacteria in culled laying chicken salami obtained at percentage of starter yeast 2% (P2). Means 2% yeast starter is an optimal point in inhibiting *Salmonella* bacteria as shown in Figure 3.

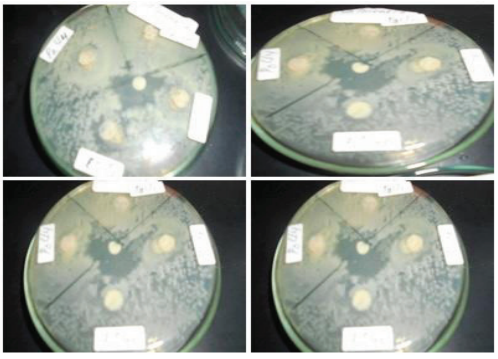

Figure 3. Inhibity of culled laying hens salami against *Salmonella* sp.

Figure 3 showed that the inhibition zone activity of laying hens salami on growth of *Salmonella* spp bacteria create a clear zone around the disc. Evidence that a test material

has antibacterial activity is indicated by the formation of a clear zone or zone of inhibition around the disc. The result of antibacterial activity of culled laying hens salami on growth of *Salmonella* sp. bacteria was obtained from each treatment of T0, T1, T2, T3 and T4 on the percentage of yeast 0%, 1%, 2%, 3% and 4%. Each treatment formed an inhibitory zone with an average diameter of 0.45 mm, 0.25 mm, 0.8 mm, 0.40 mm and 0.40 mm, although not statistically significant (P> 0.05).

It can be concluded that the yeast starter used in the study can produce bioactive components i.e antimicrobial compounds so useful as biopreservative agents. Lactic acid bacteria is a bacterium that produce lactic acid as a primary metabolite product and also produces other antibacterial substances such as hydrogen peroxide, diacetyl and antibacterial bacteriocin that can inhibit the growth of other bacteria (Tagg et al., 1976).

As it is known that some of the common pathogenic microbes contaminating meat are *Escherichia coli, Salmonella and Staphylococcus sp.* The microbial content of meat can from non hygienis animal farms and slaughter houses (Mukartini et al., 1995) as well as the long process of meat processing also allows the occurrence of microbial contamination in its processed products. Based on SNI 01-3820-1995, *Salmonella* contamination in meat sausage must be negative, and *S. aureus* up to 10^2 colonies /g.

CONCLUSIONS

Salami products (fermented sausages) are safe from *Escherichia coli* bacteria (<3 AMP / g), *Staphylococcus aureus* (<10 CFU / g), and *Salmonella* (negative). Means that salami products are feasible and safe for consumption because they are free from pathogenic bacteria and SNI 01-3820-1995 requirements for all treatments

The use of yeast and lactic acid bacteria may inhibit the growth of pathogenic bacteria such as *Escheriacia coli*, having inhibitory zone activity of each mean diameter T0 (0.275 mm); T1 (0.2 mm); T3 (0,275 mm); T3 (0.20) mm and T4 (0.275 mm); *Staphylococcus aureus* with clear zones grew in line with increasing percentage of yeast ie T0 (0.23 mm), T1 (1.60 mm), T2 (1.98 mm), and T3 (1.6 mm) and T4 (2, 33 mm) and *Salmonella* formed inhibition zone with mean diameter T0 (0.45 mm), T1 (0.25 mm), T2 (0.8 mm), T3 (0.40 mm) and T4 (0.40 mm).

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CORRELATIVE RESEARCH REGARDING THE TOTAL POLYPHENOLIC CONTENT, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF THREE TYPES OF ROMANIAN HONEY

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Abstract

Knowledge of biologically active potential of honey has made significant progress in the last decade, due to the diversification and improvement of the analysis methods regarding the content in various functional compounds. The aim of this study was to evaluate the total polyphenolic content and the antioxidant and antibacterial potential of some honey samples, establishing the correlations between the values of the examined parameters. The investigations were carried out on 7 samples of honey and their botanical origin was determined through melissopalynogical analysis. The samples belonged to the following types of honey: multifloral honey (no=4), lime tree honey (no=2), rapeseed honey (no=1). The total polyphenolic content was determined by Folin-Ciocâlteu method and the antioxidant activity by DPPH radical scavenging method. The antibacterial activity was tested on 3 bacterial strains: Staphylococcus aureus, Bacillus cereus and Enterococcus faecalis. The results showed a positive correlation between the investigated parameters. For instance, one sample of multifloral honey recorded the highest total polyphenolic content (274.65±1.85mg GAE/100g honey), correlated with the highest levels of antioxidant (12.30±0.43 mmol Trolox/100 g honey) and strong antibacterial activity.

Key words*: multifloral honey, lime tree honey, rapeseed honey, antioxidant activity, antibacterial effect.*

INTRODUCTION

In the last decades, a special attention has been drawn to antioxidants, which have been associated with multiple benefits for human health.
Nowadays, honey is considered to be one of the last remaining natural products, minimally affected by industrial technologies.
The main constituents present in honey are represented by the carbohydrates, comprising approximately 95% of its dry weight basis (Nagai et al., 2006).
In addition to this, honey contains numerous compounds such as polyphenols, enzymes (e.g., glucose oxidase, catalase), ascorbic acid, carotenoid-like substances, organic acids, Maillard reaction products, amino acids and proteins recognised as valuable antioxidants (Estevinho et al., 2008; Da Silva et al., 2016).
However, available literature suggest that the antioxidant activity of honey is mainly provided by the polyphenols, and then by the other constituents (Gheldof and Engeseth, 2002).
The term 'polyphenol' is usually defined chemically as a substance that possesses an aromatic ring bearing one or more hydroxyl substituents including functional derivatives (esters, methyl esters and glycosides). Some phenolic compounds are exceedingly widespread, while others are present exclusively in certain plant families or in particular development stages (Cheynier, 2012). Moreover, evidences confirm that among all major groups of polyphenols, only flavonoids and phenolic acids can be found in honey and they mainly exert their antioxidant activity by neutralizing free radicals, by donating an electron or hydrogen atom (Rice-Evans, 1996).
A plethora of research demonstrated that honey may be used for the treatment of various pathologies, such as colds, skin wounds and several gastrointestinal diseases and this effect can be attributed to both antibacterial and anti-inflammatory properties of honey, regarding high osmolarity, acidity and content of hydrogen peroxide. In this regard, the antibacterial activity of honey is well known

and documented as well (Weston, 2000; Taomina et al., 2001).
The aim of our research was to determine the total polyphenolic content, the antioxidant and the antibacterial activity of some Romanian honey samples. Additionally, a correlation between the above mentioned parameters was carried out.
Furthermore, we aimed to identify the botanical origin of the honey samples, by performing the melissopalynological analysis.

MATERIALS AND METHODS

Seven samples of honey were collected from the Laboratory for the Quality Control of Apiculture Products within the Institute of Life Sciences of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, where they were brought by beekeepers across Romania, in order to be investigated. All the honey samples were stored at room temperature in dark before analysis.
The melissopalynological analysis was conducted in the Cell Analysis Laboratory of the Institute of Advanced Horticultural Research of Transylvania, Cluj-Napoca, by using the method implemented by Louveaux et al. (1978) and Werner von Der Ohe et al., (2004), with microscopic slides. The examination of the microscopic preparations was realized with an optical microscope (Olympus BX 41), using the 40X lens for the identification of the pollen grains. Moreover, the images of the microscopic slides were achieved with an UC30 camera and processed with an Olympus Stream Basic software.
The total polyphenolic content was determined by a spectrophotometric method, called the Folin-Ciocâlteu method, with some modifications (Folin and Ciocâlteu, 1927; Singleton et al., 1999; Kim et al., 2003).Two g of honey were diluted in 70% methanol solution and the resulting mixture was transferred to 20 ml flasks, filled with 70% methanol solution and then, filtered. After 2 hours, 25 µl of the obtained solution, 125 µl of 0.2 N Folin-Ciocâlteu reagent and 100 µl of Na_2CO_3 were pipetted into a 96-well plate. After incubation in dark and at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 760 nm wavelength with a Biotek Synergy HT multidetector spectrophotometer.
The standard curve was produced for gallic acid and the total polyphenolic content was expressed as mg gallic acid equivalents per 100 g of honey sample (mg GAE/100 g honey sample).The results are presented as mean of four determinations ± standard deviation (Microsoft Excel, 2010).
The scavenging activity against 1.1-diphenyl-2-picrylhydrazyl (DPPH) radical of honey was evaluated according to the procedure described by Velazquez et al., (2003) and Molyneaux (2004), with some modifications. The methanolic DPPH solution was prepared extemporaneously at a concentration of 2 mg/100 ml and then, sonicated for 15 minutes. 200 µl of DPPH solution and 40 µl of the honey solution diluted in methanol 70% were added in a 96-well plate and the control test was made with 200 µl of DPPH solution and 40 µl of 70% methanol. The reaction mixture was incubated for 30 minutes at the room temperature in the dark. Absorbance was measured at 517 nm wavelength, by using a multichannel spectrophotometer. The standard curve was produced for 6-hydroxy-2,5,7,8-tetramethylchroman-2 carboxylic acid (Trolox). The results are presented as mean of four determinations ± standard deviation (Microsoft Excel, 2010). Antioxidant activity was expressed as a percent of inhibition of DPPH radical and as Trolox miliequivalents/100 g honey sample.
The testing of three reference bacterial strains sensitivity, such as *Staphylococcus aureus* ATCC 6538P, *Bacillus cereus* ATCC 11778 and *Enterococcus faecalis* ATCC 29212 was performed against the seven honey samples. It was determined according to the technique described in the Kirby-Bauer antibiogrammethod, which is currently one of the most frequently requested laboratory method; in order to obtain a conclusive result, the method should be conducted under standard conditions (Schwalbe et al., 2007). The test is based on the property of the antibiotics, respectively the active components contained in the honey samples to diffuse in solid culture media by achieving different concentration gradients, which gradually decrease from the deposition site to the edge of the diffusion zone. For the diffusometric techniques performed in Petri dishes, there is required the

usage of 90 mm diameter Petri dishes, made of glass or plastic, which have perfectly planar, clean and sterilized surfaces. In the case of dishes presenting irregularities of their surfaces, a thin layer of equalizing agar may be poured.

The culture medium used in the present research was Mueller-Hinton agar.

This growth medium must possess constant qualities from one batch to another in other to allow rapid growth of the tested germs, contain no germ-inhibiting substances or antimicrobial substances.

Before sowing the Petri dishes with the culture medium, depending on the number of the samples used, 0.5 mm wells were cut, by using a template.

From the bacterial culture tested for sensitivity to different honey samples, a suspension with a density equal to 0.5 was made by means of an electronic densitometer.1 ml of this suspension was introduced into the Petri dish with Mueller-Hinton agar and the dish was tilted in different directions in order to cover the entire surface of the medium.

The excess suspension was removed, and the Petri dish was held near the gas bulb in order to dry. Then, 20 µl of honey sample was placed in each well, the order of the samples always respecting the clockwise direction.

Micro-tablets of amoxicillin were used as positive control for bactericidal activity. Petri dishes were incubated then, for 24 hours at 37°C.

The following statistics were assessed by using Microsoft Excel, 2010: Mean, Minimum (Min.), Maximum (Max.), Standard Deviation (Std. Dev.), Coefficient of variation %.

Furthermore, Correlation coefficient (r) was calculated by using GraphPad Prism 6.0 Software in order to determine correlations between the investigated parameters (Correlation statistical function). Moreover, all the chemicals and reagents used in the present research were of analytical grade.

RESULTS AND DISCUSSIONS

The identification of the floral species of the pollen grains that are present in the composition of the analyzed honey samples was conducted by means of microscopic slides.

The images corresponding to the honey samples (Figure 1) were interpreted on the basis of photos from recent literature. Therefore, a series of features were monitored, namely the morphology and dimensions of the pollen grains, the structure of the tegument, the shape and number of germinating pores (Palmieri et al., 2017).

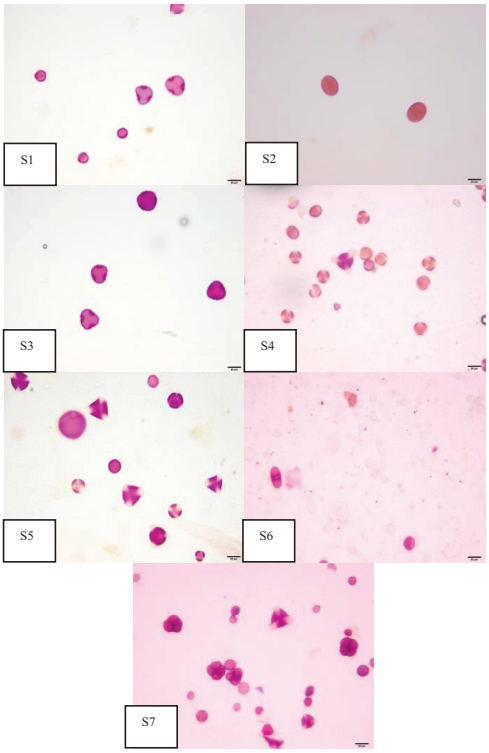

Figure 1. The microscopic image of pollen grains corresponding to honey samples S1, S2, S3, S4, S5, S6, S7 (40X; original photo)

In Table 1 are outlined the botanical families and species of the honey samples and they were classified into four groups, specifically, predominant pollen (>45%), secondary pollen (16-45%), important minor pollen (3-15%) and minor pollen (<3%).

According to the melissopalynological analysis, predominant pollen (> 45%) came from two botanical families, namely: *Brassicaceae (Brassica* ssp.*)* and *Tiliaceae (Tilia* ssp.*)*.

Secondary pollen (16-45%) originated from 7 plant families such as: *Hypericaceae, Fabaceae, Polygonaceae, Rosaceae, Salicaceae, Brassicaceae, Ericaceae* and the

important minor pollen (3-15%) and minor pollen (<3%) belonged to more than 10 plant families.

Table 1. The melissopalynological analysis of the honey samples S1-S7

Sample code (S1-S7)	Predominant pollen (>45%)	Secondary pollen (16-45%)	Important minor pollen (3-15%)	Minor pollen (<3%)
	Family-Species	**Family**-Species	**Family**-Species	**Family**-Species
S1	***Tiliaceae-*** *Tilia* ssp.	***Hypericaceae-*** *Hypericum* ssp.	***Fabaceae-*** *Trifolium* ssp. *Robinia pseudoacacia* ***Rosaceae-*** *Fragaria* ssp.	***Rosaceae-*** *Filipendula ulmaria* ***Fabaceae*** ***Asteraceae*** ***Gramineae***
S2		***Polygonaceae-*** *Fagopyrum esculentum* ***Fabaceae-*** *Trifolium* ssp	***Asteraceae-*** *Achillea* ssp. *Cirsium* ssp. *Ambrosia* ssp ***Boraginaceae-*** *Symphytum* ssp. ***Fabaceae***	***Asteraceae-*** *Taraxacum officinale* *Centaurea* ssp. *Helianthus annuus* ***Rosaceae*** ***Plantaginaceae*** *Plantago* ssp. ***Apiaceae*** ***Boraginaceae-*** *Phacelia tanacetifolia*
S3	***Tiliaceae-*** *Tilia* ssp.		***Fabaceae***	***Rosaceae*** ***Gramineae***
S4	***Brassicaceae-*** *Brassica* ssp.			***Fabaceae-*** *Vicia* ssp. *Trifolium* ssp. ***Salicaceae-*** *Salix* ssp. ***Rosaceae-*** *Prunus* ssp.
S5		***Rosaceae*** ***Salicaceae-*** *Salix* ssp. ***Brassicaceae-*** *Brassica* ssp.	***Fabaceae-*** *Robinia pseudoacacia* ***Asteraceae-*** *Taraxacum officinale*	***Fabaceae-*** *Trifolium* ssp ***Betulaceae-*** *Betula* ssp. ***Fagaceae-*** *Quercus* ssp. ***Lamiaceae***
S6		***Fabaceae-*** *Trifolium* ssp.	***Gramineae-*** *Zea mays* ***Apiaceae*** ***Asteraceae-*** *Cirsium* ssp. *Achillea* ssp. ***Plantaginaceae*** *Plantago ssp.* ***Polygonaceae-*** *Rumex* ssp. ***Gramineae*** ***Rosaceae-*** *Rubus* ssp. ***Lamiaceae*** ***Fabaceae*** ***Fagaceae-*** *Castanea sativa*	***Asteraceae-*** *Centaurea* ssp. ***Fabaceae-*** *Vicia* ssp. *Robinia pseudoacacia*
S7		***Ericaceae***	***Salicaceae-*** *Salix* ssp. ***Asteraceae-*** *Taraxacum officinale* *Centaurea* ssp. ***Onagraceae-*** *Epilobium* ssp. ***Rosaceae*** ***Fabaceae***	***Caryophyllaceae-*** *Silene* ssp. ***Fagaceae-*** *Fagus* ssp. ***Rhamnaceae-*** *Rhamnus* ssp. ***Rosaceae-*** *Rubus* ssp. ***Asteraceae*** ***Tiliaceae-*** *Tilia* ssp. ***Gramineae*** ***Apiaceae***

The synthesis of the data presented above (Table 1) revealed that two samples were classified as lime tree honeys (S1 and S3), one sample as rapeseed honey (S4) and four samples proved to be multifloral honeys, having different types and percentages of pollen (S2, S5, S6 and S7).

The Folin-Ciocâlteu method was used in order to evaluate the total polyphenolic content and the following regression equation of the gallic acid calibration curve was used: $y = 5.3634x + 0.0812$, $R^2 = 0.9991$.

The amounts of polyphenols in the honey samples ranged between 19.49±0.78 mg GAE/100g honey and 274.65±1.85 mg GAE/100g honey (Table 2).

Table 2. The total polyphenolic content of the honey samples S1-S7

Honey sample	Total polyphenolic content (mg GAE/100g honey)
S1	23.50±1.32
S2	274.65±1.85
S3	21.40±1.32
S4	19.49±0.78
S5	20.01±0.78
S6	49.93±3.87
S7	75.01±1.40

The highest content of total polyphenols was identified in honey sample S2 (multifloral honey), while S4 (rapeseed honey) emphasized the smallest amounts of total polyphenols. Large amounts of total polyphenols were also recorded in honey samples S6 (multifloral honey) and S7 (multifloral honey), while honey samples S1 (lime tree honey), S3 (lime tree honey) and S5 (multifloral honey) revealed decreased levels of total polyphenols.

Honey samples belonging to the same assortment highlighted very varied values regarding the total polyphenolic content. For instance, honey sample S2 (multifloral honey) recorded the highest value (274.65 ± 1.85 mg GAE/100 g honey), while S5, also a multifloral honey presented low amounts of total polyphenols (20.01±0.78 mg GAE/100 g honey).

The properties and composition of honey depend on several factors, such as the floral source, climatic conditions, processing, storing and handling technologies (Kaskoniene and Venskutonis, 2010; Khalil et al., 2011).

The free radical scavenging of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was evaluated by a spectrophotometric method.

Moreover, for determining the antioxidant activity, the regression equation of the calibration curve % Inhibition/Trolox concentration was used: $y = 743.88x - 13.306$, $R^2 = 0.9988$.

Thereby, antiradical activity was expressed as an Inhibition percent and Milliequivalents Trolox/100 g honey sample (Table 3).

Table 3. The antioxidant activity of honey samples (S1-S7) by DPPH method

Honey sample	Inhibition %	Mmols Trolox/100 g honey sample
S1	11.96±5.28	3.40±0.71
S2	78.19±3.19	12.30±0.43
S3	15.6±4.01	3.89±0.54
S4	8.33±1.93	2.91±0.26
S5	8.83±4.84	2.98±0.65
S6	20.59±1.62	4.56±0.22
S7	27.86±5.99	5.53±0.81

The highest antioxidant activity, expressed in both ways was recorded by a multifloral honey sample (S2), while S4, a rapeseed honey sample presented the lowest antioxidant activity. An increased antioxidant activity was also revealed by honey samples S6 and S7, both of them being multifloral honeys.

In the present study we showed that the honey samples that recorded a strong antioxidant activity also revealed an increased content of total polyphenols.

These results were in agreement with the findings of other authors. Ferreira et al. (2009) and Kaškonienė et al. (2009) have also demonstrated that polyphenol-rich honey samples have higher antioxidant activity. Therefore, it can be stated that there is a strong relationship between these two parameters (Hołderna-Kędzia and Kędzia, 2006).

The hierarchy of the honey samples was almost identical for all the seven samples from the point of view of the total polyphenolic content and antioxidant activity (Table 4).

Table 4. Total polyphenolic content and radical scavenging activity (antioxidant activity) of the analysed honey samples

Investigated Parameters		Honey Samples						
		S1	S2	S3	S4	S5	S6	S7
Total Polyphenolic Content (mg GAE/100 g honey sample)	Min-Max range	22.34-25.13	272.0-276.3	19.54-22.34	18.42-20.29	19.17-20.84	44.71-54.03	73.80-76.78
	Mean	23.50	274.6	21.40	19.5	20.01	49.93	75.01
	St. Dev.	1.32	1.85	1.32	0.78	0.78	3.87	1.40
	Coefficient of variation %	5.61	0.68	6.17	4.02	3.9	7.75	1.86
Inhibition %	Min-Max range	7.04-19.30	74.62-81.92	11.89-21.30	5.62-10.18	4.19-15.03	19.59-23.01	21.87-36.16
	Mean	11.96	78.19	15.6	8.33	8.83	20.59	27.86
	St. Dev.	5.28	3.19	4.01	1.93	4.84	1.62	5.99
	Coefficient of variation %	44.14	4.08	25.72	23.13	54.88	7.88	21.51
Mmols Trolox/100 g honey sample	Min-Max range	2.74-4.38	11.82-12.80	3.39-4.65	2.54-3.16	2.35-3.81	4.42-4.88	4.73-6.65
	Mean	3.40	12.30	3.89	2.91	2.98	4.56	5.53
	St. Dev.	0.71	0.43	0.54	0.26	0.65	0.22	0.81
	Coefficient of variation %	20.8	3.49	13.84	9.02	21.94	4.77	14.57

For instance, the most valuable honey sample was represented by multifloral honey sample (S2), which reported the highest polyphenolic content and antioxidant potential.

Honey sample S4 (rapeseed honey), on the other hand, emphasized the lowest values regarding the investigated parameters.

The correlation between the total polyphenolic content and the antioxidant activity (Inhibition % and Milliequivalents Trolox) was performed by statistical analysis, which underlined that there was a strong positive correlation between the analyzed parameters (r=0.9828702 for Total Polyphenolic Content/Inhibition %; r=0.9828702 for Total Polyphenolic Content/Trolox; r=0.9999996 for Inhibition %/Trolox; $p<0.05$) (Table 5).

Table 5. Correlation between total polyphenolic content and radical scavenging activity of the analysed honey samples (correlation coefficients (r) value)

Parameters	Total Polyphenolic Content	Inhibition %	Trolox
Total Polyphenolic Content	-	0.9828702	0.9829022
Inhibition%	0.9828702	-	0.9999996
Trolox	0.9829022	0.9999996	-

Regarding the bactericidal activity of the honey samples S1-S7, the interpretation of the results was made on the basis of the diameters of the lysis zones, expressed in mm (Table 6).

Table 6. The bactericidal activity of honey samples (S1-S7)

Samples	Bacterial strains- diameters of lysis zones, expressed in mm		
	Staphylococcus aureus	*Bacillus cereus*	*Enterococcus faecalis*
S1	13.04	11.90	0
S2	20.98	12.85	14.37
S3	10.51	9.70	9.09
S4	0	7.43	7.65 PI
S5	0	0	0
S6	12.53	9.67	6.34
S7	15.64	10.46	12.36 PI
Positive control (Amoxicillin)	26.49 RC	R	18.05

RC= resistant colonies; R= resistant strain; PI= partial inhibition.

The most intense bactericidal activity on the *Staphylococcus aureus* strain (Figure 2) was observed in honey sample S2 (multifloral), the

diameter of the lysis zone approaching that of the positive control (amoxicillin).

It should be noted that the secondary pollen (16-45%) of the honey sample S2 belongs to the species *Fagopyrum esculentum* (Watanabe et al., 1997) and the genus *Trifolium* (Jerković et al., 2016), recognized in the literature for their strong antibacterial and antioxidant effects.

In the multifloral honey sample S5, the bactericidal activity was absent, while in honey samplesS6and S7 (both multifloral), the bactericidal activity was poor towards intermediate. Figure 2 also highlighted the presence of a synergic effect between lime honey and multifloral honey.

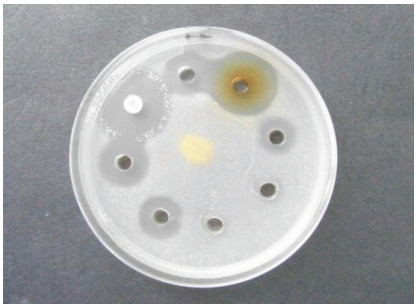

Figure 2. The bactericidal activity of honey samples (S1-S7) on the *Staphylococcus aureus* strain

The bactericidal activity against the strain of *Bacillus cereus* was absent in the honey sample S5 and decreased in the others, the lysis zone ranging from 9.67 mm (S6) to 12.85 mm (S2).

However, the examined honey samples (excepting honey sample S5) showed better bactericidal activity than the positive control (Figure 3).

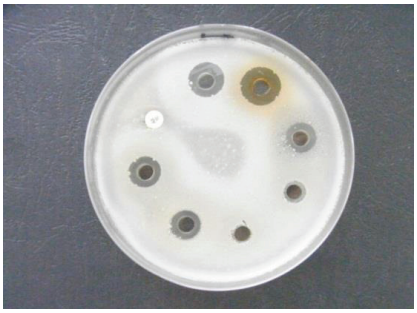

Figure 3. The bactericidal activity of honey samples (S1-S7) on *Bacillus cereus* strain

Only two of the fourmultifloral honey samples presented an increased bactericidal activity on the *Enterococcus faecalis* strain, namely honey samples S2 and S7 (Figure 4).

The two lime tree honey samples and the rapeseed honey sample indicated a low bactericidal activity on the three international reference strains used in the present research.

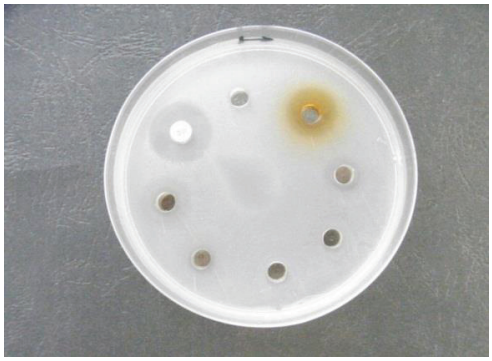

Figure 4. The bactericidal activity of honey samples (S1-S7) on *Enterococcus faecalis* strain

CONCLUSIONS

The melissopalynological analysis allowed to highlight the botanical origin of the honey samples, with the predominant plant species and the secondary species, many of the samples not being in conformity with the beekeeper's statement. According to the results obtained in the melissopalynological analysis, three of the seven honey samples were found to be monofloral and four were multifloral honeys, with different types and percentages of pollen. From the category of multifloral honey,two samples were lime tree honeys and one sample was a rapeseed honey.

We have shown a close positive correlation between the total polyphenolic content, antioxidant and antibacterial activity. In addition to this, the multifloral honey sample S2 recorded the highest content of total polyphenols, the strongest antioxidant activity and presented an extremely effective bactericidal activity against all three strains tested. Moreover, the honey samples S4 (rapeseed honey) and S5 (multifloral honey) have obtained the lowest values regarding the investigated parameters.

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EVALUATION OF RAW MILK QUALITY GATHERED FROM NORTH EAST AREA OF ROMANIA

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Abstract

This paper presents the data of a study on the commercial quality of fresh cow`s milk gathered from the North East area of Romania. Raw cow milk quality have been surveyed on samples collected from 126650 dairy cows (breeds Friesian, Simmental, Brown and Pinzgau) from 2458 farms from four counties in north eastern region of Romania. The samples were collected in sterilized plastic bottles of 50 ml preserved with bronopol 0.2 %, kept at refrigerating conditions till analysis. Analyses of raw milk included microbiological and physico-chemical parameters like bacterial count, somatic cell count, fat, protein, casein, lactose, urea, dry matter, density and pH. The results of the researches carried out indicated that all the raw milk collected fully complied with the en-force regulations concerning the physico-chemical quality features but for the safety hygienic ones, including the bacterial count (BC) and somatic cells count (SCC) the values found were higher.

Key words*: raw milk, microbiological features, proximate composition, cattle.*

INTRODUCTION

Milk is a natural product with a complex chemical composition (Pereira, 2014; Ivancia et al., 2019), being one of the most complete foods in nutritional terms; being rich in essential nutrients for growth and maintenance of a healthy life (Vilela, 2002; Marcondes et al., 2014).

The importance of adding milk to the human diet is because of its richness in proteins, fats, carbohydrates (lactose), mineral salts, vitamins, which provide immunologic protection and essential nutrients to its consumers (Sordillo et al., 1997; Oliveira et al., 1999; Matte et. al., 2014). The chemical composition is rather complex. Thus it provides an optimal environment for microorganism development (Filimon et al., 2011, Raţu et al., 2018).

Milk chemical composition is influenced by breed, season, physiological condition, animal individuality, lactation stage, feeding, body condition score, sanitary conditions of the mammary gland, interval between lactations, and the moment of milking at which the sample is collected (Miller et al., 1970; Fox and Mcsweeney, 1996; Lane et al., 1997; Bernabucci et al., 2002).

Most of the dry matter in milk is represented by nitrogenous substances, most of them (95%) being proteins and 5% being non-protean nitrogenous compounds (Harding, 1995; Bille et al., 2009).

Milk proteins are ranked as quality proteins (Raţu et al., 2019) with a good biological value and digestibility (97% to 98%) similar to fish meat proteins, rapid absorption and utilization in the body (Schaafsma, 2000).One of the most important proteins is casein (Bos et al., 2000).

It is well known that the fresh raw milk contains bacteria and somatic cells. These are the milk's biological constituents (Schutz et al., 1994). The numbers of these biological constituents varies according to production conditions like the animal's health and hygiene during milking, hygiene of the milking equipment, preserving and transporting the milk and the milk products (Turner et al., 1990; Maciuc et al., 2017). These microorganisms have an important role in the alteration and

contamination of milk (Filimon et al., 2011; Sakar, 2016).

Due to its chemical composition coupled with its high water content, a pH close to neutral, raw milk was recognized as a source of food-borne illness and disease (Sakar, 2016) and epidemiological reports on food-borne outbreaks due to consumption of raw milk infected with potential pathogens have been reported (Oliver et al., 2009).

Temperature control is critical to prevent milk alteration, because of the growth and multiplication of diverse microorganisms resulting in its early deterioration (Lues et al., 2010; Sakar, 2016).

Having in view the new regulations imposed by EU in 2016 (REGULATION (EU) 2016/1012), the current study aimed to present the evolution and the actual stage of raw milk quality from the north east area of Romania.

MATERIALS AND METHODS

The samples were gathered from 126650dairy cattle from 2458 exploitations' from 4 counties situated in the north east region of Romania. Samples were collected in sterilized plastic bottles of 50 ml during the official control of productive performances, which took place at every 28 days, in alternative ways (in first month at morning milking, and in the next month at evening milking). Each sample was previously preserved with bronopol 0.2%, labeled with a unique code and was also mentioned the animals identification number. The samples were kept at refrigerating conditions till the moment in which were delivered to analysis laboratory, and analysed in a maximum of a week from the moment in which the samples were brought to laboratory.

Analyses of milk physico-chemical composition included fat, protein, casein, lactose, urea, dry matter, density and pH were realized in according with AOAC norms (2019) using the Fourier Transformed Infrared technique (FTIR), performed with Lacto Scope (Delta Instruments). Microbiologically speaking, were analysed the following features: bacterial count (BC) using Bactoscope device, and somatic cell count (SCC) performed with Soma Scope device, the obtained results being multiplied by 1000. Before analysis samples were heated into a water bath till a temperature of 38ºC.

The software used for statistical analysis was SPSS. We calculated the average, standard deviation, coefficient of variation.

RESULTS AND DISCUSSIONS

In total a number of 309809 of raw milk samples were analyzed, data from the analysis were summarized in total (Table 1 and 2) and after separated by year and county and by year, county and season(Tables 3-6).

Table 1. Microbiological features of raw milk samples

Specification	Bacterial count (ufc/ml)	Somatic cell count (scc/ml)
$\bar{X}$	182.48	457.19
$s_{\bar{x}}$	387.78	834.03
V (%)	192.29	182.42

*Results have to be multiplied by 1000

Table 2. Proximate composition of raw milk samples

Specification	Fats (%)	Proteins (%)	Lactose (%)	Dry matter (%)	Urea (mg/100 g)	Casein (g/l)	Density (g/l)	pH
$\bar{X}$	3.84	3.366	4.72	12.61	25.337	26.42	1029.52	6.64
$s_{\bar{x}}$	0.78	0.34	0.24	0.68	8.94134	2.93	0.20	0.05
V (%)	20.29	10.29	5.25	5.39	35.28	11.11	0.01	0.85

Regarding the microbiological features of raw milk we can observe that the values for the both indicators exceed the maximum values permitted by national regulatory. We can conclude that producers did not fully respect the good hygiene practices, both during milking, storage or transportation of the raw milk. Analysing the data presented in Table 2, we can observe that the average values for all the determined parameters are similar with the ones mentioned in the specialty literature.

Table 3. Microbiological and proximate composition of raw milk samples in 2017

Specification		Botoşani	Iaşi	Neamţ	Suceava
Bacterial count (ufc/ml)	$\bar{X} \pm s_{\bar{x}}$	192.36±73.25	206.25±64.32	177.41±56.48	185.27±74.34
	V%	165.83	234.58	174.21	165.92
Somatic cell count (scc/ml)	$\bar{X} \pm s_{\bar{x}}$	513.85±890.46	510.01±888.15	400.59±819.94	383.49±710.35
	V%	173.29	174.14	204.68	185.23
Fats (%)	$\bar{X} \pm s_{\bar{x}}$	3.68±0.74	4.10±0.90	3.98±0.71	3.95±0.82
	V%	20.33	21.98	18.00	20.72
Proteins (%)	$\bar{X} \pm s_{\bar{x}}$	3.44±0.36	3.48±0.35	3.45±0.31	3.36±0.40
	V%	10.62	10.05	10.16	12.08
Lactose (%)	$\bar{X} \pm s_{\bar{x}}$	4.75±0.23	4.75±0.22	4.75±0.25	4.63±0.28
	V%	4.90	4.67	5.32	6.00
Dry matter (%)	$\bar{X} \pm s_{\bar{x}}$	12.56±0.12	12.98±0.98	12.91±0.98	12.75±0.81
	V%	1.02	7.61	7.65	6.36
Urea (mg/100 g)	$\bar{X} \pm s_{\bar{x}}$	24.40±7.74	24.41±7.80	19.27±10.95	25.09±7.46
	V%	31.75	31.98	56.85	29.74
Casein (g/l)	$\bar{X} \pm s_{\bar{x}}$	27.20±2.75	27.53±2.73	26.88±2.83	26.25±3.28
	V%	10.13	9.93	10.55	12.51
Density (g/l)	$\bar{X} \pm s_{\bar{x}}$	1029.27±0.20	1029.33±0.87	1029.83±0.87	1029.95±0.88
	V%	0.01	0.08	0.07	0.08
pH	$\bar{X} \pm s_{\bar{x}}$	6.64±0.05	6.64±0.06	6.65±0.10	6.64±0.12
	V%	0.85	1.01	1.60	1.87

Table 4. Microbiological and proximate composition of raw milk samples in 2018

Specification		Botoşani	Iaşi	Neamţ	Suceava
Bacterial count (ufc/ml)	$\bar{X} \pm s_{\bar{x}}$	184.36±73.45	194.45±72.34	172.14±63.13	175.47±74.34
	V%	165.83	134.58	154.41	165.84
Somatic cell count (scc/ml)	$\bar{X} \pm s_{\bar{x}}$	583.16±1004.94	406.15±853.68	475.05±761.63	433.94±819.49
	V%	172.32	210.18	160.34	188.84
Fats (%)	$\bar{X} \pm s_{\bar{x}}$	3.90±0.71	4.24±0.73	3.64±0.71	3.43±0.81
	V%	18.24	17.30	19.70	23.83
Proteins (%)	$\bar{X} \pm s_{\bar{x}}$	3.10±0.32	3.43±0.36	3.48±0.33	3.37±0.37
	V%	10.85	10.67	9.85	11.24
Lactose (%)	$\bar{X} \pm s_{\bar{x}}$	4.73±0.19	4.66±0.22	4.75±0.24	4.64±0.29
	V%	4.17	4.92	5.19	6.32
Dry matter (%)	$\bar{X} \pm s_{\bar{x}}$	12.74±0.97	12.72±0.69	12.35±0.90	12.18±0.89
	V%	7.68	5.49	7.31	7.34
Urea (mg/100 g)	$\bar{X} \pm s_{\bar{x}}$	21.94±9.75	24.55±7.91	29.14±7.87	27.65±6.25
	V%	44.48	32.25	27.00	22.61
Casein (g/l)	$\bar{X} \pm s_{\bar{x}}$	26.54±2.58	26.88±2.90	26.48±2.87	26.43±2.84
	V%	9.74	10.82	10.85	10.74
Density (g/l)	$\bar{X} \pm s_{\bar{x}}$	1029.15±0.89	1029.70±0.76	1029.22±1.06	1029.73±1.03
	V%	0.08	0.07	0.07	0.10
pH	$\bar{X} \pm s_{\bar{x}}$	6.65±0.10	6.68±0.16	6.63±0.13	6.59±0.13
	V%	1.55	2.51	2.08	2.07

From the data presented in Table 3, for year 2017, we observe that the analysed samples had a very good homogeneity regarding the following characters: lactose, dry matter, density and pH (for all the counties). A medium homogeneity was recorded for protein and casein content, also for all the counties. The obtained values were inhomogeneous for the following characteristics: bacterial count, somatic cell count, as well as for fat and urea content. The results obtained from the analysed samples gathered in 2018 show a very good homogeneity for lactose, dry matter, density and pH; a good homogeneity for protein and casein content and an in-homogeneity for bacterial count, somatic cell count, fat and urea content. A possible explanation of those recorded data could be that the small farmers didn't fully respect the welfare conditions for animals. In Tables 5 and 6 are presented, on seasons, data regarding raw milk microbiological and proximate composition gathered, in 2017 and 2018.

Table 5. Variation of microbiological and proximate composition of raw milk samples in 2017 seasons

Specification		Bacterial count (ufc/ml)	Somatic cell count (scc/ml)	Fats (%)	Proteins (%)	Lactose (%)	Dry matter (%)	Urea (mg/100 g)	Casein (g/l)	Density (g/l)	pH
Botosani											
Spring	$\bar{X}$	142.32	562.92	3.83	3.38	4.25	12.32	22.90	25.20	1029.13	6.66
	$s_{\bar{x}}$	140.34	1222.14	0.66	0.30	0.12	0.92	8.88	2.32	0.80	0.08
	V(%)	134.25	161.04	12.35	9.21	3.62	2.34	39.90	9.23	0.02	1.33
Summer	$\bar{X}$	152.32	403.40	3.62	3.25	4.21	12.12	26.66	25.20	1029.14	6.61
	$s_{\bar{x}}$	150.34	723.99	0.66	0.29	0.20	0.92	11.44	2.31	0.96	0.13
	V(%)	189.44	139.45	19.39	9.29	4.45	2.12	42.91	9.00	0.09	2.06
Autumn	$\bar{X}$	131.12	324.99	4.01	3.49	4.69	12.43	21.40	22.43	1029.20	6.66
	$s_{\bar{x}}$	142.44	593.60	0.23	0.31	0.20	0.99	5.21	2.56	0.92	0.10
	V(%)	136.25	129.63	19.32	9.96	4.39	2.66	26.69	9.35	0.08	1.50
Winter	$\bar{X}$	112.12	692.55	3.99	3.43	4.29	12.33	19.12	26.52	1029.11	6.65
	$s_{\bar{x}}$	121.14	1166.39	0.69	0.33	0.19	0.94	9.41	2.55	0.86	0.08
	V(%)	104.52	169.64	12.39	9.26	3.95	2.30	44.00	9.60	0.08	1.31
Iaşi											
Spring	$\bar{X}$	216.27	536.03	4.089	3.41	4.79	12.90	25.33	26.98	1029.37	6.66
	$s_{\bar{x}}$	237.43	888.94	0.90	0.30	0.19	0.97	8.86	2.41	0.87	0.07
	V(%)	206.33	165.83	22.09	9.06	4.01	7.54	35.01	8.94	0.08	1.12
Summer	$\bar{X}$	266.72	487.96	3.87	3.27	4.72	12.64	24.86	26.10	1029.21	6.63
	$s_{\bar{x}}$	273.22	949.42	0.81	0.32	0.20	1.04	7.64	2.59	0.87	0.05
	V(%)	202.23	194.56	20.95	9.87	4.41	8.25	30.73	9.93	0.08	0.81
Autumn	$\bar{X}$	266.27	248.26	4.26	3.59	4.673	13.11	22.66	28.64	1029.24	6.65
	$s_{\bar{x}}$	273.55	464.07	0.86	0.35	0.25	0.91	5.97	2.88	0.82	0.06
	V(%)	226.22	186.93	20.30	9.96	5.37	6.97	26.37	10.06	0.07	0.97
Winter	$\bar{X}$	109.37	728.13	4.12	3.591	4.790	13.039	24.647	28.06	1029.45	6.62
	$s_{\bar{x}}$	337.34	1058.57	0.95	0.32	0.21	0.97	7.82	2.46	0.91	0.06
	V(%)	222.56	145.38	23.13	8.98	4.43	7.48	31.75	8.78	0.08	0.93
Neamţ											
Spring	$\bar{X}$	176.27	325.51	3.82	3.53	4.80	12.67	22.00	25.42	1029.75	6.63
	$s_{\bar{x}}$	187.43	713.01	0.71	0.34	0.22	0.77	10.20	2.95	0.75	0.13
	V(%)	106.33	219.04	18.49	9.72	4.61	6.05	46.35	11.59	0.07	1.95
Summer	$\bar{X}$	266.72	483.84	4.26	3.35	4.66	13.64	27.80	24.51	1030.01	6.67
	$s_{\bar{x}}$	178.11	853.08	0.70	0.33	0.22	0.98	11.06	2.63	0.85	0.13
	V(%)	101.13	176.32	16.40	9.90	4.74	7.22	39.79	10.71	0.08	1.98
Autumn	$\bar{X}$	186.27	375.65	4.07	3.42	4.65	13.11	23.61	27.16	1029.81	6.62
	$s_{\bar{x}}$	278.55	924.27	0.71	0.34	0.25	1.06	10.91	2.91	0.88	0.15
	V(%)	126.21	246.05	17.35	9.79	5.45	8.12	46.21	10.70	0.09	2.31
Winter	$\bar{X}$	79.87	378.64	3.98	3.46	4.76	12.92	19.27	26.89	1029.83	6.65
	$s_{\bar{x}}$	87.84	717.46	0.72	0.35	0.25	0.99	10.96	2.84	0.80	0.11
	V(%)	112.56	189.49	18.00	10.16	5.33	7.66	56.86	10.55	0.08	1.60
Suceava											
Spring	$\bar{X}$	163.76	435.31	3.71	3.22	4.76	12.48	23.68	24.98	1030.04	6.66
	$s_{\bar{x}}$	166.52	811.62	0.81	0.37	0.23	0.87	7.75	3.00	0.89	0.12
	V(%)	181.50	186.45	21.68	11.50	4.83	6.98	32.75	12.01	0.09	1.86
Summer	$\bar{X}$	202.76	428.76	4.16	3.28	4.63	13.00	26.05	25.57	1030.00	6.63
	$s_{\bar{x}}$	116.57	756.29	0.79	0.33	0.30	0.69	6.92	2.57	0.90	0.12
	V(%)	165.99	176.39	18.93	9.94	6.39	5.30	26.56	10.05	0.09	1.74
Autumn	$\bar{X}$	137.76	284.42	4.02	3.62	4.65	12.80	25.36	28.48	1029.79	6.68
	$s_{\bar{x}}$	121.57	535.60	0.81	0.41	0.30	0.77	7.59	3.24	0.83	0.12
	V(%)	136.63	188.31	20.12	11.40	6.50	6.05	29.92	11.38	0.08	1.87
Winter	$\bar{X}$	102.76	260.96	3.94	3.57	4.70	12.73	26.26	27.95	1029.90	6.61
	$s_{\bar{x}}$	161.53	361.38	0.78	0.41	0.28	0.78	7.11	3.23	0.86	0.14
	V(%)	151.63	138.48	19.87	11.47	5.90	6.09	27.08	11.56	0.08	2.18

For raw milk collected in Botoşani County (2017, all seasons) we obtained very homogenous character for lactose and casein content, density and pH. For bacterial count and somatic cell count those characters were inhomogeneous. The rest of the features had a very good or good homogeneity depending on season. For the rest of counties the results show a good to very good homogeneity for all the studied characters with the exception of bacterial and somatic cell count which were inhomogeneous.

Table 6. Variation of microbiological and proximate composition of raw milk samples in 2018 seasons

Specification		Bacterial count (ufc/ml)	Somatic cell count (scc/ml)	Fats (%)	Proteins (%)	Lactose (%)	Dry matter (%)	Urea (mg/100 g)	Casein (g/l)	Density (g/l)	pH
Botosani											
Spring	$\bar{X}$	162.72	761.97	3.83	3.28	4.75	12.56	22.90	25.70	1029.13	6.66
	$s_{\bar{x}}$	140.76	1227.14	0.66	0.30	0.17	0.92	8.88	2.37	0.80	0.08
	V(%)	136.25	161.04	17.35	9.21	3.67	7.34	38.80	9.23	0.07	1.33
Summer	$\bar{X}$	192.72	703.40	3.62	3.25	4.71	12.27	26.66	25.70	1029.14	6.61
	$s_{\bar{x}}$	150.76	973.89	0.66	0.28	0.20	0.87	11.44	2.31	0.96	0.13
	V(%)	136.25	138.45	18.38	8.79	4.45	7.17	42.91	9.00	0.09	2.06
Autumn	$\bar{X}$	131.12	324.89	4.01	3.48	4.69	12.93	21.40	27.43	1029.20	6.66
	$s_{\bar{x}}$	142.64	583.60	0.73	0.31	0.20	0.99	5.71	2.56	0.92	0.10
	V(%)	112.25	179.63	18.32	8.96	4.38	7.66	26.69	9.35	0.08	1.50
Winter	$\bar{X}$	192.12	687.55	3.99	3.43	4.78	12.93	19.123	26.57	1029.11	6.65
	$s_{\bar{x}}$	121.16	1166.38	0.69	0.33	0.18	0.94	8.41	2.55	0.86	0.08
	V(%)	106.52	169.64	17.38	9.76	3.85	7.30	44.00	9.60	0.08	1.31
Iaşi											
Spring	$\bar{X}$	186.27	639.12	4.02	3.40	4.68	12.64	24.13	26.48	1029.83	6.71
	$s_{\bar{x}}$	237.83	1148.65	0.76	0.32	0.24	0.75	7.72	2.58	0.76	0.27
	V(%)	203.33	179.72	18.99	9.47	5.14	5.96	32.01	9.77	0.07	4.10
Summer	$\bar{X}$	253.72	482.81	4.51	3.16	4.63	12.77	30.79	24.81	1029.61	6.64
	$s_{\bar{x}}$	273.22	931.18	0.58	0.34	0.21	0.66	8.47	2.65	0.75	0.09
	V(%)	202.23	192.86	12.96	11.01	4.71	5.17	27.52	10.70	0.07	1.42
Autumn	$\bar{X}$	203.27	230.22	4.40	3.57	4.63	12.78	22.25	28.06	1029.65	6.69
	$s_{\bar{x}}$	273.55	529.71	0.68	0.33	0.20	0.62	6.35	2.60	0.72	0.07
	V(%)	223.22	230.09	15.52	9.25	4.49	4.87	28.55	9.29	0.07	1.11
Winter	$\bar{X}$	109.37	253.29	4.12	3.53	4.71	12.71	22.60	27.69	1029.68	6.65
	$s_{\bar{x}}$	337.38	528.095	0.74	0.34	0.23	0.72	6.54	2.78	0.79	0.09
	V(%)	222.53	208.49	18.17	9.75	5.00	5.70	28.94	10.04	0.07	1.36
Neamț											
Spring	$\bar{X}$	174.27	537.62	3.64	3.42	4.75	12.41	29.40	26.47	1029.25	6.64
	$s_{\bar{x}}$	157.43	795.23	0.72	0.34	0.25	1.01	8.02	3.05	1.08	0.13
	V(%)	104.33	147.92	19.69	9.85	5.33	8.14	27.28	11.52	0.11	2.03
Summer	$\bar{X}$	244.72	322.66	3.64	3.41	4.78	12.36	29.18	26.99	1029.24	6.63
	$s_{\bar{x}}$	175.11	311.47	0.70	0.34	0.23	0.90	7.73	3.16	1.05	0.14
	V(%)	101.13	96.53	19.34	9.91	4.85	7.28	26.51	11.70	0.10	2.07
Autumn	$\bar{X}$	144.27	405.93	3.63	3.42	4.75	12.34	29.03	26.64	1029.22	6.63
	$s_{\bar{x}}$	275.55	567.41	0.73	0.34	0.24	0.91	7.89	2.82	1.07	0.14
	V(%)	124.21	139.78	20.08	9.89	5.07	7.38	27.18	10.58	0.10	2.10
Winter	$\bar{X}$	72.57	724.86	3.66	3.43	4.74	12.38	29.10	25.63	1029.19	6.63
	$s_{\bar{x}}$	57.54	1217.45	0.71	0.33	0.27	0.88	7.84	2.22	1.03	0.14
	V(%)	102.54	167.96	19.43	9.74	5.63	7.14	26.95	8.66	0.10	2.12
Suceava											
Spring	$\bar{X}$	152.46	564.60	3.95	3.16	4.63	12.12	42.40	26.06	1029.99	6.46
	$s_{\bar{x}}$	116.30	844.79	0.97	0.55	0.22	0.66	0.55	4.31	2.02	0.18
	V(%)	131.60	149.63	24.48	17.44	4.81	5.45	1.29	16.55	0.20	2.74
Summer	$\bar{X}$	192.46	361.39	3.54	3.33	4.68	12.21	28.21	25.88	1029.48	6.54
	$s_{\bar{x}}$	116.57	550.84	0.76	0.32	0.26	0.99	6.14	2.51	0.89	0.12
	V(%)	151.67	3035.19	21.47	9.70	5.57	8.08	21.77	9.69	0.09	1.90
Autumn	$\bar{X}$	164.46	660.94	3.29	3.38	4.63	12.04	28.16	26.20	1029.90	6.62
	$s_{\bar{x}}$	116.57	1087.42	0.80	0.36	0.29	0.89	6.13	2.57	1.08	0.15
	V(%)	151.68	164.53	24.26	10.76	6.36	7.40	21.77	9.79	0.11	2.20
Winter	$\bar{X}$	182.46	292.46	3.61	3.41	4.64	12.39	27.08	26.87	1029.62	6.59
	$s_{\bar{x}}$	116.58	555.80	0.82	0.39	0.31	0.90	6.16	3.08	1.03	0.13
	V(%)	151.68	190.04	22.84	11.49	6.62	7.24	22.76	11.47	0.10	2.02

From the data presented in table 6 (for all seasons from year 2018) the same conclusions, as in 2017, could be drawn. The bacterial and somatic cell count were inhomogeneous while the rest of the features (fat, protein, lactose, dry matter, urea, casein content, as well as density and pH) had a good to very good homogeneity depending on season.

CONCLUSIONS

Milk gathered had a poor microbiological quality with values of BC and SCC close to or even above the threshold of actual sanitary regulations. This was due to the fact that producers did not fully respect the hygiene practices, during milking, storage or transportation of the raw milk.

However, all the raw milk collected fully complied with the en-force regulations of the European Union concerning the physico-chemical quality features but for the safety hygienic ones, including the bacterial count (BC) and somatic cells count (SCC) the values found were higher. Cleaning and disinfection of milking equipment is one of the critical control points for determining the hygienic quality of raw milk.

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THE INFLUENCE OF AUXILIARY MATERIALS ON HARDNESS, HEAT TREATMENT LOSSES AND SENSORY PROPERTIES OF THE MEAT PRODUCTS

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Abstract

The use of additives in the meat industry must ensure that obtained products are appreciated by consumers, provided good yields are achieved. Thus, the work follows the influence of water, salt, polyphosphate and fat additions, on heat treatment losses, hardness, as well as sensory properties of meat products. Analyzing the experimental results it has been found that with the increase in the percentage of water and fat added, cooking losses increased, while the hardness decreased. Increasing the addition of salt has determined to a decrease in heat treatment losses and increased hardness of thermally treated products. The addition of polyphosphates increasing has generated to a decrease in heat treatment losses and hardness of products. By following the degree of sensory satisfaction, in general, increasing the addition of water, salt, polyphosphates has led to appreciated products, with the exception of the increase in added fat that has led to a decrease in the satisfaction of consumers.

Key words*:meat products, heat treatment losses, hardness.*

INTRODUCTION

Meat and meat products are an important source of food for humans through complex chemical composition. Apart from the high nutritional value, the sensory value of the meat is very important, and the valorisation of the meat in superior meat products from the sensory point of view, implies the study of the factors that generate this quality (technologies, used additives, equipment's, etc.) (Banu, 2002; Georgescu et al., 2019).

On the other hand, the transformation of meat into meat products must be done under optimum conditions, ensuring a correlation between the quality obtained and costs, the technological losses (boiling, smoking, storage) directly influencing these costs.

The impact of various additives on the quality of the products and the behaviour to the heat treatment was studied. Liu (2000) showed that the proteins of vegetable origin used to obtain meat products have improved the emulsification and water retention capacity of the heat-treated products. Gadekar (2016) the cooking yield of goat meat product significantly improved due to the addition of textured soy protein.

The use of modified starch in meat mixtures improved the stability of the emulsion and reduced the separation of fats (Aktaş N., Gençcelep H., 2006), and Cierach et al (2014) showed that the use of starch reduces heat treatment losses, improving sensory properties, texture and stability of the colour. Trout et al. (1986) considers that polyphosphates and pH are responsible for 80% of water binding capacity and Glorieux S. (2017) studied the reduction of phosphates in meat products in order to limit the impact on health, but without affecting the quality of the products. Zhuang (2016) showed the reduction of heat treatment losses and the increase of the hardness of salted and kneaded meats as the salt concentration increased.

The paper aims to study the impact of the addition of water, fat, salt and polyphosphates on the hardness of meat products, the sensory properties related to the texture and the implications on the losses to heat treatment.

MATERIALS AND METHODS

The study used emulsions obtained from chilled pork, fat (10%, 20%, 30%), water (10%, 20%, 30%), salt (1%, 2%, 3%), sodium nitrite (0.013%) and polyphosphates (0%, 0.3%, 0.5%), the additions being related to meat. Samples with a diameter of 2 cm and a length of 10 cm were boiled at 70^0C for 10 minutes in the thermal center of the product and thermostated for 12 hours at 4^0C. The analyzed parameters were the hardness of the products and losses registered during the heat treatment.
For the determination of the hardness of the products, appreciated by the cutting force, the samples with diameters of 2 cm that have been cut at the texturemeter TA-XT Plus. The cutting was performed perpendicular to the longitudinal axis of the samples.
After the heat treatment, the samples were cooled to 20^0C, weighed and the losses were expressed as juice and fat.

RESULTS AND DISCUSSIONS

The influence of fat addition

Studying the influence of the addition of fat on the quality of meat products, we can say that the fat used has a positive influence, if: the protein tissue of fatty tissue is not fragile and abundant. in this sense, the fat should not contain surplus connective tissue, and the one surrounding the fat cell must be sufficiently resistant, so that at least one damaged fat cell is crushed; the fat is not "oily" at the temperatures used in the technology of manufacture of sausages, because the oily phase expelled from the fat cells forms films on the surface of the meat granules, thus preventing the migration of water to the surface of the rod in the drying processes, prevents the paste from being bound, no consistency is achieved. It's recommended to use strong fat; the fat has a high degree of freshness, the enzymatic hydrolysis of the lipids can lead to a change in the taste, which becomes soapy, and the consistency of the product becomes soft.
Analyzing the losses to the heat treatment registered for the products with different additions of fat, we could find the following:
- the increase of the fat concentration in the meat composition has led to an increase of the losses due to the heat treatment, which indicates that with the increase of the added fat concentration decreases the stability of the emulsion formed during the chopping – mixing;
- thus, the losses increased from 14.49% for the samples with 10% fat, to 29.3% for the samples with 20% fat, respectively 30.28% for the samples with 30% fat (Table 1, Figure 1).

Table 1. Variation of cooking loss depending on the fat and water added

The type of additions		Cooking loss, %
Addition of fat, %	10	14.49
	20	29.3
	30	30.28
Addition of water, %	10	18.71
	20	24.68
	30	27.71

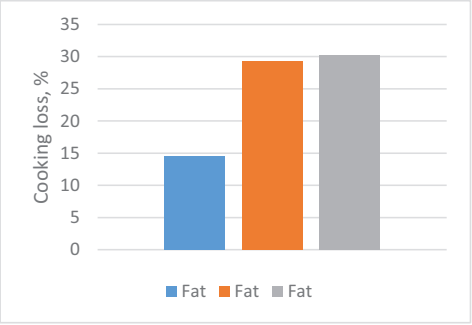

Figure 1. Cooking loss variation depending on the added fat

Analyzing the hardness of the heat-treated samples, it was found that with the increase in the amount of added fat, the cutting forces of the analyzed samples decreased, thus: the samples with a fat content of 10% had a hardness of 1.92 kgf, those with 20% fat 1.51 kgf, respectively 1.12 kgf for samples with 30% fat (Figure 2).
The high hardness values of the low fat samples may be because these samples had higher amounts of muscular tissue and consequently higher concentrations of structural proteins extracted in the fluid phase of the composition, which established bonds between particles, creating a strong and stable matrix after heat treatment.
Sensory analysis of the samples with different fat additions showed that the most appreciated, in terms of taste, succulence, appearance in the

section, were the samples with 10% and 20% fat. The composition with 10% added fat had a good succulence, pleasant taste, which is due to the stability of the emulsion given by the muscular test and the smaller losses recorded during the heat treatment. The composition with 30% fat had air voids, cracks inside the product, islands of fat.

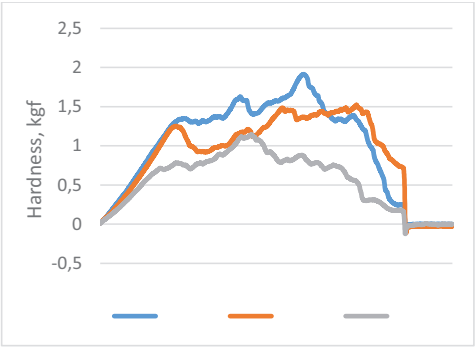

Figure 2. Hardness variation depending on the added fat

Sensory analysis of the samples with different fat additions showed that the most appreciated, in terms of taste, succulence, appearance in the section, were the samples with 10% and 20% fat. The composition with 10% added fat had a good succulence, pleasant taste, which is due to the stability of the emulsion given by the muscular test and the smaller losses recorded during the heat treatment. The composition with 30% fat had air voids, cracks inside the product, islands of fat.

The influence of water addition

Overall, losses from heat treatment are influenced by: the meat pH and correlated with it by the capacity of water retention; the diameter of the heat treated product; the presence or absence of the membrane; the addition of additives that increase the capacity of hydration and water retention (NaCl, polyphosphates); the meat structure that characterizes a certain biochemical phase after slaughter; the temperature and boiling time.

The assessment of the losses due to the heat treatment showed that they vary directly in proportion to the percentage of water added (Figure 3).

The samples with 20% and 30% water recorded higher losses, respectively 24.68% and 27.71%, compared with those with 10% water, which had 18.71% losses. The water retention capacity depends on the pH of the meat, the increase of the pH determines the increase of the water retention capacity.

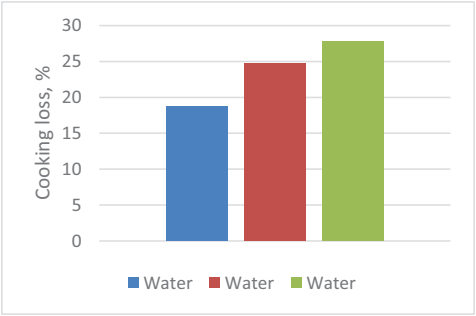

Figure 3. Cooking loss variation depending on the addedwater

The speed of reaching the final pH also influences the water holding capacity. If the pH drop is rapid, while the musculature maintains a temperature higher than 35°C, a denaturing of the myofibrillary proteins occurs and the conformation of the actomyosin molecule changes.

By precipitating the sarcoplasmic proteins over the myofibrillary ones, the groups involved in fixing the water molecules are masked, which diminishes the water retention capacity.

Up to a certain limit, increasing the amount of added water causes the adhesion of the composition to increase, as a result of the passage in electrolytic solutions of a larger quantity of structural proteins. When are added too large quantities of water, the adhesiveness of the product decreases.

Up to a certain limit, increasing the amount of added water causes the adhesiveness of the composition to increase, as a result of the passage in the electrolytic solution of a larger quantity of structural proteins.

When too large quantities of water are added, the adhesiveness of the product decreases.

The analysis of the variation of the cutting force according to the percentage of water added shows that as the percentage of water increases, the cutting force of the heat-treated samples decreases.

Thus, samples with 10% water recorded a hardness of 2.14 kgf, those with 20% water recorded a value of 1.76 kgf, and those

with 30% water recorded a value of 1.4 kgf (Figure 4).

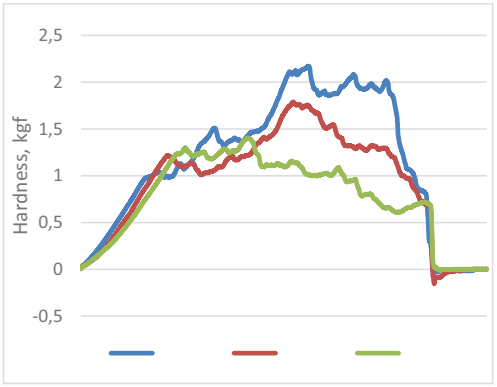

Figure 4. Hardness variation depending on the added water

Compared to the 10 and 20% water samples, the 30% water addition sample has a more pleasing appearance, taste and color, does not show air and gelatin holes. The sample with the addition of 20% water has a well-defined odor and pleasant taste. The 10% water sample had low succulence and the composition did not bind well. Inappropriate binding of the composition with 10% water was explained by the fact that no optimal extraction medium was created for the structural proteins that would ensure a good paste binding.

The influence of salt addition

The addition of NaCl to the meat compositions determines, besides the improvement of the sensory and preservative properties, the increase of the apparent viscosity and the shear stress, the increase of the ionic strength of the environment, which in turn favors the extraction of myofibrillary proteins in the liquid phase.

Increasing the concentration of solubilized proteins favors water binding and reduction of boiling losses, with the improvement of the succulence of the finished products (Figure 5).

Heat losses ranged from 32.65% for 1% salt samples, to 24.44% for 2% salt samples and to 14.59% for 3% salt samples (Table 2).

The variation of the cutting force was directly proportional to the salt concentration of the mixture, with the mention that the differences between the values were not major. Thus, at 1% salt the cutting force was 1.77 kgf, at 2% salt the cutting force was 1.92 kgf, and for 3% salt the cutting force was 2 kgf (Figure 6).

Table 2.Variation of cooking loss depending on the salt and polyphosphates added

The type of additions		Cooking loss, %
Addition of salt, %	1	32.65
	2	24.44
	3	14.59
Addition of polyphosphates, %	0	22.26
	0.3	19.50
	0.5	17.23

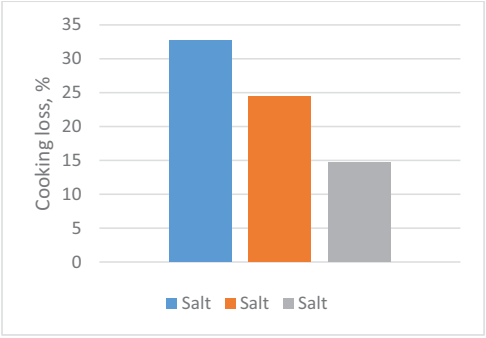

Figure 5. Cooking loss variation depending on the added salt

The increase of the extraction of structural proteins, favored by the increase of the salt concentration, favors the establishment of a greater number of links between the components of the system.

Sensory analysis of the samples showed that the composition with 1% salt added has an inadequate elasticity, consistency and succulence (it's crushed when cut). The 3% salt composition has the best sensory properties (consistency, juiciness, section appearance and smell).

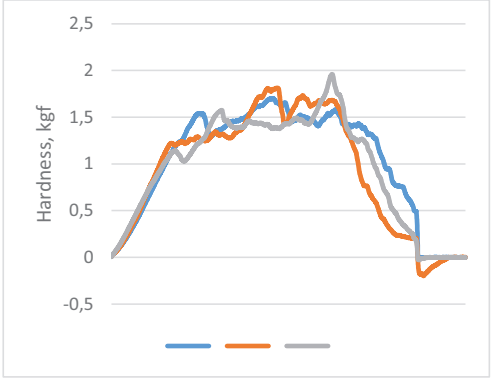

Figure 6. Hardness variation depending on the added salt

The influence of polyphosphates addition

Analyzing the influence of polyphosphates on boiling losses it was found that the highest losses were recorded for the polyphosphate-free compositions.

Polyphosphates favor the binding of water to muscle proteins, which has repercussions on the sensory characteristics, the quality of the emulsions and of finished product yield. As polyphosphate addition increases, water losses are lower: 17.23% for 0.5% polyphosphate samples, 19.50% for 0.3% polyphosphate samples and 22.26% for non-polyphosphate samples (Table 2, Figure 7).

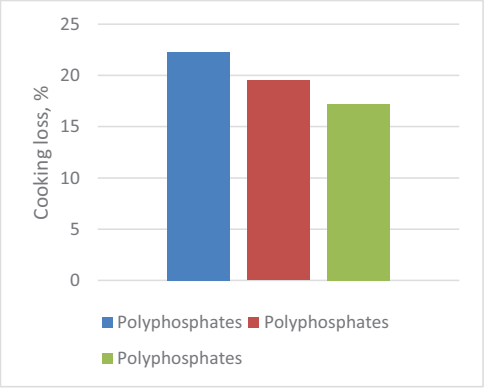

Figure 7. Cooking loss variation depending on the addedpolyphosphates

The addition of polyphosphate resulted in a decrease in the cutting force, with significant differences between samples with and without polyphosphate. The polyphosphate-free composition has the highest cutting force of 2.24 kgf because it has lost most of the water. The sample with 0.3% polyphosphate had a cutting force of 1.51 kgf and the sample with 0.5% polyphosphate, which had a cutting force of 1.24 kgf (Figure 8).

By the addition of polyphosphates the pH of the meat increases, the meat proteins are brought to a pH higher than the isoelectric point, which increases the hydration capacity.

Polyphosphates are capable of forming complexes with $Ca^{2+}$and Mg^{2+} ions which (exist in meat in amounts ranging from9 to 20 mg/100 g of muscle tissue) form bridges between the electrically charged groups of protein chains, thereby reducing the hydration capacity and water retention (Banu, 1997).

The formation of polyphosphate - Ca^{2+}/Mg^{2+} complexes leads to a relaxation of the structure of the miofibrilar proteins, that is, the increase of the capacity of hydration and water binding.

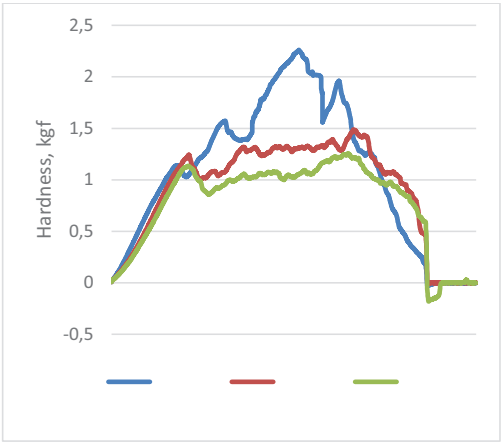

Figure 8. Hardness variation depending on the addedpolyphosphates

The addition of polyphosphates favors the dissociation of the actomyosin complex into actin and myosin, which have a higher hydration capacity.

Sensory analysis of the samples without the addition of polyphosphate emphasized that they do not show succulence and elasticity. The compositions with the addition of 0.3 g and 0.5 g polyphosphate have the corresponding consistency, elasticity and succulence.

CONCLUSIONS

Following the analysis of the obtained results, it was found that with the increase of the fat and water concentration of the compositions the losses to the heat treatment increase and the hardness of the products decreases. The addition of salt and polyphosphates caused the decrease of heat treatment losses. The increase of the salt addition caused the hardness increase, and the increase of the concentration of polyphosphates caused to decrease the hardness of the cooked products. All the additives used have generally improved the sensory characteristics of the obtained products. The increase in the concentration of added fats has led to a decrease in consumer satisfaction.

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SPECIFIC GLUTEN-BASED FLOURS RECOMMENDED IN THE GLUTEN-FREE DIET

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Abstract

The bakery industry is based on the unique properties of gluten proteins from wheat and some other cereals flour. Those unique viscoelastic properties are responsible for the specific characteristics of different wheat flour foods. Porosity, elasticity, texture and structure of these products are attributes that make bakery products based on wheat flour more attractive for consumers. Unfortunately, the incidence of gluten intolerance or celiac disease among the Romanian population is increasing in the last few years and the only treatment of the disease is in fact a very strict control of the diet in order to eliminate any source of gluten from the diet. In this regard, developing of new, gluten-free bakery products ensures a wider range of product for ill people and at the same time to ease their life by providing industrial products which make the meal preparing time more efficient spent. The present paper presents the gluten-free alternatives to wheat flour products for baking industry.

Key words*: alternative flours, celiac disease, gluten-free diet, gluten-free flour.*

INTRODUCTION

Consumers, food producers and health professionals are uniquely influenced by the popularity of the gluten-free diet. These consumer demands and expectations have led food manufacturers to continually adapt and improve the processing formulas and techniques used to make gluten-free products.

In this paper, we aim to provide a clear picture of the current motivations behind using gluten-free diets, as well as the technological and nutritional challenges of the diet as a whole. the characteristics of alternative flours, hydrocolloids, stabilizing substances and fiber sources have been shown to play a complex role in imitating the functional and sensory effects of gluten in gluten-free products.

However, the quality of gluten-free alternatives is often inferior to gluten-containing products.

The bakery industry is based on the unique properties of gluten proteins present in wheat and some other cereals.

The unique viscoelastic properties are responsible for the characteristics of different wheat flour foods. The processing of wheat flour products is based on the ability of protein substances to develop and form the gluten network. Gluten-based protein substances are important in the properties and behavior of the dough, the bread volume and structure, the making of pasta.

Among the urban population, in recent years, the incidence of gluten intolerance has increased from year to year. Celiac disease is an autoimmune disease triggered by the ingestion of gluten in genetically predisposed individuals. The only treatment is based on a strict, gluten-free diet for life.

As the prevalence of celiac disease is increasing, it is urgently necessary to have a better knowledge of the foods recommended for this diet.

MATERIALS AND METHODS

In this paper, I would like to present alternatives to wheat flour used in bakery.

The impact of celiac disease on people's daily lives, especially health-related quality of life, has been regularly investigated. Some research shows that a gluten-free diet can significantly improve the quality of life after the diagnosis of celiac disease.

RESULTS AND DISCUSSIONS

What is intolerance? Symptoms of food intolerance cause problems especially in the gastrointestinal area. These appear after a large time lag from the table, even after 72 hours. This "incompatibility" is actually indigestion, because the aggressor is a food ingredient that the body cannot properly and fully handle. (eg lactose intolerant)

What is cross allergy? Extremely bizarre, for some patients what manifests as a food allergy is actually the fever of the fan. For example bee pollen contains common choices with certain foods.

The gluten problems

Celiac disease (CD)

Celiac disease is a severe autoimmune disease, and it can damage a person's digestive system. It is vital for people to be aware that celiac disease only affects around 1 % of the population. The only treatment for celiac disease is a strict gluten-free diet (Health Canada, 2012).

Gluten intolerance or non-celiac gluten sensitivity (NCGS)

Gluten sensitivity is not an autoimmune disorder, nor does it cause damage to the small intestine. NCGS or gluten sensitivity is defined as "*a clinical entity induced by the ingestion of gluten leading to intestinal and/or extra-intestinal symptoms that improve once the gluten-containing foodstuff is removed from the diet, and celiac disease and wheat allergy have been excluded*". Symptoms are highly variable, and are often similar to those of celiac disease, making diagnosis a challenge (Pulse Canada, 2011). Some estimates put the prevalence of gluten intolerance at between 0.5 % and 13% of the population. Treatment is adherence to a gluten-free diet. (Market and Markets Gluten-Free Products Market by Type, 2018)

Should gluten be reduced or eliminated?

People need to be aware that celiac disease affects only 1% of the world's population and is transmitted hereditary. The most frequent incidents are found for the sensitivity to non-celiac gluten, which according to some estimates have prevalence between 0.5% and 13% of the population.

Gluten intolerance is a condition that can be very difficult to identify and easily associated with other conditions. To combat these conditions a gluten-free treatment is recommended. It's just that there is a wave of opinions that suggest that gluten has negative health implications. This new tendency to remove gluten from the total diet for both sufferers and healthy people is not beneficial for any party (consumer and industry).

Also, there is very little research that suggests that excluding gluten from a diet will benefit the health of people who do not have a condition such as celiac disease or gluten intolerance.

Reducing gluten intake from the diet

Gluten is completely reduced in the diet of people with celiac disease as soon as a doctor has diagnosed the condition, but for people who suffer from gluten intolerance it is gradually reduced and total elimination is not recommended.

It may not be beneficial for everyone with gluten intolerance to completely remove their gluten from their diet, as people's symptoms may vary depending on their severity.

It is possible that some people may consume small amounts of gluten without symptoms.

Most people with gluten intolerance want to eliminate gluten from their diet, but this must be done gradually. (Sainsbury et al., 2011; Biagi et al., 2009).

New trend - gluten free products contained a lot of products with less gluten; gluten-free products; dietary fiber-rich products; using unconventional ingredients, etc.

In the processing of cereals, gluten is the combined fraction of gliadin (prolamine) and glutenin (glutelin) of wheat.

The protein fraction of gluten is represented as a three-dimensional network, extremely important in food processing. Gluten from wheat flour forms a three-dimensional protein network, based on proper hydration and mixing. These network forming properties are used in kneading to create viscoelastic dough matrices.

Since gluten is the essential structure-building protein in wheat-based foods, its elimination presents a major challenge for formulators.

Gluten-free options currently in use include: flours (rice, sorghum, quinoa, amaranth, teff, soy, buckwheat, pea, bean, chickpea, lentil, protein insect); starches (tapioca, corn, potato, arrowroot); whey powder and egg; gums; emulsifiers and dough conditioners.

Rice flour is found under different types, depending on variety and granularity. Following the milling and processing of the rice, a powder with different granules of rice is obtained. It is recommended to combine it with other types of flour, up to 50%, especially those that are rich in protein to balance the texture and to obtain a proper structure. The rice flour is finer and softer; dough is extremely malleable, easy to process. It is not recommended to get bread from 100% rice flour because it results in granulated products and a crumbly texture. Partial replacement of rice flour with chestnut flour results in lower hardness, increased specific volume, and better color and sensory properties. High chestnut flour recipes had low quality. In pastry-confectionery is used to obtain products for diabetics, biscuits, sticks, creams, home-made sweets, tarts, cakes, puddings, caramel or syrup expanded in chocolate, various garnitures etc.

Corn flour. Corn seeds are milled to a corresponding degree of crushing. It has a yellow-golden or orange color, sometimes even whitish, with a non-uniform grain which, in mastication, produces a characteristic, sweet, hazelnut taste. It is a flour rich in fiber, vitamins and mineral salts (riboflavin, niacin, folic acid thiamine and iron). In bakery, corn flour is mixed with other gluten-free meals (under 25%), preferably rice and sorghum, buckwheat or amaranth, for consistently baked products. It borrows an excellent product texture. It is used for making meat-based fillers, mixed with other flours for various assortments of bread and pastries, tortillas, waffles, pancakes, bread and various desserts.
Notes: Appetizer biscuits with corn flour, cookies, pizza

Hemp flour does not contain gluten substances and forms consistent dough, so it is recommended to use it in mixtures with other types of flour, except for sticks, cakes or biscuits. It is an excellent source of protein, it contains all the essential amino acids, rich in dietary fiber. It gives a pleasant hazelnut flavor to bread products, muffins, cakes and pancakes. Hemp seed meal (protein powder) can be used in any kind of dough for making bread, noodles, pasta, gnocchi, cakes, biscuits, etc., culinary products, desserts. It is ideal in the vegan diet and can substitute for meat.

Millet flour is obtained by grinding millet seed. Milled seeds are very small and can be yellow, white, gray or red. It is a flour rich in vitamin B complex, mineral salts (magnesium, iron, potassium, phosphorus) and is a good source of fiber and protein. The millet flour has a light beige or yellow color, a discrete taste, similar in texture to rice flour.
Millet flour is recommended to be used, in one meal, in a ratio of max. 25%. As a gluten-free meal, it is indicated both in children's diet and in special diets. Bread with millet has a delicate taste, and is easy to digest, is rich in protein and minerals, but can have a slightly sweet taste. Bread must be freshly eaten as it hardens very quickly. It can be used to thicken sauces and soups, for bread and pastry specialties, desserts, pancakes, biscuits, cakes, etc.

Sorghum flour. The sorghum meal has a finer texture, but it is recommended that it be combined with other gluten-free flours. It has high protein, iron and fiber content, as well as antioxidants. The red and white flour is found on the market, has a slightly sweet taste and gives a whole wheat appearance to baked products. It is used in mixtures of 25-30% with other gluten-free flours, for making cakes, biscuits or gluten-free bread. Various bakery products, both leavened and uncooked, soups, popcorn, can be used in the preparation of fermented beverages or can be cooked in the form of flakes, wholly or syrupy extract.

Konjac Flour or Konjac Powder is a product obtained from the root of the Konjak plant, *Anorphophallus Konjac*, is a perennial plant originating in the subtropical and tropical areas of East Asia, Japan and China to the south in Indonesia. Konjac flour is a natural product with a taste close to neutral (most frequently is associated with the taste of salty), non-

allergenic main component of the fiber of *glucomannan* (about 40%), which absorb a lot of water and eliminate the feeling of hunger. This type of flour has a high fluid absorption capacity, rich fiber content, creates a satiety feeling and has a zero caloric content. It can be used as a gelling agent or thickening agent (sweet or salted, sauces), and desserts (creams, soups, puddings and jellies). The way of use is as simple as that of starch or other similar ingredients.
It is used for making cakes, improves dough texture, pearled pasta, replacing couscous or rice.

Amaranth flour. It is made from amaranth seeds, rich in protein. Amaranth flour is characterized by a protein content nearly twice as high as wheat (up to 19%), but with a very balanced protein composition. The flour has a strong nutty taste, a complex and very dense flavor, difficult to work with. Amaranth meal is recommended to combine with moist ingredients such as eggs, butter and dairy products. It is suitable for mixtures containing brown sugar or maple syrup. Because of the distinct taste, moderately, about 10-20% of a flour mixture is used. It is not recommended to get bread from 100% amaranth meal, because the baked products may have a bitter taste and may get too early. It is used in the preparation of bread by direct method, cakes, tops (Alencar, 2017, Machado Alencar, 2015).

Quinoa seed and flour. This cereal is benefic in balanced diet and is excellent source of protein. Quinoa flour increases loaf volume and yields a more homogeneous crumb structure, while not affecting product taste (Alencar, 2017; Machado Alencar, 2015). Quinoa flour does not contain gluten and can be used to make dough and cakes. This flour is a specific flavor that can be contoured by the addition of walnut, cinnamon or cardamom.

Chia seed and flour It is made from Chia seed from the *Salvia hispanica* plant, the super nutrient rich in nutrients, especially Omega 3, fiber, calcium and protein, and absorbs a large amount of water. More often used chia seeds are used. Chia flour does not adversely affect loaf volume and crumb firmness (Miñarro, 2012; Moreira, 2013).

Buckwheat flour or sarazin black wheat (popular name in Eastern Europe). Buckwheat (common buckwheat *Fagopyrum esculentum*, tartar buckwheat, bitter *Fagopyrum tataricum*) is not a proper grass, but is part of the *Polygonaceae* family, and the bean is shaped like a small pyramid. Buckwheat beans have a triangular characteristic of tetrahedron and have a dark brown or black peel. Decorated they are brown or light green.
Buckwheat beans may be whole or may be ground as flour. The milling results in a very fine, soft touch to the touch, pleasant taste and a special flavor. When processing, it absorbs a large amount of water. It is a very grainy cereal, a strong nutty taste, bitter, slightly sweet, easy to digest. It is usually used in combination with other types of flour.
In the buckwheat is also a toxic substance, *phagopyrin*, which causes some people the phenomenon of photosensitisation.
Buckwheat flour can be prepared as such or mixed in varying proportions with wheat flour. The dough is flavourful and savory. (Mariotti, 2013) Dehulled buckwheat flour improved the baking performance of commercial mixtures, whilst puffed buckwheat flour had a clear effect on water availability and the interaction between the matrix biopolymers. Buckwheat flour is recommended for the preparation of: pancakes, thickening of sauces, blending (15-25%) with other types of flour for the preparation of various types of bakery products, biscuits, pancakes, noodles, jelly production.

Chestnut flour. Chestnut provides two distinct products of chestnuts paste and chestnut flour, which are used in the food industry. Chestnut meal is a light brown sweet flour, obtained by drying and fine grinding of edible chestnuts. Chestnut flour is ideal for thickening pudding sauces, and in flour mixes it is used for bread, muffins, cake tops or various desserts. It is used to make chalva, chocolate, cakes and candies, even bread (mixed with wheat flour); it can replace, if necessary, potato (chestnut purée). Chestnut meal can be prepared with butter and milk to successfully replace potato purée. A 20% chestnut flour is used in a basic mixture.

Added in a too high percentage, chestnut flour, print the products an unpleasant taste of earth (Paciulli, 2016).

Coconut flour is made from fresh coconut pulp in dry and skimmed pastry and then finely crushed. The texture is very similar to that of wheat flour. Coconut has a low carbohydrate level, ideal for making bread and pastries. Coconut is rich in fiber, about 38.5% fiber, which is the highest percentage of other flour. Coconut is hypoallergenic. Up to 15-25% of coconut meal is used in mixtures, but other preparations can be obtained at a ratio of 100%. Coconut is gluten-free, has a great flavor and is perfect for baking, but it should be taken into account that in large quantities, it requires additional liquid ingredients (Trinidad, 2006).

Carob flour. The carob flour is obtained from the fruits (the carob tree) of the *Ceratonia siliqua* tree, spontaneously grown or grown in the Mediterranean and the Near East. The carob meal is obtained by drying and fine milling of the pods. It has a subtle color and taste of caramel, somewhat similar to cocoa. Rich in pectins, quality soluble fiber, and sugars with thickening properties, carob powder has the ability to regulate intestinal transit, recommended both in constipation and diarrhea. In addition, carob sugar is slowly absorbed and causes a steady, beneficial glycemia for the body. The carob meal is very suited to cocoa powder, is naturally sweet, flavored and ideal in sweet foods, being one of the natural and healthy additives in bakery products, ice cream, salad dressings and other foods. Carob germ flour loaves have the lowest volume Carob germ flour is a good alternative to wheat flour to produce viscoelastic dough and high quality gluten-free bread (Smith, 2012). It is an important ingredient for pastry products, successfully replacing cocoa. Being naturally sweet and thickening, the cinnamon powder can be used to prepare cakes and other sweets, especially when it is desirable to reduce the amount of sugar or to increase the consistency of the creams (Miñarro, 2012).

Teff flour. It is obtained from *Eragrostis tef* or Ethiopian millet. It is marketed as ground, whole or prepared flour (Campo, 2016).

Teff has a unique nutty flavor, easy to molasses. Teff flour contains a form of starch that helps regulate blood sugar levels, helping to maintain weight. It is combined with other types of flour, but not more than 25% of any flour mixture. Teff meal can be consumed just like any other grain: as a main course, with berries at breakfast, it offers a pleasant taste of hazelnut cookies, cakes, pasta, pancakes and waffles.
In Ethiopia flour is fermented 1-3 days to make "Injera", a sour-dough-type flat bread.

Vegetable flour. Chickpea flour yields the highest volume and the softest crumb. Soy flour alters the textural properties and color of the bread.

One of the *additives* often used as a processing aid and/or quality-improving minor ingredient, is dietary fiber. The addition of dietary fiber does not only compensate for the nutritional loss of dietary fiber when excluding wheat flour or whole meal from the product recipe, but it also introduces an ingredient with excellent water-binding, viscosity-increasing, and even gel-forming capacities. As a result, product thickening and texturizing characteristics are re-introduced in the gluten-free process (Korus, 2015; Martínez, 2014; Pastuszka, 2012; Sciarini, 2017).
Hydrocolloids are polymers that display thickening properties through the binding of water. As a result, the viscosity of the gluten-free "dough/batter" is enhanced and gas is better retained in the "dough" matrix, which increases bread loaf volume and improves loaf crumb structure. (Demirkesen, 2010; Dizlek, 2016; Hager, 2013; Morreale, 2018; Prakriti Jnawali, 2016). The most popular hydrocolloids are xanthan gum, hydroxypropyl methyl cellulose (HPMC), and the same pectin, guar gum, locust bean gum, agarose, tragacanth gum, cress seed gum, and carboxymethyl cellulose (Cappa, 2013; Demirkesen, 2010; Lazaridou, 2007; Liu, 2018; Moreira, 2013; Naji-Tabasi, 2014; Nicolae, 2016).

CONCLUSIONS

Celiac disease is an increasingly common autoimmune condition that affects the intestine

and has multiple systemic manifestations. Despite the increase in rates of diagnosis, most people with celiac disease remain undiagnosed.
A concern of the food industry is finding new ingredients to get these products.
It is extremely easy to recommend the replacement of gluten as an essential ingredient in many products. But it is not enough just to replace a gluten-free ingredient in a recipe, because there are technology problems, requirements for improving sensory characteristics, and the quality of gluten-free products is not adequate compared to the concern for a gluten-free diet.
Restaurants should include gluten free products as an option for this special category of consumers.
Specialists in gluten-free products have to be formed.

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RESEARCH REGARDING THE USAGE INFLUENCE OF SOYA FLOUR AND FOOD ADDITIVES ON BREAD QUALITY

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Abstract

The use of soy flour in bakery products improves the handling of the dough, the bread core is lighter in color and softer. In these conditions, the research carried out aimed to test the possibility of replacing a part of the wheat flour with enzymatically active soybean meal, associated with ascorbic acid (0.005% of the amount of flour) and sodium stearoyl-2-lactate (0.4%), as stabilizer and emulsifier. During the experimental period, 4 recipes were made for bread making, respectively a control sample and 3 experimental samples, in which different quantities of enzymatically active soybean meal (5%, 10%, 15%) were introduced. There was an improvement in the hydration capacity of the dough, in proportion to the amount of the added soy flour. As a result, it can stated that soybean meal increases the absorption capacity of the water and increases the durability of bread freshness.

Key words*: active enzymatic soy flour, alimentary additives, bread.*

INTRODUCTION

The introduction of enzymes in bakery products began in 1886, the purpose being to improve the rheological qualities of bread (Mizrahi, 1967).

One source of bread enzymes was the enzyme-activated soy flour containing lipoxygenase. The purpose of adding the soybean meal was to whiten the flour and respectively the breadcrumbs, to increase the tolerance to mixing and to improve the bread volume and internal structure (Johnson and Myers, 1995).

In soy there are two types of enzymes, respectively type I (optically active at pH 9; thermally stable at 69°C for 25 minutes and inactive with linoleic acid ester and carotenoids) and type II (optically active at pH 6.5, active against linoleic acid ester). Only type II contributes to the improvement of the bakery features. The soybean enzyme called lipoxidase II has been identified as the isoenzyme responsible for the bleaching of carotene (Doxastakis et al., 2002).

The second function of lipoxygenase in the dough is to improve the mixing tolerance and the processing properties of the dough (Ribotta et al., 2005).

Sanful and Darko (2010) investigated the effects of enzymatically active soybean meal and found that it greatly increased the tolerance to chewing.

This capacity was dependent on the quality of the substrate that depended on the amount of fat in the flour.

A more important practical effect of the action of lipoxygenase is to improve the rheology of the dough, to strengthen it during baking and in the oven, resulting in an improved bread volume (Shafali and Sudesh, 2004).

Tripathi et al. (2019) appreciated that both lipids and oxygen were needed to improve the volume of bread.

The action of lipoxygenase can lead to unwanted flavors in bread (Johnson and Myers, 1995).

The purpose of the paper was to test the possibility of using the enzymatically active soybean meal in order to replace the wheat flour from the technological process of bread making to reduce the gluten content.

Soya flour is quite often used in the bakery industry, but in Romania it has not been legally established the recommended quantity to be used.

MATERIALS AND METHODS

The main raw materials used were wheat flour type 650, soybean meal, baker's yeast, additives that were used during the experimental period.
As food additives, ascorbic acid 0.005% of flour and sodium stearoyl-2-lactate (E481) 0.4% were used as stabilizer and emulsifier.
During the research, 4 bread recipes were analyzed, namely a control sample and 3 experimental samples, in which different quantities of enzymatically active soybean meal, ascorbic acid and sodium stearoyl-2-lactate were introduced.
In general, the following ingredients were used for the fabrication of bread:
-1.5 kg of flour for leaven and 1.5 kg of flour for dough, obtaining a total of 3 kg destined for each of the three samples, as well as for the control sample;
- water in the amount of 0.830 l in leaven and 0.830 l in dough;
- yeast 45 g in leaven for each sample;
- salt 45 g in dough.
Soybean flour was not added to the control batch, while in the experimental batches the soybean meal was replaced by 5% wheat in experimental batch I, 10% in batch II and 15% in batch III. To the experimental batches were added food additives, respectively ascorbic acid in the proportion of 0.005% of the amount of flour and stearoyl-2-sodium lactate in proportion of 0.4% of the flour.
On each experimental batch, 6 loaves were obtained, on average, which were obtained by the indirect process. The experiment was repeated 2 times.
The main phases of the technological process are the following:
- preparation and dosing of raw materials;
- preparation of the dough by the two-phase method with leaven and dough. The kneading time of the leaven was of 4 minutes, and the fermentation time of the leaven was of 120 minutes;
- the kneading of the dough aims to obtain a homogeneous mixture of the raw materials and at the same time a dough with good rheological properties. Kneading the dough took 4 minutes. The appreciation of the end of the dough kneading is made organoleptic, respectively the well kneaded dough is homogeneous, consistent, dry when kneaded, elastic and easily separates from the kneader's arm;
- fermentation of the dough, during which a series of physico-chemical, biochemical and microbiological transformations take place, which have an important influence on the physico-mechanical and technological properties of the dough; fermentation time of 40 minutes for the control sample, and in the other samples of 30 minutes;
- the repetition of dough kneading aims to eliminate part of the carbon dioxide accumulated in the dough, which slows the activity of the yeasts and the pressure of the gas bubbles, increasing the breaking resistance of the dough. The duration of kneading repetition in our case (protein surplus flour) is of maximum 1 minute;
- dividing the dough consists of dividing the dough into pieces;
- the preparation of the predrying dough pieces has the role to restore the physical properties of the dough partially destroyed during the division; takes 3-5 minutes;
- modeling the dough aims to give a shape of the dough that the bread has to have;
- the final drying is done in a warm and humid environment, the temperature being of 30-35°C, and the relative humidity of the air of 75-85%, conditions that prevent the surface drying, the formation of an unwanted crust that leads during the baking to the cracking of the surface and enhanced fermentation. The final drying is done in specially arranged rooms or in rake depots (wooden boards). The determination of the end of the fermentation was made organoleptic (soft, raised, elastic, and after a finger press on the surface, it gradually returns to the original form);
- the baking was done for 25 minutes for the experimental samples, as well as for the control sample at a temperature of 230-240°C;
- the storage of the bread is done in order to cool the bread in optimum conditions and to maintain its quality during storage.
The obtained bread was packed, after 3 hours since removing it from the oven, in closed polyethylene bags.
In order to determine the quality of the used flour, moisture (SR90:2007), crude protein (SR 91:2007) and crude ash (ISO 2171:2010) were determined.

The main rheological characteristics of the dough were determined with the help of the farinograph, respectively the hydration capacity, the development of the dough, the stability of the dough, the elasticity of the dough in Brabender units (Bu), the softening of the dough in Brabender units (Bu), the power of the flour.
The physical-chemical indicators of the bread obtained during the experimental period were determined according to the standard SR 91:2007.
In order to assess the results obtained, the Student's test was used to assess the significance of the differences between the average values, and the analysis of the variant was done with the ANOVA program.

RESULTS AND DISCUSSIONS

The flour quality indicators used to obtain the bread analyzed during the experimental period are presented in Table 1.

Table 1. Flour quality indicators used in experiments

Indicators	M.U.	Wheat flour	Soy flour
Humidity	%	10.81	11.05
Crude protein	% SU	9.95	31.96
Ash	% SU	0.65	2.73

Following the analysis carried out on the flour samples, the following quality indicators were obtained:
- the humidity ranged between 10.81% for the wheat flour and 11.05% for the soy flour, being located between the nominal values;
- the proportion of ash related to the dry matter was of 0.65% for the wheat flour and 2.73% for the soybean flour, which is a source of mineral elements;
- the protein content related to the dry substance was of 9.95% in the wheat flour, and in the soybean one was of 31.96%, which is also an important source of lysine for the human consumer.
The rheological indicators of the dough made during the experimental period are presented in Table 2.
The hydration capacity of the dough was of 58.4% in wheat flour, and the addition of soy flour resulted in an increase in values, proportional to the added quantity, the differences being significant for the experimental batch 3 ($P < 0.05$).
The dough development took place in 2-3 minutes for the control batch, the values remainning close to the experimental batch (2.1-2.2 minutes).
The stability of the dough was of 4.2 minutes for the control batch, the addition of soy flour causing an increase of this rheological indicator.
The elasticity of the dough registered the value of 138 Brabender units, and the softening degree of the dough of 110 Brabender units, both parameters being influenced in an increasing way by the addition of soy flour.
The power of the flour was between 35-39, values that were within appropriate limits, which allowed the experience to unfold.
The physical-chemical indicators of the bread obtained by the baking samples, after 3, 24, 48 and, respectively, 72 hours after the exit from the oven, are presented in Table 3.
Bread volume. At 3 hours after baking, the lowest value is bread from the experimental batch E1 (312.27 cm^3/100 g product), using 5% soybean meal and food additives. Sample 2, which used 10% soybean meal and food additives, has the highest value (355.32 cm^3/100 g product).

Table 2. The rheological indicators of the dough made during the experimental period

Indicators	M.U.	Batch			
		control	E1	E2	E3
Hydration capacity	%	58.4±2.05	63.4±3.12	66.7±2.89	68.1±1.96
Dough development	Minutes	2.3	2.2	2.1	2.1
Dough stability	Minutes	4.2	4.7	5.1	5.3
Dough elasticity	Bu	138±7.32	1198.05±	139±7.58	156±6.26
Softening dough	Bu	110±5.14	115±7.29	121±8.45	127±6.74
The power of flour	-	37	39	37	35

Table 3. Physical-chemical indicators of the bread obtained during the experimental period

Physico-chemical indicator	Baking time (hours)	Control batch	E1 batch	E2 batch	E3 batch
Volume cm^3/100 g product	3	335.12	312.27	355.32	336.24
	24	330.34	296.16	332.51	325.62
	48	310.07	309.27	327.16	310.46
	72	326.21	309.52	334.29	325.58
	3-72	325.43	306.80	337.32	324.47
Height (H), cm	3	10.31	10.11	10.97	10.42
	24	10.24	10.20	10.43	9.89
	48	9.83	10.43	10.31	9.97
	72	9.67	10.32	10.43	10.10
	3-72	10.01	10.26	10.53	10.09
Diameter (D) cm	3	15.55	15.41	15.53	15.57
	24	15.50	15.12	15.26	15.21
	48	15.22	15.19	15.19	15.14
	72	15.01	15.29	15.21	15.17
	3-72	15.32	15.25	15.30	15.27
H/D	3	0.66	0.66	0.71	0.67
	24	0.66	0.67	0.68	0.65
	48	0.64	0.69	0.68	0.66
	72	0.65	0.67	0.68	0.66
	3-72	0.65	0.67	0.69	0.66
Porosity, %	3	81.32	76.11	79.35	78.75
	24	80.89	78.25	81.14	79.27
	48	79.65	78.44	81.07	79.36
	72	81.02	78.89	80.65	80.42
	3-72	80.72	77.92	80.55	79.45
Elasticity, %	3	93.12	95.32	95.24	93.56
	24	90.64	89.52	92.18	91.19
	48	91.35	90.12	92.22	91.31
	72	92.71	96.31	96.15	94.10
	3-72	91.95	92.82	93.95	92.54
Humidity, %	3	42.24	40.84	40.81	40.55
	24	42.10	41.24	41.32	42.05
	48	42.34	40.77	41.37	41.48
	72	42.22	41.69	41.91	41.63
	3-72	42.22	41.14	41.35	41.43

At 24 hours, the volume of all samples decreased, the bread from the experimental batch 1 having the smallest volume 296.16 cm^3/100 g product, and the bread from group E2 had the highest volume (332.51 cm^3/100 g product).

At 48 and 72 hours after baking, it is found that the largest volume is the one of the breads used in which have been added food additives and 10% soybean meal.

As a mean value, it is observed that the largest volume was the one of the breads from the experimental batch E2, which were used food additives and soybean meal 10%, the increase compared to the control being of about 3.52%.

Bread height. It was observed that at 3 hours after baking, the height of the control sample was of 10.31 cm, being an average value. An increase in bread height was observed for the experimental batch E2, and the lowest one was for the batch E1. After 24 hours of baking, batch E2 had the highest height, while the breads in the batch E3, the lowest one.

The same situation is observed after 48 hours and 72 hours after baking: sample no.4 increases the most (by 0.9%), the highest breads being those obtained by adding additives and soy flour 10%.

Bread diameter. There is a decrease in the bread diameter as several hours pass from baking, the values recorded being relatively close and statistically insignificant ($P>0.05$).

Height/diameter ratio. The highest value of the ratio was registered for the experimental batch E2, but, on average, the differences between the average values were insignificant ($P>0.05$).

The *porosity of the bread* varied within relatively narrow limits in the control and experimental batches E2, decreasing in the experimental batch E3 by 1.57% and in the experimental batch E1 by 3.47% compared to the control batch.

Bread core elasticity has the highest values for the experimental batch E2 (93.95%), to which 10% soybean flour was added.

The humidity of the bread obtained from wheat flour with the addition of soybean flour and additives decreased compared to the control batch (by 2.56% in batch E1, 2.06% in batch E2 and by 1.87% in batch E3).

Otegbayo et al. (2018) stated that soy enrichment of bread creates a dense food nutrient that is important for health. By adding 5% soy flour in the produced bread, it has a greater nutrient value, similar to the one of wheat flour, being liked by consumers. The 5% soy flour addition is benefic for nutritional value and increases the consumer acceptability through its sensory properties. Also, the bread had a decreased anti-nutritients level, being safe for the consumer.

Sana et al. (2012) advised to replace the wheat flour with a proportion of soybean flour that is greater than 7% in order to obtain a high nutritional bread with sensorial qualities.

CONCLUSIONS

Enzymatically active soybean meal which contains lipoxygenase can be used as a substitute for a portion of wheat flour, provided that an additive of food additives (ascorbic acid 0.005% and stearoyl-2-sodium lactate 0.4%) is used.

The hydration capacity of the dough was improved by the addition of soybean meal which caused an increase in values, proportional to the added quantity.

The stability, elasticity and softening of the dough have been influenced in an increasing way by the addition of soybean meal and food additives.

The highest volume, the best ratio of bread height and diameter, porosity, elasticity were positively influenced by the use of 10% soybean meal with an addition of food additives.

In all the samples with the addition of soybean meal, the humidity was diminished, favoring the preservation of the bread for a longer time.

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EFFECT OF SHELL INJURY ON HAEMOCYTE CONCENTRATION AND SHELL REGROWTH OF GIANT AFRICAN LAND SNAIL (*ARCHACHATINA MARGINATA*)

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Abstract

The effect of shell injury on growth and haemocyte concentration were evaluated in this study. Thirty-two (32) snails between 130-180g were randomly divided into four (4) treatments with eight (8) replicate each. The four treatments include: T1 (control), T2, (1 cm shell damage) T3 (2 cm shell damage) and T4 (3 cm shell damage). Haemolymph was collected on weekly basis for four weeks. Parameters monitored were total haemocyte count and shell growth. Result showed that shell injury/damage had significant effect (P<0.001) on total haemocyte count and shell growth. It can be concluded from this study that shell injury had influence on immune response of the animal, although compensatory growth was recorded after week four of the experiment. It can be recommended from this study that irrespective of level of shell damage used in this study, adequate attention should be given not to kill the animal as the process compromise the total haemocyte count which is responsible for immune defense of the animal. It is therefore recommended that adequate care and proper hygiene must be maintained in other not allow opportunistic infection since immune cells (haemocytes) are compromised in other not cause economic loss due to unforeseen mortality.

Key words*: Archachatina marginata, haemocyte, Land Snail, Shell growth, Shell injury.*

INTRODUCTION

Land snails generally have shell which protects them from physical damage, predators and dehydration (Ademolu et al., 2015). Similarly, the shell housed the animal especially during unfavorable condition. The shells are twisted into spiral level known as whorls. The whorls are largest at the base and each one gets progressively smaller as it gets to the tip, known as the apex. The snail shell has a large opening called aperture (Adamowicz and Bolaczek, 2003). Due to current trend of intensive rearing of snails to meet up with demand, there is need for cage culture or semi-intensive rearing of this animal. During intensive rearing, snails at times try to escape from their rearing vicinity and thus fall off from some height and as such break their shell. This occurrence put snails at a great danger depending on the site of injury. It could also lead to haemolymph loss which may result to death of this animal if such injury is much. In most occasions, the damage to shell calls for the process of healing which require regrowth of the damaged part and this may be energy demanding and costly (Jonathan, 1990). Studies have also shown that wound healing process requires the activity of macrophages which promote angiogenesis and collagen formation (Leibovich and Ross, 1975; Polverini et al., 1977; Hunt et al., 1984; Kovacs and DiPietro, 1994).

For invertebrate like mollusks, shell formation is known to be a complex process which involves deposition of both organic and inorganic materials (Wilbur, 1983).

The shell formation process comprises of shell mineralization known to be in succession of compartments (Crenshaw, 1972: Saleuddin and Petit, 1983).

The first to be reckoned with is the mantle cavity which secrete the molecules that form the shell, followed by periostracum (with mostly organic layer) and the extrapallial cavity into which the outer fold epithelium secretes calcifying mixture of proteins, glycoproteins and calcium carbonate ($CaCO_3$) (Mutvei, 1980; Fenget al., 2000; Marin and Luquet, 2004; Dalbeck et al., 2006; Marie et al., 2011; Marin et al., 2012).The longitudinal section of a shell is made up of a multilayer of calcium carbonate in two or more concentric layers, which are usually covered by an external layer (Saleuddin

and Petit, 1983). Below the periostracum is an inner nacreous layer, followed by inner primastic (Marie et al., 2011).

During rearing of snail under intensive system, damages in shell do occur due to climbing of housing facility by this animal and such may lead to economic loose due to mortality. It therefore becomes very important to understand the influence of this damage on immune status of this animal within specific period of time and to monitor recovery period depending on the level of damage. The aim of this study is to evaluate the effect of shell injury on haemocyte concentration and shell regrowth of Giant African Land snail (*Archachatina marginata*).

MATERIALS AND METHODS

Experimental Site

The research was carried out at the Snail Research Unit of the College of Animal Science and Livestock Production (COLANIM), Federal University of Agriculture, Abeokuta, Ogun State. Abeokuta lies between the rain forest vegetation zone of Western Nigeria on latitude $7^0$10'N, longitude $3^0$2'E and altitude 76m above sea level. The climate is humid with a mean annual rainfall of 1,037mm, an average temperature of 34.7^{0}C and an imminent average humidity of 82% throughout the year (Google earth 2017).

Materials

A total of thirty-two (32) snails (*Archachatina marginata)* between 130-180 g were purchased from local market. The snails were kept in plastic cages (30cm by 40cm by 24cm). Feeding trough, watering trough, sensitive scale, plier, eppend of tube, syringe and needle (5 ml), ruler, Vernier caliper and concentrate feed were used during this study. Marker and masking tape was also used for proper identification.

Snails and their management

The plastic cages along with the plastic feeders and drinkers were cleaned before the arrival of the snails and the commencement of the experiment. Feed and water were also provided *ad libitum* throughout the period of the experiment. Four weeks was set aside for the acclimatization of the snails before the commencement of the experiment. The experiment lasted for six (6) weeks.

Experimental Design

Thirty-two snails used for this experiment were randomly assigned into four (4) different treatments with 8 replicates for each treatment.

Treatment 1: No shell damage (control)
Treatment 2: 1 cm shell damage
Treatment 3: 2 cm shell damage
Treatment 4: 3 cm shell damage

All snails in both groups were treated equally in terms of feeding and drinking water provision. Composition of feed used was given in Table 1.

Table 1. Composition of experimental diets (g/100g)

Ingredients	Quantity (g)
Maize	50
Wheat offal	27.5
Groundnut cake	12.25
Soy bean meal	4
Bone meal	3
Oyster shell	3
Salt	0.25
Total	100

Shell damage/Injury

The snails were cleaned with damp foam in order to remove the dirt on them. The snails were weighed on a sensitive scale before the damaged of the shells. The snails in each treatment (1, 2, 3, 4) were brought out of cages, a ruler was placed on the tip of shell and white board marker was used to mark out the part to be damaged as 0 cm, 1 cm, 2 cm and 3 cm. After marking out, a plier was used to cut out the part as marked to be damaged. Shell growth was measured weekly for six weeks using Vernier caliper (Figure 1).

Collection of Haemolymph

Haemolymph was collected from the anterior portion of the head region after full extension of the foot muscle with the aid of syringe and needle. Haemolymph was collected from the control group and other treatment levels (1 cm, 2 cm and 3 cm) immediately after shell damage and stored in eppendof tube for haemocyte count. Haemolymph collection was also carried out on weekly basis.

Total Hemocyte Count

Haemolymph from eight snails per treatment were selected from the four groups of snails with damaged shell (control, 1, 2, and 3 cm). A dilution of 1:19 was made with the aid of 5% eosin solution which was loaded into improved haemocytometer. Haemocyte found in the four squares were counted. Thereafter, numbers of cells counted were multiplied by a conversion factor (50,000) to obtain the total haemocyte count.

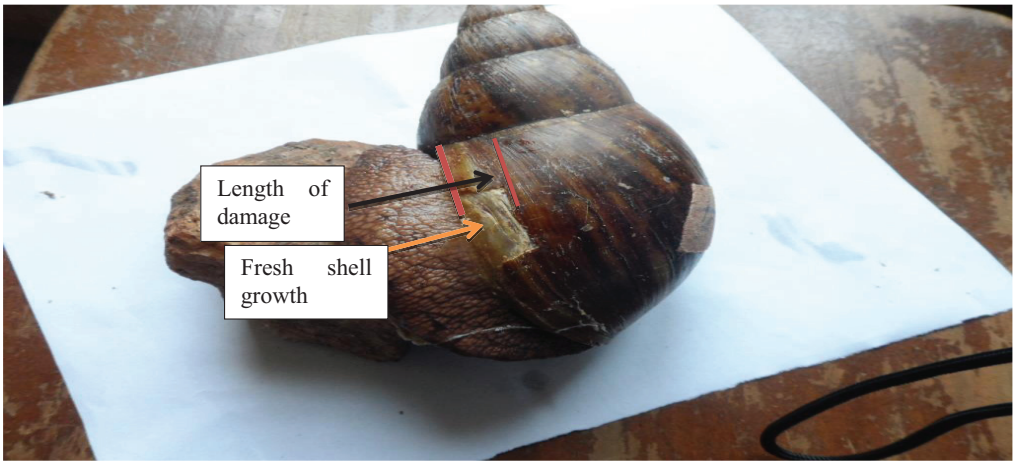

Figure 1. Portion of shell damage and regrowth

Statistical Analysis

The data generated from this experiment was subjected to a least square analysis of variance using the SYSTAT Statistical package (SYSTAT, 1992) in randomized complete block design (RCBD). Significant treatment means were separated using Duncan multiple range test (Gomez and Gomez, 1984). Model used for this experiment is stated below.

$Yij = \mu + T_{i} + W_{ij} + (TW)_{ij} + \sum ij$

Where,

Yij = Dependent Variables

μ = Population mean

Tí = effect of levels of shell damage (I = 1-4)

$W_{[j}$ = effect of weeks of haemolymph collection (I = 1-4)

∑ij = random error

RESULTS AND DISCUSSIONS

Result of summary of analysis of variance showing the effect of shell injury on haemocyte count of Giant African Land snail is shown in Table 2. Different levels of shell damage had significant effect on haemocyte count (P<0.001), while effect of week on haemocyte count during the shell damage was not significant (P>0.05).

Significant effect seen in haemocyte count is as a result of anti-inflammatory responses which are very common during injury in many animal models. Allograft inflammatory factor-1 (AIF-1) which is an interferon inducible calcium-binding cytokine has been associated with inflammatory response in mollusks (Liet al., 2013).

Table 2. Analysis of variance (ANOVA) showing the effect of shell injury on haemocyte count of Giant African Land snail (*Archachatina marginata*)

Source	Degree of freedom	Mean square
Treatment	3	330945.650***
Week	3	2425.117NS
Error	73	399960543

P< 0.001***

Studies had also shown that macrophages which facilitate wound healing, angiogenesis and collagen formation are found at the site of injury (Leibovich and Ross, 1975; Polverini et al., 1977; Hunt et al., 1984; Kovacs and DiPietro, 1994). Inflammatory response is vital to body injury, wound repair and immune response (Ottaviani et al., 2010). In mollusk, especially in snails, haemocytes are the analogue of various types of immune cells

found in vertebrate and as such, they are known to be released whenever there are challenges in the system of this animal.

Table 3 shows the least square means of effect of shell damage on haemocyte count of Giant African Land snail.

The control group had the highest number of means compared to other levels which were not significantly different from each other.

This observation is an indication that damages of shell at any magnitude compromise immune status of this animal which is largely represented by total haemocyte population.

Table 3. Least square means showing the effect of shell injury on haemocyte count of Giant African Land snail (*Archachatina marginata*)

Parameter	Lease square means($\times 10^6/mm^3$)	S.E.M (±)
Control(undamaged shell)	345.200^a	44.719
1 CM Shell damage	107.000^b	44.719
2 CM Shell damage	109.400^b	44.719
3 CM Shell damage	114.500^b	44.719

Legend: CM: Centimeter

Means within the same column having different superscript differs significantly (P<0.001).

Haemocytea are known to be the chief immune effect or cells which perform diverse immunological activities such as phagocytosis, encapsulation and cytotoxicity (Ray et al., 2013). If damages to shell could affect the population of these cells, then it means that any other challenge at this moment of injury may be very dangerous to the survival of the animal. Jonathan (1990) reported that experimentally shell-damaged snails had higher rate of mortality than did uninjured snails. Also, Ray et al.(2013) reported that exposure of two species of snails (*B. begalensis* and *L. marginalis*) to cypermethrin and fenvalerate lead to haemocyte density shift and morphological damage. All these reports are testifying to the fact that both physical and chemical damage could compromise the population of haemocytes which are known to be responsible for immune activities in the system of this animal.

Table 4 shows least square means showing effect of shell injury on weekly haemocyte count of Giant Africa Land snail (*A. marginata*). Result showed that haemocyte count was not significantly different (P>0.05) across the three weeks of collection.

The implication of this observation is that quick adjustment within the system of the animal had taken place thus nullifying effect of the damage within the three weeks of the study. Least mean square showing effect of different levels of shell damage on growth after damage is shown in Table 5.

Table 4. Least square means showing the effect of shell injury on weekly haemocyte count of Giant African Land snail (*Archachatina marginata*)

Week	Lease square means($\times 10^6/mm^3$)	S.E.M (±)
0	149.400	44.719
1	172.400	44.719
2	152.600	44.719
3	149.700	44.719

Table 5. Least square means showing the effect of different levels of shell growth after injury

Parameter	Lease square means	S.E.M (±)
Control(no shell damage)	0.175^c	0.057
1 cm shell damage	0.241^{bc}	0.057
2 cm shell damage	0.347^{ab}	0.057
3 cm shell damage	0.444^a	0.057

Means within the same column having different superscript differs significantly (P<0.001).

It was obvious that snails with 3 cm shell damage had the highest regrowth of 0.444 cm, followed by 1 cm and 2 cm shell damage which were not significantly different from each other (0.241 vs 0.347 cm) while the control had the least growth (0.175 cm).

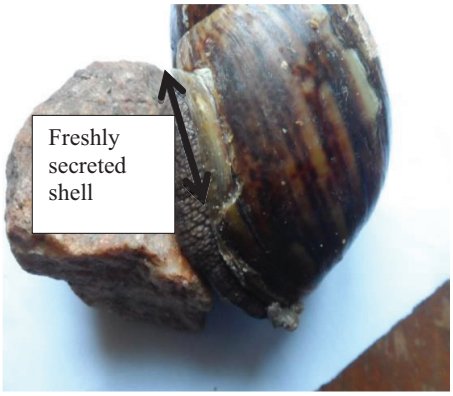

Figure 2. Freshly secreted shell after shell damage

Figure 2 shows the freshly secreted shell after shell damage.

The observation made in this study may be as a result of calcium and phosphorous mobilization from the body of the animal to compensate for the losses that occur during shell damage procedure.

According to Jonathan (1990), this process of shell repair is highly energy demanding.

It was also reported that experimentally damaged shells grew significantly more new shell than the undamaged ones (Jonathan, 1990).

This assertion is in line with the observation made in this study. Mollusks shell formation has been reported to be complex and involves deposition of calcium carbonate ($CaCO_3$) which is known to be an inorganic material mixed with organic material (Hare, 1963; Wilbur, 1983).

CONCLUSIONS

This study has shown that shell injury had significant effect on haemocyte concentration. Irrespective of the level of shell damaged used in this study, total haemocyte count reduced compared to the control group.

This observation is an evidence of immunosuppression and this call for adequate care during this period of shell injury. If adequate care is not taken during this period of injury, opportunistic infection may kill the animal as haemocyte play crucial role in the immune defense of this animal.

The implication of this study is that snail farmers should maintain hygienic environment with adequate care during any eventuality of shell damage under intensive method of production.

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DETECTION OF *AEROMONAS SALMONICIDA* IN FISH SAMPLES FROM LAKE OHRID BY CULTURE AND POLYMERASE CHAIN REACTION METHODS

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Abstract

Aeromonas salmonicida is a bacterial pathogen that causes infection mainly in Salmonidae family. The objective of this ongoing study was to isolate and identify the bacterial pathogen in Lake Ohrid, since the main fish species that populate this aquatic body belongs to the Salmonidae family. Salmo letnica and Salmo ohridanus are the main species where we have focused our analyses. These fish species not only are one of the main catches but also one of the main foods in the region around Lake Ohrid. Sampling was carried out during spring and autumn season in 2017. Material from liver and skin mucus from each sample was used for the inoculation of the TSA medium. The incubation period was between 5-7 days at 18°C which was followed biochemical test. PCR was performed by using MIY primer (MIY1 5'-AGCCTCCACGCGCTCACAGC-3' and MIY2 5'-AAGAGGCCCCATAGTGTGGG-3'). All the data were analyzed with Sigma Plot 12.5. A multiple Comparison Procedures (Tukey Test) between groups, water temperature, liver bacterial colonies and skin colonies ($p<0.05$) was performed.

Key words: *A. salmonicida, MIY primer, PCR, Sigma plot 12.5, TSA medium.*

INTRODUCTION

Lake Ohrid is a unique transboundary aquatic body due to the endemic fish species that populate it, which belongs to *Cyprinidae* and *Salmonidae* family. It has a significant importance in biological and economic aspects for the regions that surround this natural aquatic body. According to recent studies in Lake Ohrid were determined 21 native fish species, seven of them are considered endemic species (Talevski et al., 2009) such as *S. ohridanus* and *S.letnica* (Karaman, 1924). These two fish species belongs to Ohrid brown trout and represent one of the main catches and commercials fishes in the region (Mitchell et al., 2010). But over the last decade the stock of *Salmo letnica* has been constantly decreasing (Jordanova, 2016). Water pollution may be the main cause for such decline of this stock species although it's important to mention that there is a lack of published data for the wild stock situation through the years. As it's worth to mention that there are a few publications regarding fish pathology in Lake Ohrid, especially in reference with fish parasites (Dimovska et al., 2013). The study is focused on the detection of a bacterial pathogen, *Aeromonas salmonicida* that cause severe infection mainly on Salmonidae fish. In the last decade it has been shown that *A. salmonisada* can cause severe infection in other fish species that don't belong to Salmonidae family, including carp (*Cyprinus carpio*), eel (*Anguilla Anguilla*) (Rivera et al., 2014). Data shows that *A. salmonicida* infection can be transferred *via* water from infected fish to healthy fish (Skrodenyte-Arbaciauskiene et al., 2012). Furunculosis, the disease caused from *A. salmonicida*, represent an acute to chronic condition inflicted heavy losses in wild and cultured stocks fish (Sudheesh et al., 2012). Affected fish often show skin ulcerations, lethargy and in appetence (Wiklund and Dalsgaard, 1998). Hemorrhages may occur at the bases of fins and the abdominal walls, heart

and liver (Menanteau-Ledouble et al., 2016). Enlargement of the spleen and inflammation of the lower intestine are common features of chronic infections, but in acute outbreaks fish may die rapidly with few signs. The major route of transmission appears to be via infected fish and contaminated water (Hastings and Ellis, 1988). Although the disease causes mortality of all ages, the most serious losses occurs during spring-autumn in the sea water farms.

Clinical outbreaks and mortality appear to be triggered by stress factors such as crowding, poor water quality, fright, high temperature and physical trauma (Pekala-Safinska, 2018). By taking into consideration the information above, our research is mainly focused in isolation and detection of *A. salmonicida* in Salmonidae species (*S. ohridanus* and *S. letnica*) of Lake Ohrid by combining conventional methods and PCR technique.

MATERIALS AND METHODS

Materials: Samples that belongs to Ohrid trout (*Salmo letnica* and *Salmo ohridanus*) were collected in spring (April-May) and autumn (September-October) 2017. In total were collected 77 samples, mainly female and male adult individuals. The fish were capture from licensed fisherman in three sites as shown in Figure 1. These sites are mostly frequented from tourist near an urban community with multiply contaminations inputs. The first site was located in Lin village (n=21), the second in Udenisht (n=14) and the third site in Pogradec city (n=42). **Methods:** To evaluate the condition of fishes samples the Fulton condition factor (CF) was calculated according the formula: CF= 100 x BW (g) ÷ FL^3 (cm) (Jordanova et al., 2016; Nash et al., 2006). For each fish the total length (TL), fork length (FL) and body weight (BW) were measured. A visual evaluation for external and internal lesion after the dissection was made and recorded. For the isolation of bacterial pathogen, *A. salmonicida*, we used Trypton Soy Agar (TSA) medium which was inoculated with diluted mucus taken from each fish samples and from material taken from the liver. The plates were incubated for 4-7 days at the temperature of 18°C.

PCR analysis: DNA templates were prepared by lysing three to four bacteria from colonies, that grow in TSA medium after the incubation period, in 20µl lysis tampon (50 mM KCL, 10mM Tris pH 8.3, 2.5mM $MgCl_2$, 0.45% NP-40, 0.45% Tween 20 and milli-Q H_2O) (Tanaka et al., 2012; Rivera, 2015). The lysates were incubated at 95°C for 5 minutes and only 1 µl from the lysates is used for PCR mix volume. The PCR mixture contained 0.25 µl of Taq polymerase, 2.5µl of Buffer 10X, 2µl of $MgCl_2$, 0.25µl of dNTP 100mM, 0.5µl of each primer 200µM, 18µl of milliQ H_2O and 1µl of DNA template. The primer used for the amplification are MYI primer (MIY1 5'-AGCCTCCACGCGCTCACAGC-3' and MIY2 5'-AAGAGGCCCCATAGTGTGGG-3') (Byers et al., 2002). The mix volume was held for 2 min at 94°C, then amplified for 35 cycles (denaturation for 30s at 94°C and annealing and elongation for 1 min at 68°C) followed by a final extension for 3 min at 68°C. The PCR product size was 512bp. For the separation of the DNA fragment a 1% gel agarose was prepared and the DNA fragment runs at 100V for 45 min.

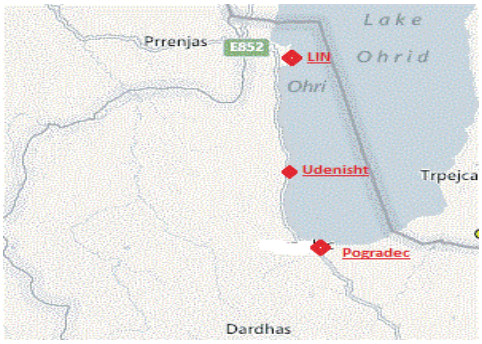

Figure 1: Google map where there are mark the three samples site: Lin village, Hudenisht and Pogradec

RESULTS AND DISCUSSION

For each fish samples we have recorded the morphometric data, external and internal symptoms if present. The Fulton condition factor has been calculated for each sample in order to evaluate the condition of the fishes that we have analyzed. For the Salmonidae species the Fulton factor values usually fall in the range 0.8 to 2. The result obtained show that 27% (n=21) of samples has CF value lower than

1.70% (n=54) of samples has a CF value between 1 and 2 and only 0.3% (n=2) has a CF value greater than 2. We have hypothesized that the health condition of the fish is related with the number of the bacterial colonies present in the liver, skin and with external/internal symptoms if observed. In the Table 1 it shows the output of the ANOVA analysis and whether there is a statistically significant difference between our group means (FC and liver bacterial colonies, bacterial skin colonies and fish symptoms present or not). A multiple comparisons analysis is performed to determine if there is a statistically difference within groups mentioned above. A Schefffe post hoc test revealed a statistically significance within Liver colonies and CF value lower than 1 (8.0476 ± 3.77460) and CF value between 1 and 2 (6.0556 ± 2.56549) (p=0.035) with a confidence level 95% (α=0.05). Also the test show a statistically significance within fish symptoms and CF value lower than 1 (4.7143 ± 0.46291) and CF value between 1 and 2 (4.3889 ± 0.49208) (p=0.037) with a confidence level 95% (α=0.05). There was no statistically significant difference within skin colonies and CF value lower than 1 and CF value between 1 and 2 (Figure 2). Also to determine if there is a correlation between the fish symptoms that we observed and the total number of the bacteria present in liver or skin we perform Person Correlation (2-tail) with statistical program SPSS 25.00. The data show that there is a positive correlation between fish symptoms and liver colonies, statistically significant (p=0.001) with a confident level 99% (α =0.01) and between fish symptoms and bacterial skin colonies statistically significant (p=0.002) with a confident level 99% (α =0.01). A Pearson Correlation (2-tailed) between fish symptoms and Fulton condition factor was also performed with SPSS 25.0 statistical program and show a negative correlation (R^2=-0.326) between the two variables, statistically significant (p=0.004) with a confident level 99% (α =0.01). Since the main objective of our research was to determine the presence of *A. salmonicida*, and data shows that the incidence of this bacterial pathogen is mainly in spring and autumn, we calculated with Sigma Plot 12.5 if there is a significance difference between water lake temperature in the time of sampling and the total number of bacterial colonies present in liver and skin (Figure 3). According to the literature there is a strong relation between the effect of temperature on pathogen multiplication and host immune mechanisms (Groberg et al., 1978).

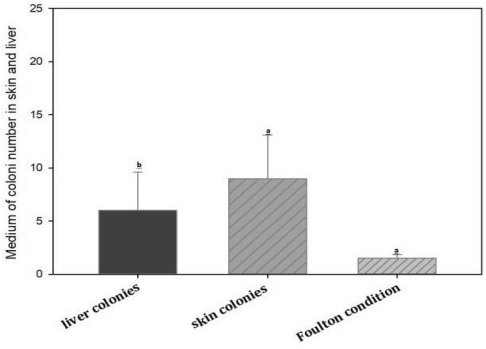

Figure 2. The differences in the median values among the treatment groups. The labels in different marks shows the difference in standart deviation between groups (Sigmaplot 12.5, Tukey test, statistically significant p<0.05)

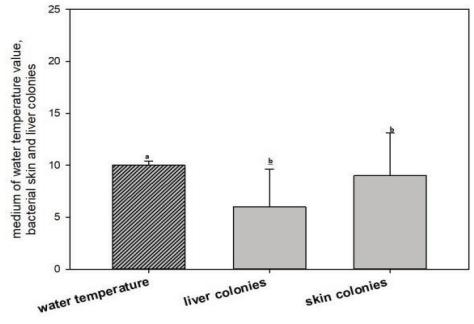

Figure 3. The differences in the median values among the treatment groups. The labels in different marks shows the difference in standard deviation between groups (Sigmaplot 12.5, Tukey test, statistically significant p<0.05)

The isolation of *A. salmonicida* in TSA medium resulted negative after morphological and biochemical tests. It doesn't observed dark brown bacterial colonies, typical of *A. salmonicida*. But instead we have isolated rod yellow, smoothly colonies that belongs Pseudomonas genus (Figure 4). We assume that the presence of these bacterial species in almost all the samples is related with the water quality of Ohrid Lake.

Table 1: ANOVA results perform with statistical program SPSS 25.0

		Sum of Squares	df	Mean Square	F	Sig.
Liver colonies	Between Groups	60.734	2	30.367	3.546	.034
	Within Groups	633.786	74	8.565		
	Total	694.519	76			
Skin colonies	Between Groups	81.526	2	40.763	3.140	.049
	Within Groups	960.786	74	12.984		
	Total	1042.312	76			
Fish symptoms	Between Groups	2.050	2	1.025	4.430	.015
	Within Groups	17.119	74	.231		
	Total	19.169	76			

* We can see that the significance value is 0.034 therefore, there is a statistically significant difference in the mean of liver colonies and Fulton condition factor (FC); skin colonies and Fulton condition Factor p=0.049; fish symptoms and Fulton condition factor p=0.015.

a)  b) 

Figure 4. a) *Salmo letnica* dissection, evaluation of internal organs.
b) Bacterial colonies after inoculation of TSA with liver material and skin mucus diluted material.

For each sample we have performed PCR amplification which resulted negative for the presence of *A. salmonicida*.

A. salmonicida represent a bacterial pathogen not only in fishes but also it is classified as a foodborne pathogen in human (Novotny et al., 2014). The salmonidae species (*Salmo letnica* and *Salmo orhidanus*) that we have selected for our research represent one of the main foods for the population that lives in the region around Ohrid, but also an important food attraction for tourist. Not only it's of a great importance having recorded data for the stock fish population of Ohrid brown trout but also knowing fish health situation. It is worth to mention that there are many publications about the water pollution of Lake Ohrid from chemical and biological hazards and their effect on fish health (Mali, 2014; Aliu et al., 2011; Lokovska et al., 2019).

Also after the fish dissection there are no sign, typical to the furunculosis such as a widespread hemorrhaging to a greater or less degree in the internal organs (liver, kidney etc.) (Bruno and Ellis, 1996).

CONCLUSIONS

In this article it has presented the first accumulated results, of an ongoing study, for the detection of *A. salmonicida* in fish samples from Ohrid Lake. We assume that the negative result obtained with culture medium and PCR are related with the relative small sample (n=77) that we have analyzed. The morphophysiological data that we have obtained from our fish samples (*S. letnica* and *S. ohridanus*) shows that we are dealing in general with healthy fish (n=54, 70% in total). No external signs that may lead to furunculosis disease such as darkening of the skin, reddening of the fin basis have been observed.

From the other hand we have observed sign in some fish samples (n=36 with external/internal

signs) that in general are not related with *A. salmonicida* infection, to mention a few white spot, weight drop, liver hyperplasia etc.

Since there are no publication (in our knowledge) about the isolation and identification of *A. salmonicida* in Salmonidae fish of Ohrid Lake, makes it difficult from our part to compare our data or to reach in a final conclusion. Since this is a ongoing study we are still analyzing fish samples from Ohrid Lake with the main objective detection of *A. salmonicida*.

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INDUCTION AND RECOVERYTIMES OF ANESTHESIA IN CYPRINIDS USING VARIOUS DOSES OF CLOVE OIL (*EUGENIA CARYOPHYLLATA*)

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Abstract

Anesthetics are widely used in fish farming. Their purpose is to reduce the mobility and stress of fishes due to manipulation. In the present study we tested the efficiency of clove oil (Eugenia caryophyllata) in different doses [4.70 mL anesthetic/50 liter of water (0.094 mL/Lwater); 2.36 mL anesthetic/50 liter of water (0.0472 mL/Lwater); 1.82 mL anesthetic/50 liter of water (0.0364 mL/Lwater)] in carp (Cyprinus carpio) and in Prussian carp (Carassius gibelio) with different body mass. Anesthesia induction times were monitored. At each of these stages the respiratory rate was monitored by recording the opercular movements. The results show a direct correlation between the fish size and the induction times of anesthesia. Regardless of species, larger and heavier specimens require longer periods for anesthesia. Instead, recovery times in larger-body specimens were shorter. Different doses have influenced the duration of anesthesia induction and recovery times, the most effective dose being 4.70 mL/50 liter of water followed by 2.36 mL/50 liter of water. The dose of 1.82 mL/50 liter of water did not induce anesthesia.

Key words: *efficiency, fish anesthesia, natural anesthetics, opercular movements.*

INTRODUCTION

Anesthetics are synthetic or natural medicinal products used for the temporary loss of sensations. In terms of mechanisms of action, they are classified under general, regional and local anesthesia. General anesthetics lead to total immobility, amnesia, sleep, analgesia, unconsciousness, and a reduced autonomic response to harmful stimuli.

Regional and local anesthetics interrupts neuronal conduction by inhibiting the influx of sodium ions, thus blocking painful sensations in the regions where they are administered. In fishery practice, anesthetics are widely used (Zahl et al., 2012), when the fish must be immobilized for a longer period of time. In general, fish are anesthetized when invasive surgical procedures or biological sampling (blood samples, different types of tissues) are required. Anesthetics are also used in situations which require fish handling during transport, sorting, artificial breeding, or administration of vaccines. Different types of anesthetics and methods of anesthesia are used for the purpose of stunning the fishes (electronarkosis, hypotermia, etc.), eliminating stress-causing situations (Robb and Kestin, 2002; Zydlewski et al., 2008; Wilson et al., 2009).

Administration of anesthetics can be done in several ways: by immersing fish in short-term baths containing anesthetics or by subcutaneous or intramuscular injection. The anesthetics used are found in a wide range, both natural (Rezende et al., 2017; Hoseini et al., 2018) and synthetic anesthetics. These generally present a period of persistence in the fish body, especially at the musculature level, which is why after administration, a period of quarantine it is necessary before the fish are destined for human consumption and in accordance with EU regulations (Nicolae et al., 2018; Totoiu et al., 2018). The anesthetics most commonly used in fish are: MS-222 (tricaine methanesulfonate) (Küçük, 2018), Benzocaine (Fabiani et al., 2013), Propiscin (Kazuń and Siwicki, 2012),Quinaldine (Sneddon, 2012), 2-Phenoxyethanol (Varkey and Sajeevan, 2014),

Metomidate, Clove Oil (Palić et al., 2006), Aqui-S^{TM} (Javahery and Moradlu, 2012) and carbon dioxide (Bernier and Randall, 1998).
Anesthetics efficiency is dependent on several factors as the size of the doses administered or the size of fish, water temperature and species (Skår et al., 2017). Although so far many studies have been conducted on the use of anesthetics in aquaculture, there are still many unknown data on their efficiency. Induction of anesthesia should be done in accordance with the purpose of research or the manipulation of fish in such a manner that the correct dose it's used (Ferreira et al., 2018). Any error can lead to the induction of a state of stress to the fish, with all the following consequences or in the worst case scenario, the death of the fish. Clove oil is a dark brown liquid obtained by the distillation of strains, leaves and flower buds of *Eugenia caryophyllata*, with the active component which is eugenol [2-Methoxy-4-(prop-2-en-1-yl)phenol] in a proportion of 85-95%, along isoeugenol [2-Methoxy-4-(prop-1-en-1-yl)phenol] andmethyleugenol [1,2-Dimethoxy-4-(prop-2-en-1-yl)benzene]. It has a wide range of uses: as an antioxidant (Ghadermazi et al., 2017), antimycotic (Estrada-Cano et al., 2017), antibacterial (Xu et al., 2016) and as an anesthetic. Since the clove oil has a certain amount of persistence time in the organism of which is subject to the anesthesia (Zhao et al., 2017), its use is prohibited in fish that are used for human consumption or in fish who are going to be released into the natural environment (Gueretz et al., 2017).

MATERIALS AND METHODS

The dose efficiency (0.094 mL/L water; 0.0472 mL/L water; 0.0236 mL/L water) of clove oil was tested on 2 groups of carp (*Cyprinus carpio*) and 2 groups of Prussian carp (*Carassius gibelio*) with different body size and mass (Table 1).

Table 1. Body weight average values of the experimental groups and dispersion indices

Clove Oil 0.094 mL/Lwater				
Experimental Group	Abr.	$X \pm s_x$	V%	s
Common carp	Group 1.1	150.8 ± 1.374	9.11	13.737
Common carp	Group 1.2	442.4 ± 14.413	32.58	144.133
Prussian carp	Group 1.3	100.6 ± 1.679	16.69	16.787
Prussian carp	Group 1.4	240.8 ± 10.12	42.03	101.199
Clove Oil 0.0472 mL/Lwater				
Experimental Group	Abr.	$X \pm s_x$	V%	s
Common carp	Group 2.1	153.2 ± 1.108	7.23	11.077
Common carp	Group 2.2	441.4 ± 14.882	33.71	148.818
Prussian carp	Group 2.3	98.6 ± 1.601	16.24	16.009
Prussian carp	Group 2.4	241.6 ± 9.911	41.02	99.11
Clove Oil 0.0236 mL/Lwater				
Experimental Group	Abr.	$X \pm s_x$	V%	s
Common carp	Group 3.1	157.6 ± 1.292	8.19	12.915
Common carp	Group 3.2	428.6 ± 5.42	12.65	54.201
Prussian carp	Group 3.3	98.4 ± 1.264	12.85	12.641
Prussian carp	Group 3.4	229 ± 4.205	18.36	42.048

The experiment was carried out in the Aquaculture Laboratory of UASVM Cluj-Napoca. Fish anesthesia was done by successively immersing the specimens in a clove oil solution at a water temperature of 21 °C in 50L basins. Throughout the experiment, the dissolved oxygen level was maintained at 8 mg O_2/L of water. Since the clove oil is hardly miscible in water, having a density of 1.040 – 1.067 g/cm^3 (Nowak et al., 2012), it has previously been mixed into a separate container by vigorous stirring with a smaller amount of water. Afterwards, it was discharged and homogenized in the experimental pools. Following the induction of total anesthesia, the fish were transferred to fresh water pools which

had the same water temperature. Prior to anesthesia (24 hours), the feeding of fishes was stopped. The times for induction and recovery of anesthesia (MI – mild imbalance; LD – lateral decubitus; TA – total anesthesia; FSR – first signs of recovery; TR – total recovery; OR – opercular rate) were monitored by filming the experiment with a Nikon Coolpix P540 digital camera. Based on the filming, anesthesia and recovery times and phases were then chronometer and the database for the analysis of the results regarding the relationship between body mass and induction and recovery times (seconds) from anesthesia was established for the two different species.

RESULTS AND DISCUSSIONS

In Figures 1, 2 and 3 the anesthesia induction times for the experimental groups, respectively the opercular rate (respiratory movements) in each phase of anesthesia are represented. In each figure the average values and the standard error for each phase are presented.

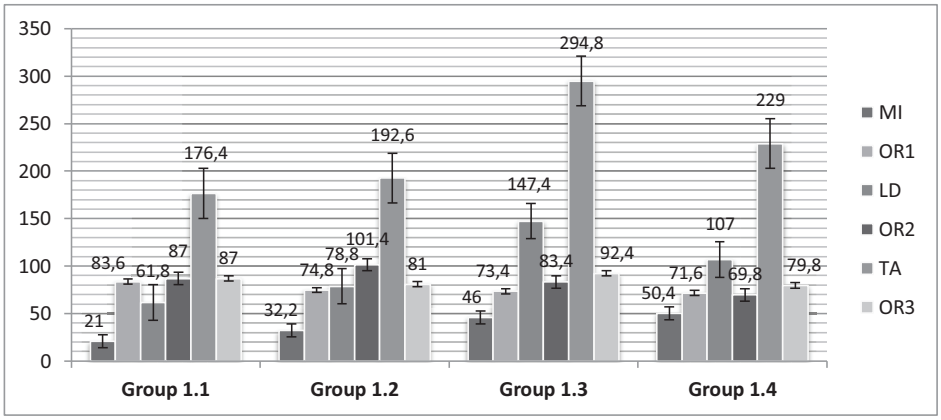

Figure 1. Induction times (seconds) of anesthesia and the rate of operculum movements to the four groups (average values of time and standard error) (Clove Oil doses – 0.094 mL/L water)

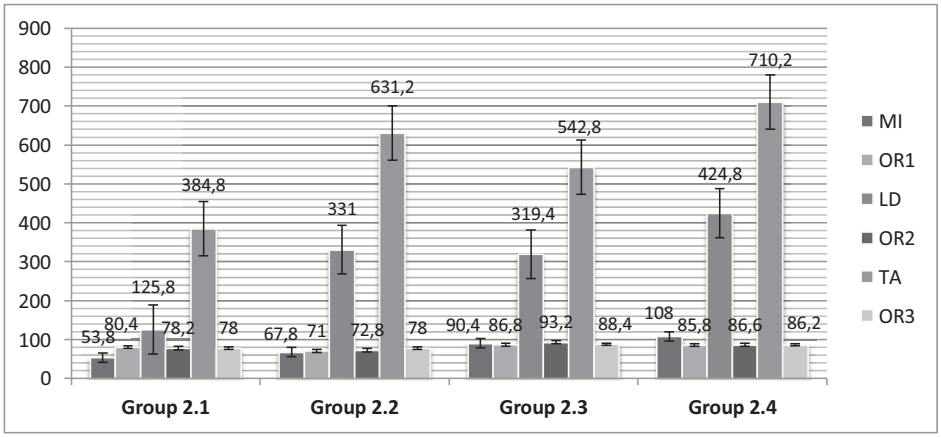

Figure 2. Induction times (seconds) of anesthesia and the rate of operculum movements to the four groups (average values of time and standard error) (Clove Oil doses – 0.0472 mL/L water)

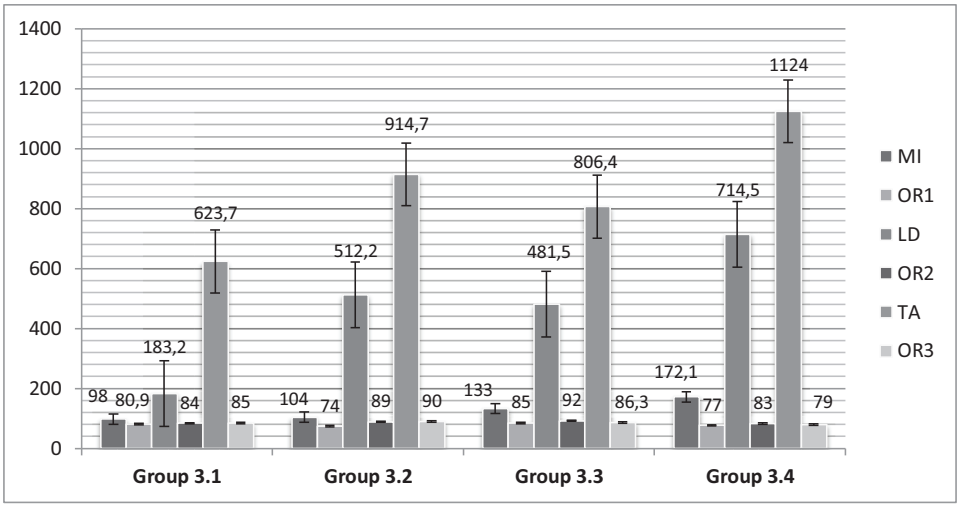

Figure 3Induction times (seconds) of anesthesia and the rate of operculum movements to the four groups (average values of time and standard error) (Clove Oil doses – 0.0236 mL/L water)

From graphical representation, it can be observed that anesthesia induction times increase as anesthetic doses are lower, as found in other studies (Hseu et al., 1998).Thus, in the case of carp with lower body mass (group 1.1, 2.1, 3.1) total anesthesia (TA) is induced at 176.4 (1.1) 384.8 sec (2.1) to 623.7 sec (3.1). It can be seen therefore, that with halving the dose of clove oil, the total times of induction of anesthesia doubles. The same situation was observed for the other phases of anesthesia (mild imbalance MI and lateral decubitus LD). The first signs of imbalance were observed at 21 sec (1.1), 53.8 sec (2.1) and 98 sec (3.1), and the lateral decubitus phase it was observed at 61.8 sec (1.1), 125.8 sec (2.1) and 183.2 sec (3.1).Similar results were obtained in the other experimental groups, and it seems to be a general rule that with the increase in anesthetic dose, the induction times of anesthesia decrease. By reference to the species and size of the specimens, it was also observed that in the case of the smaller specimens (group 1.1, 2.1, 3.1 and 1.3, 2.3, 3.3), anesthesia is induced more rapidly as compared with larger specimens (group 1.2, 2.2, 3.2 and 1.4, 2.4, 3.4).However, an atypical situation was observed in experimental group 1.3 (small Prussian carp specimens with a body weight of 100.6 ± 1.679 g) where the induction times of anesthesia were higher compared to the large Prussian carp specimens (group 1.4) with a body mass of 240.8 ± 10.12 g. Thus, in group 1.3, the induction phases of lateral decubitus and total anesthesia were longer (LD = 147.4 sec; TA = 294.8 sec) compared to group 1.4 (LD = 107 sec, TA = 229 sec). This situation was observed only at the clove oil concentration of 0.094 mL/L water. In the other two concentrations of anesthetic (0.0472 mL/L water, 0.0236 mL/L water), induction time of anesthesia were greater in specimens with higher body mass.

The opercular rate (OP1, OP2, OP3), corresponding to the respiratory rate, regardless of the anesthesia induction phase and the experimental group, was more pronounced than the species limits (carp and Prussian carp). In the experimental group 1.1, the opercular rate corresponding to the first phase of anesthesia (OP1) presented 83.6 movements / minute. At the lateral decubitus phase, the opercular rate (OP2) was 87.0 movements / minute, the same rate being maintained for total anesthesia (OP3 = 87.0 movements / minute). In the experimental group 1.2 and the 0.094 mL clove oil/L water concentration, the opercular rate also ranged within normal limits, but an acceleration was observed in the lateral decubitus phase, which returned to lower values at the time of total anesthesia installation (OP1 = 74.8 movements / minute, OP2 = 101.4 movements / minute, OP3 = 81.0 movements / minute).In other studies on the

induction of anesthesia in fish (Al-Hamdani et al., 2010) were obtained lower values of respiratory rate (34.2 - 43.3 movements / minute). It seems that clove oil induces hypoxia conditions (Stecyk and Farrel, 2002), with similar respiratory and opercular rates being obtained (7.8±1.7 – 10.7±1.8 / min^{-1}). Similar values were obtained for opercular rate at the use of lower doses of anesthetic (Group 2.1 = 78.0 – 80.4movements/min; Group 2.2 = 71.0 – 78.0 movements/min.; Group 2.3 = 86.8 – 93.2 movements/min.; Group 2.4 = 85.8 – 86.6 movements/min.; Group 3.1 = 80.9 – 85.0 movements/min.; Group 3.2 = 74.0 – 90.0 movements/min.; Group 3.3 = 85.0 – 92.0 movements/min.; Group 3.4 = 77.0 – 83.0 movements/min.).

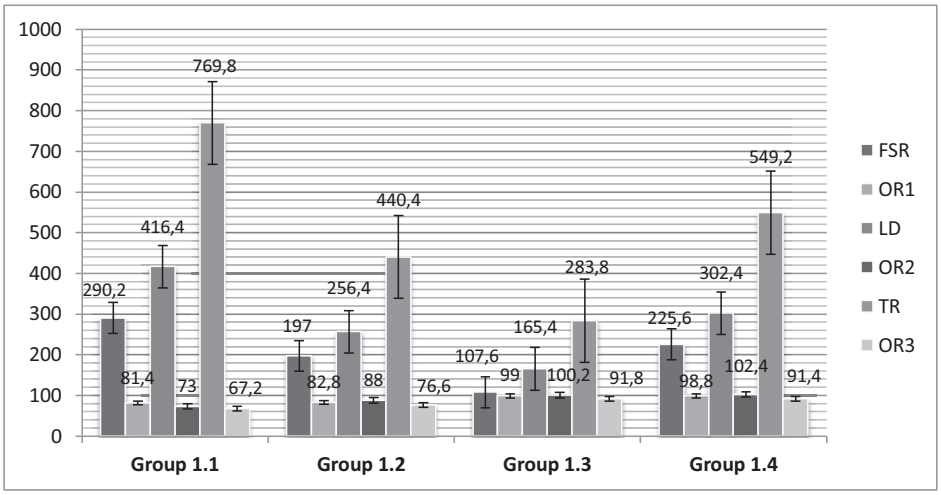

Figure 4. Recovery times (seconds) of anesthesia and the rate of operculum movements to the four groups (average values of time and standard error) (Clove Oil doses – 0.094 mL/L water)

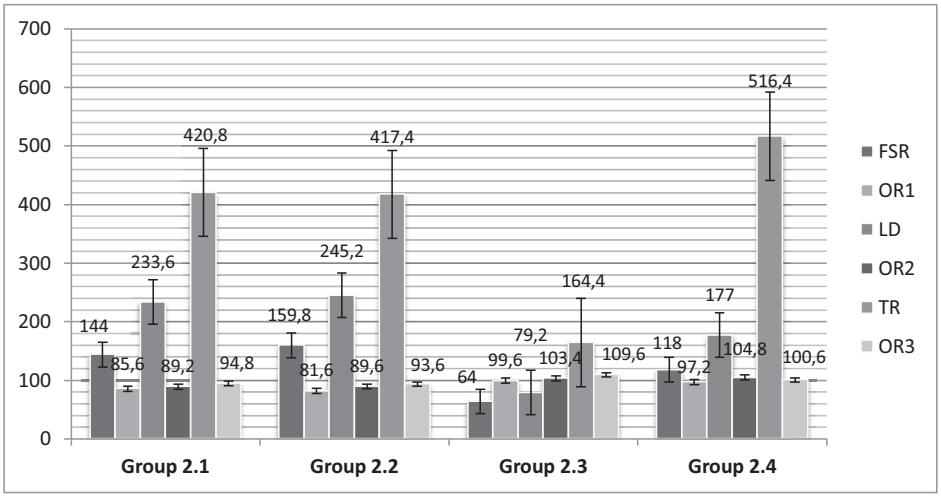

Figure 5. Recovery times (seconds) of anesthesia and the rate of operculum movements to the four groups (average values of time and standard error) (Clove Oil doses – 0.0472 mL/L water)

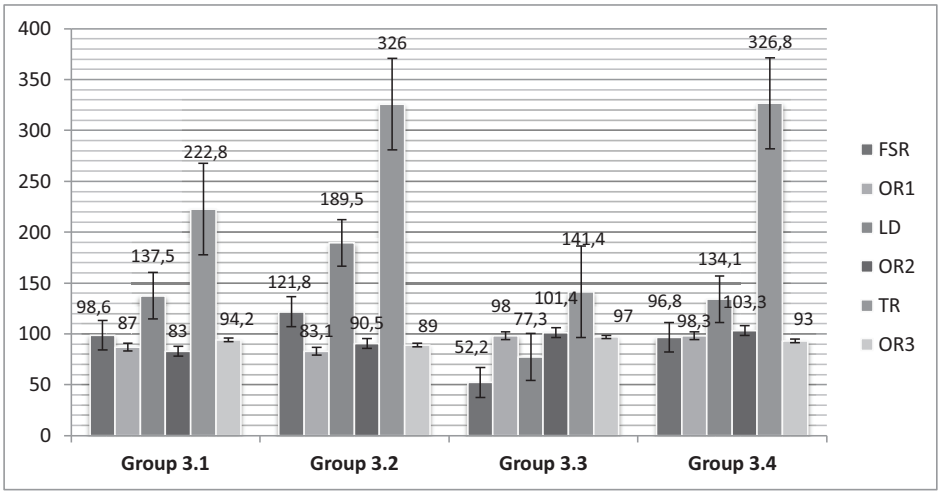

Figure 6. Recovery times (seconds) of anesthesia and the rate of operculum movements to the four groups (average values of time and standard error) (Clove Oil doses – 0.0236 mL/L water)

Recovery from anesthesia times is shown in Figures 4, 5 and 6. As a general rule, it can be seen that the longer they are, the concentration of anesthetic is higher. Thus, for groups 1.1, 1.2, 1.3 and 1.4 (Clove Oil doses = 0.094 mL/Lwater), total recovery times were: Group 1.1 TR = 769.8 sec; Group 1.2 TR = 440.4 sec.; Group 1.3 TR = 283.8 sec.; Group 1.4 TR = 549.2 sec. In the case of carp, total recovery times were longer than in the Prussian carp. In experimental group 1.3 (Prussian carp BW = 100.6 ± 1.679 g), the recovery times are approximately equal to the induction anesthesia times (TA = 294.8 seconds vs. TR = 283.8 seconds). For group 2.1, 2.2, 2.3, 2.4 (Clove Oil doses = 0.0472 mL/Lwater), the recovery times were: Group 2.1 TR = 420.8 sec.; Group 2.2 TR = 417.4 sec.; Group 2.3 TR = 164.4; Group 2.4 TR = 516.4 sec. Similarly, it can be seen that in group 2.3 (Prussian carp BW = 98.6 ± 1.601 g), the average recovery times were the shortest. For experimental group 3.1, 3.2, 3.3, and 3.4 (Clove Oil doses = 0.0236 mL/Lwater), total recovery times were as follows: Group 3.1 TR = 222.8 sec.; Group 3.2 TR = 326.0 sec; Group 3.3 TR = 141.4 sec.; Group 3.4 TR = 326.8 sec.

Operculum movements during recovery from anesthesia were within normal range, but varied according to the doses of anesthetics used and species. Thus, in carps, anesthetized at doses of 0.094 mL/L water (Group 1.1 BW = 150.8 ± 1.374 g), the opercular rate at the time of the first signs of recovery (FSR) was 81.4 movements / min, and for group 1.2 (BW = 442.4 ± 14.413 g) this was 82.8 movements / min. For both groups of carps, at the time of total recovery, the respiratory rate was lower (Group 1.1 TR-OR3 = 67.2 movements / min vs. Group 1.2 TR-OR3 = 76.6 movements / min.).In Prussian carps, anesthetized at doses of 0.094 mL/Lwater, the opercular movements showed at the time of the first signs of recovery the following frequencies: Group 1.3 (BW = 100.6 ± 1.679 g) FSR-OR1 = 99.0 movements/min., Group 1.4 (BW = 240.8 ± 10.12 g) FSR-OR1 = 98.8 movements / min. When total recovery installed, the opercular rates were 91.8 movements / min.(Group 1.3), respectively 91.4 movements / min. (Group 1.4).

In Group 2.1, 2.2, 2.3, 2.4 anesthetized with 0.0472 mL/L water Clove oil, an increase in the opercular rate was observed from the start of recovery to the time of total recovery. Thus, for group 2.1 (carp BW = 153.2 ± 1.108 g), the frequency of the opercular rate at the time of the first signs of recovery (FSR) was 85.6 movements / min, so that at the moment of total recovery (TR), the frequency would reach 94.8 movements / min. For group 2.2 (carps BW = 441.4 ± 14.882 g) OR1-FSR = 81.6 movements / min. vs. OR3-TR = 93.6 movements / min. Similar situations were also observed for

Prussian carps in group 2.3 (BW = 98.6 ± 1.601 g) OR1-FSR = 99.6 movements / min vs. OR3-TR = 109.6 movements / min, respectively group 2.4 (BW = 241.6 ± 9.911 g) OR1-FSR = 97.2 movements/min. vs. OR3-TR = 100.6 movements/min.

The increase of the respiratory frequency rate at the dose of 0.0472 mL/L Water Clove Oil is the physiological response of fish subjected to a long time under the influence of anesthetics.

For the group 3.1, 3.2, 3.3, 3.4, the frequency of the opercular movements did not show very large differences from the start of recovery (FSR) to the total recovery (TR), thereby indicating the inefficiency of a small dose of anesthetic.

CONCLUSIONS

Anesthetics administered to fish when they are manipulated or suffer invasive interventions, have a beneficial effect by reducing stress. However, the correct dosing of anesthetics is extremely important, and must be effective both in duration and effect.

The results of our study demonstrate the efficacy of the 0.094 mL/Lwater Clove Oil dose, both in terms of anesthesia induction and recovery times and respiratory rate.

The dose of 0.0472 mL / Water Clove Oil is efficient but induces a state of stress to fish due to long induction of anesthesia and recovery times. We do not recommend using the 0.0236 mL / Water Clove Oil dose due to the extremely long anesthesia induction times.

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PRELIMINARY RESULTS REGARDING THE EFFECTS OF DIETARY-PROTEIN LEVELS ON THE GROWTH PERFORMANCE AND FEED EFFICIENCY OF COMMON CARP FRY

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Abstract

This experiment aimed to determine the optimum dietary protein level for common carp fry (Cyprinus carpio L.), with the average initial body weight of 2,98 g ±0,05 g (W) and the total length of 5,42±1,17mm (TL). The experiment was conducted for 31 days in a recirculating aquaculture system (RAS). The fish were divided into four experimental groups, in duplicate: V1-30% crude protein and 7% lipids, V2-44% crude protein and 22% lipids, V3-45% crude protein and 16% lipids, and V4- 50% crude protein and 14% lipids.Weight gain (WG) and SGR significantly increased ($p<0.05$) as dietary protein levels increased.Feed conversion recorded the best values at 50% protein diets, the values being considerably lower than those from V1, V2,and V3 variants. Based on the obtained data, it was estimated that the optimal level of protein for fry carps weighing between 2,9 g and 7 g was 50%.

Keywords:*protein level, growth performance, common carp, recirculating aquaculture systems (RAS).*

INTRODUCTION

Aquaculture is increasingly contributing to world food production, being the fastest food producing sector worldwide, and the cheapest source of animal protein with total global production of 73.8 million metric tons (FAO, 2016).

Common carp (*Cyprinus carpio L.*) is one of the main aquaculture species in many European, Latin American and Asian countries, and is estimated that the global fishery and aquaculture production reached approx. 4,556 million tons in 2016 (FAO, 2018). The fast growth rate (Mohapatra and Patra, 2014) high environmental tolerance, ease of handling, tolerance to high stocking densityand ability to utilize effectively artificial diet (Kirpichnikov, 1999) has made it one of the most culture species from Romanian farms.In our country, carp aremainly raised in earth ponds and represent around 33.36% of the fish population structure (Annual report of National Agency for Fisheries and Aquaculture, 2016).

Taking into consideration the growing demandfor carp consumption, it is necessary to develop new technologies, to increase production processes. One of the methods would be to obtain carp larvae outside the breeding season and to grow them during the winter season in recirculating aquaculture systems (RAS), in optimum conditions, and later provided growing in earth ponds (Kristan et al., 2012). This offers an opportunity to increase carp production significantly and reduce the production cycle length, a fact which has the effect of lowering costs and contribute to the profitability of the fish farms.

Although common carp is one of the most frequently cultured freshwater fish species, many aspects regarding the aquaculture in RAS systems arenot studied enough, and therefore, it is essential to determinate the optimal protein requirements for each stage of development in these culture systems.

Because protein is the most expensive macronutrient in the fish diet, the feeding cost accounts 40-50% of the production cost (Steven, 2011) and a key factor for the successful development of carp aquaculture it may be reducing the cost of food.

In this context, thepresent study aimed to investigate the effects of different dietary protein levelson growth performanceof fry carp.

MATERIALS AND METHODS

Experimental design. The experiment was carried out at the Romanian Center for the

Modeling of Recirculating Aquaculture Systems (MoRAS), the facility of University Dunărea de Jos, Galați, Romania, during 31 days. The recirculating system is composed of eight rearing units (water volume of 600 L each), mechanical filter, biological filter, UV lamp for water sterilization and disinfection, pumps, components for the management of dissolved gases (oxygen and carbon dioxide), independent electrical generator and was previously described in the paper of Andrei (2017).

One thousand six hundred fry carps with almost similar body weight and size (mean weight 2.98±0.05g) were randomly distributed in duplicate groups at the rate of 200 fish per trough for each experimental trial. The fish were hand fed, in three meals per day (09:00, 14:00 h, and 18:00 h) in split-rations at 3.2 % body weight (BW), or 10 g kg^{-1} metabolic weight. Four experimental variants were designed to contain four levels of protein: V1-30% crude protein, V2-44% crude protein, V3-45% crude protein, and V4-50% crude protein. Ingredients and nutrient contents of the experimental diets are presented in Table 1.

Table1. Proximate composition of experimental diets

Ingredients	U.M	Diet 1	Diet 2	Diet 3	Diet 4
Crude protein	%	30	44	45	50
Crude lipids	%	7	22	16	14
Ash	%	8	7.2	7	
Raw cellulose	%	5	1.8	2	2
Lysine		-	-	-	2.5
Phosphorus	%	0.8	1.02	1	1
Copper	mg/kg	30	5	5	6
Calcium	%	1.2	1.7	1.3	-
Sodium	%	0.2	0.3	0.3	-
Vitamin A	IU/kg	10 000	10 002	10 000	20 000
Vitamin D3	IU/kg	1 800	1 463	-	2 000
Vitamin E	mg/kg	60	200	200	200
Vitamin C	mg/kg		250	150	200

The physicochemical parameters of water (temperature, dissolved oxygen,and pH) were recordeddaily, with the sensors from the system and once a week, the nitrogen compounds ($N\text{-}NO_2^-$, $N\text{-}NO_3^-$, $N\text{-}NH_4^+$,) were monitored using Spectroquant Nova400 type spectrophotometer, compatible with Merk kits. The water sample for analysis was collected early in the morning before the feeding was done.

Calculations

Fish were weighed and measured individually at the beginning, and the end ofthe experiment and the following variables were calculated:

- Weight Gain (W) = Final Biomass (Wt) - Initial Biomass (W0) (g)
- Food Conversion Ratio (FCR) = Total feed (F) / Total weight gain (W) (g/g)
- Specific Growth Rate (SGR) = 100 x (lnWt - ln W0) / t (% BW/d)
- Relative Growth Rate (RGR) = (Wt -W0)/ t /BW) (g/ kg/.d)
- Protein efficiency ratio (PER) = Total weight gain (W) / amount of protein fed (g)
- Daily Weight Gain (DG) = (Wt – W0) / number of experimental days (g day^{-1})

Statistical analysis

One-way ANOVA was used to compare the effects of dietary protein level on the performance and feed utilization. When a significant effect was found, Duncan'stest for multiple comparisons of means was performed. The data are presented as mean±SD of the replicate groups. All statistical analyses wereconducted using SPSS version 2.0 and were assessed at a significance level of $p< 0.05$.

RESULTS AND DISCUSSIONS

Water quality

All the water parameters (Mean±SD) were suitable for the rearing of common carp (water temperature – 20.70±0.82°C, dissolved oxygen – 7.93±0.27 mg L^{-1}, pH – 7.93±0.09 pH units, nitrites – 0.06±0.02 mg L^{-1}, nitrates 18.11±3.59 mg L^{-1} and ammonia 0.13±0.07 mg L^{-1}), and were notsignificantly affected by the dietary-protein levels (ANOVA, $p> 0.05$).

Growth performance

The growth performance data of fry carp fed thediets containing various protein levels for 31 days is presented in Table 2.

The initial mean weight of the carpfrywas not significantly different($p>0.05$) for each group.

Survival of each group was over than 95%, and there was no significant difference among treatments ($p > 0.05$).

Table 2. The growth rate and feed utilization of carp fry fed at different levels of protein

Parameters	V1	V2	V3	V4
Initial biomass (g)	597.50±7.95	595.00±4.24	593.00±0.0	595.50±3.54
Final biomass (g)	914.50±7.78	1110.00±31.11	1142.50±27.58	1227.50±45.96
The initial number of fish	200	200	200	200
Total weight gain (g)	317.00 ± 12.73^{a}	515.00 ± 35.36^{b}	549.50 ± 27.58^{b}	632.00 ± 49.50^{c}
Survival (%)	95.25 ± 0.35^{a}	98.75 ± 1.77^{a}	96.25 ± 1.77^{a}	95.75 ± 3.18^{a}
Mean initial weight (g $fish^{-1}$)	2.99 ± 0.02^{a}	2.98 ± 0.02^{a}	2.97 ± 0.00^{a}	2.98 ± 0.02^{a}
Mean final weight (g $fish^{-1}$)	4.80 ± 0.06^{a}	5.62 ± 0.06^{b}	5.93 ± 0.03^{c}	6.41 ± 0.03^{d}
Daily biomass growth rate (g day^{-1})	10.23 ± 0.41^{a}	16.61 ± 1.14^{b}	17.73 ± 0.89^{b}	20.39 ± 1.60^{c}
Individual weight gain (g $fish^{-1}$)	1.81 ± 0.08^{a}	2.64 ± 0.08^{b}	2.97 ± 0.03^{c}	3.43 ± 0.04^{d}
FCR	2.74 ± 0.11^{a}	1.69 ± 0.12^{b}	1.58 ± 0.08^{b}	1.38 ± 0.11^{c}
SGR (% day^{-1})	1.37 ± 0.05^{a}	2.01 ± 0.11^{b}	2.11 ± 0.08^{b}	2.33 ± 0.14^{c}
PER	1.220 ± 0.05^{a}	1.35 ± 0.09^{a}	1.41 ± 0.07^{a}	1.46 ± 0.11^{a}

(Mean value of 2 replicates ± SD); mean values in the same raw with different superscript are significantly different ($p<0.05$)

The final weight of the fish, Weight gain, specific growth rate (SGR%) and feed conversionratio (FCR) were found to be significantly affected ($p<0.05$) with the increase of dietary protein level in the diets, while the protein efficiency ratio (PER) showed no significantly different ($p>0.05$) between the four experimental variants.

At the end of the experiment, significant differences ($p<0.05$) were registered in the final weight. The lowest individual weight was obtained in the 30% crude protein, and the highest weight was recorded in the 50 % crude protein.

The statistic comparison of the final fish weight (Duncan test) emphasizing four distinct groups of individuals based on their weight. Thus, in V1 variant, the mean individual final weight was 4.80±0.06g $fish^{-1}$, in V2 5.62±0.06g $fish^{-1}$, in V35.93±0.03 g $fish^{-1}$, respectively 6.41±0.03 in V4 (Figure 1).

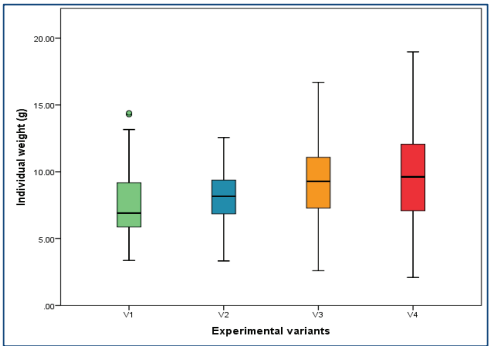

Figure1. The variation of the average individual weight – median, minimum, maximum values and quartiles registered at the end of the experiment for all experimental variants

The maximum weight gain for carpwas obtained with the diet containing 50% dietary protein level and was significantly different ($p<0.05$) from that achieved by the fish fed a 45%, 44%, and 30% protein diet.

Regarding the feed conversion ratio (FCR) it was observed a significantly decreased as the dietary protein level increased, and ranged from 2.74 to 1.38. The best FCR was obtained atV4 (50% crude protein), followed by V3 and V2 (45% and 44% protein diets), with no statistically significant differences among these two experimental variants ($p>0.05$).

Significant differences ($p<0.05$) were recorded in the daily biomass growth rate, and ranged from 10,23 g day^{-1} in V1 case, to 20.39g day^{-1} in the V4.

The specific growth rate (SGR) of frycarp fed varying levels of dietary protein showed a significantly increasing tendency with increasing dietary protein level ($p<0.05$).

So, SGR was found as 1.37±0.05, 2.01±0.11, 2.11±0.08, 2.33±0.11 % day-1 for V1, V2, V3, and V4, respectively.

According to Lovell (1989), dietary protein is considered to be of crucial importance in fish nutrition and feeding. Therefore sufficient supply of dietary protein is required for rapid growth.

In the present study, the increasein the levels of dietary protein contenthad a significant effect on the growth rate, feed conversion ratio,and specific growth rate. The growth andconversion efficiencies gradually increased with the increase of dietaryprotein levels from 30% to 50% protein containing diet. The best growth parameters were obtained when fish were fed at

50% protein containing diet, the growth rate being significantlydifferent to those groups that were fed at 30%, 44% and 45% protein diet. This may be due to the increase in protein utilization and digestibility with the increase indietary protein level up to 50%.

These findings are in agreement with those obtained by other authors. Dabrowski (1977) foundthe highest gain at grass carp at the optimum dietary protein level (45,56%). In a recent study Khan and Maqbool (2017) reported that the optimum dietary protein level for optimum growth and efficient feed utilization for *Cyprinus carpio var. specularis*, (with the mean, weight, and length of 1.50 ± 0.02 g; 4.5 ± 0.05 cm), is 41.5%. Also, Aminikhoei (2015) conducted a study for Israeli carp (average body weight, 1.3 ± 0.02 g) in order to determine the optimal dietary protein levels (20, 30, 40, and 50%), and found that the diet containing 40% crude protein is optimal for the growth and effective protein utilization. Also, others studies conducted to determine the dietary protein requirements of common carp reported dietary protein requirements ranging from 30% CP in the case of pond-reared fish and fry to over 45% CP in the case of fry and fingerlings (Inayat and Salim, 2005).

In a study conducted in ponds, Mocanu (2015), used extruded feed, with 48% crude protein, in the first 30 days of growth (individual weight of fish 1 g), then feed the carp for 90 days with 30% and 35% crude protein and found that growth performance was significantly improved at 48% crude protein and 35% crude protein.

However, the protein requirements among the fish species are influenced by fish size or age, culture conditions, and nutrient interactions in experimental diets such as protein and non-protein energy levels and further studied are needed to determine the optimal dietary protein for every life stage of carp.

CONCLUSIONS

Feed quality directly influences the growth and quality of fish meat. To choose the right feed, an account is taken of both its protein and energy content. In this case of study, the experimental diet containing 50% protein resulted in the best weight gain, specific growth rate and feedconversion ratio for the fry carp, with the mean weight between 2.9 and 7 g.

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EFFECTS OF FEEDING LEVELS ON GROWTH PERFORMANCE, AND BODY COMPOSITION OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*, WALBAUM 1792)

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Abstract

In this study we investigate the effect of different feeding levels (FL1-2.5%BW day^{-1}, FL2- 3%BW day^{-1}, FL3-3.5% BW day^{-1}, FL4-4 %BW day^{-1}, FL5-4.5 %BW day^{-1} and, ad libitum-FL6) on the growth performance, morphologic indexes and body composition of rainbow trout (Oncorhynchus mykiss) reared in a recirculating aquaculture system. 360 juvenile rainbow trout (34.17±0.11 g, mean±SD) were randomly distributed to 12 rearing units for 44 days. At the end of the trial period, the ANOVA test showed that growth performance was significantly affected by feeding levels ($p<0.05$). Final mean body weight, weight gain, and specific growth rate(SGR) increased with increasing feeding level. Feed conversio rate (FCR) was below 0.7 for all groups, except FL6 were FCR registered a value of 0.83 (g/g). The protein efficiency ratio was significantly affected by the feeding level ($p<0.05$) and the best value was obtained for FL1 group (2.59±0.05 g/g). Significant changes ($p<0.05$) in ash, protein, lipids, and water content were observed at the end of the experimental period. Protein and lipids contents of rainbow trout meat increased with increasing feeding level, while moisture content and ash significantly decreased. Also, this increasing trend was observed in terms of the hepatosomatic index, but without significant differences between groups ($p>0.05$). The viscerosomatic index of fish the FL1, FL2, and FL3 was significantly lower than those from FL4, FL5, and FL6. Based on the obtained results, it is recommended, from an economic point of view, a feeding level of 2.5 % BW day^{-1} as optimum for rainbow trout, reared in a recirculating aquaculture system, from 30g to 130 g.

Key words: *Feeding rate, growth performance, rainbow trout, body composition.*

INTRODUCTION

Feeding management practices (feeding level, feeding frequency or feeding delivery system) affect fish conversion rates and fish growth, size heterogeneity and has a significant contribution on feasibility and economics of commercial success of aquaculture (Du et al., 2006).

The main goal of trout aquaculture is to maximize growth at minimal cost because feed represents at least 40-60% of the total production costs (Hurye et al., 2010; Wendy et al., 2013). Of the feeding practices, feeding level is the most important variable and influences growth and feeds conversion rates. When fish are overfed, the digestive efficiency and the fish growth is reduced (Sanver, 2005) and poor water quality can occur (Mihelakakis et al., 2002; Serap and Fikret, 2009). On the other hand, underfed fish do not reach maximal growth, and may exhibit aggressive behavior due to limited feed availability, and the variability of fish sizes increases (Dwyer et al., 2002).

Feeding level ranges from the maintenance feeding level (at which fish neither gain nor lose weight), to the maximum feeding (maximum amount of fed the fish can consume). Over this range, feeding level reaches a maximum point beyond fish cannot consume the fed and the efficiency of feed conversion is reduced and weight gain no longer increases in direct proportion with the feeding level (Lovell, 1998).

The effects of feeding levels on fish growth and feed conversion efficiency have been studied for rainbow trout (Sanver, 2005; Kok and Siau, 2006; Bureau et al., 2006), but the results of these studies are quite variable because there are several factors which influence the feeding level, such as fish size (Mihelakakis et al., 2002), temperature (Azevado et al., 1998; Bailey and Alanärä, 2006), rearing systems (Cho et al., 2003).

In this context, the purpose of the present study

was to determinate the optimum feeding level on growth performance, feed efficiency and body composition of rainbow trout reared in a recirculating aquaculture system, from 30 g to 130 g.

MATERIALS AND METHODS

Experimental design and feeding trial. The experiment was carried out in a Recirculating Aquaculture System at „Dunărea de Jos" University, Galați, România. The experimental system consisted of 12 glass tanks, with a capacity of 132 L, each. 360 Trout fingerlings brought from fish farm Prejmer, Braşov, România, were stocked into a rearing tank for two weeks as an acclimatized period and then randomly distributed in the rearing units in such a manner to create homogenous groups with similar class frequencies and number. At the beginning of the experiment, the initial average weight±SD of fish was 34.17±0.11 g. All the fish were fed with extruded pellets with 54% protein content and 18% lipids (Table 1).

Table 1. The composition of the experimental diet

Composition	U.M	Quantity
Crude protein	%	54
Crude lipids	%	18
Cellulose	%	1
Ash	%	10
Phosphorus	%	1.4
Digestible energy	Mj/kg	19.4
Vitamin A	UI	14000
Vitamin D3	UI	2300
Vitamin E	mg	250
Vitamin C	mg	500
Lysine	%	3.5
Methionine	%	1.5
Cystine	%	0.7
Ingredients: Fish meal, fish oil, hemoglobin, full-fat soy, soybean oil, wheat gluten, sunflower meal, wheat,and wheat products.		

Fish were fed twice daily at 9 a.m. and 6 p.m. at different feeding levels (FL1-2.5%BW day^{-1}, FL2- 3%BW day^{-1}, FL3-3.5%BW day^{-1}, FL4-4 %BW day^{-1}, FL5-4.5%BW day^{-1} and, ad libitum-FL6). In the *ad libitum* feeding, fish were fed until the first two or three pellet remains to the bottom of the rearing units, and usually, this action lasted for one hour. Fecal matter was removed by siphoning every morning, before feeding and if in the *ad libitum* feeding remains any uneaten feed, this was filtered, dried and weighed in order to quantify the exact amount of consumed feed.

All experimental variants were performed in duplicate.

The main physicochemical water parameters, temperature, pH and dissolved oxygen (DO), were measured daily with a Hach-Lange equipment Sc 1000. Nitrogen compounds (N-NO_2^-, N-NO_3^-, N-NH_4^-) were determined periodically with the Spectroquant Nova 400 type spectrophotometer, using kits from Merk. Water temperature was 17.37±1.08˚C, the oxygen content was 7.46±0.256 mg L^{-1} and pH was 6.93±0.206 pH units. Regarding the dynamics of the nitrogen compounds, the average values of nitrite, nitrate, and ammonium were, 0.09±0.05 mg L^{-1}, 142.251±40,935 mg L^{-1}, and 0.115±0.075 mg L^{-1}. Although nitrate concentrations were higher, many authors reported that concentrations around 200 mg L^{-1} may be acceptable for fish growth, with no serious consequences on the short term (Colt and Armstrong, 1981).

At the end of the feeding trial all the fish were individual weight and the growth and feed utilization parameters were calculated using the following standard formulas: weight gain (WG) = final body weight (W_{t1}) – initial body weight (W_{t0}) (g), feed conversion ratio (FCR) = total feed (F)/total weight gain (W), specific growth rate (SGR (%body weight day^{-1})) = [(Ln W_{t1} – Ln W_{t0})/t] × 100, coefficient of variation (%), protein efficiency ratio (PER) = total weight gain (WG)/amount of protein fed (g). For all equations, W_{t0} and W_{t1} are the initial and final body weight of fish and t is the experimental period of the trial.

Sample preparation and analysis of biochemical composition. At the end of the study, a sample of eight fish was taken from each experimental group randomly for the determination of body composition and for body condition indices such as Hepato-somatic index ((HSI)=100 (liver weight (g)/ body weight (g)) and Viscero-somatic index ((VSI)=100 (visceral weight (g)/body weight (g)). The determinations of biochemical composition of fish were performed on muscle tissue samples according to AOAC (2000): Proteins were determined with Gerhardt type equipment by using the Kjeldahl method (N × 6.25), fats were determined by Soxhlet solvent

extraction method (petroleum ether) with Raypa extraction equipment, 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. One-way ANOVA was used to compare the effects of feeding level on the growth performance and feed utilization. To determine the significant differences among the experimental variants, Duncan's test for multiple comparisons of means was performed. All statistical analyses were conducted using SPSS version 21 and were assessed at a significance level of $p < 0.05$.

RESULTS AND DISCUSSIONS

Survival percent and growth performance of juvenile rainbow trout fed six different levels of feeding are presented in Table 2. No mortality was observed in any of the groups during the entire experimental period. At the beginning of the experiment, the normal distribution of the fish population (in terms of body weight and individual length) was statistically analysed and confirmed by statistical tests. The initial mean weight was between 34 ÷ 34.28 g and the total length was between 14.58 ÷ 14.72 cm, with no statistical differences between the experimental variants (ANOVA, $p>0.05$).

At the end of the experiment, statistically significant differences were found between the fish weight and length from the six experimental variants (ANOVA, $p<0.05$). Generally, body weight and length increased in response to increasing of feeding levels.

In fact, Duncan's analysis divided the final weight of fish into five distinct groups (FL1; FL2; FL3; FL4, respectively FL5, and FL6) (Figure 1).

Significant differences ($p<0.05$) were registered in the final length of the fish.

Fish from FL1 have a significantly lower length than those from FL2 and FL3, while in the FL4, FL5 and FL6 groups, feeding level did not significantly influence the total length of the fish.

Table 2. Growth performance of juvenile rainbow trout fed with various feeding levels

Feeding levels	Initial body weight (IBW) (g/fish)	Initial body length (IBL) (cm/fish)	Final weight (FBW) (g/fish)	Final length (FBL) (cm/fish)	Individual weight gain (g/fish)	Survival (%)	SGR	FCR	PER
FL1	34.22±5.62^{a}	14.59±0.92^{a}	87.33±14.18^{a}	19.83±1.31^{a}	53.12±1.88^{a}	100±0.0	2.13±0.04^{a}	0.71±0.02^{a}	2.62±0.08^{a}
FL2	34.08±6.41^{a}	14.66±1.11^{a}	94.63±15.62^{b}	20.26±1.34^{b}	60.56±0.54^{b}	100±0.0	2.32±0.01^{b}	0.74±0.01^{b}	2.50±0.02^{b}
FL3	34.21±5.32^{a}	14.60±0.76^{a}	104.07±15.83^{c}	20.73±1.05^{b}	69.86±0.84^{c}	100±0.0	2.53±0.02^{c}	0.75±0.01^{b}	2.46±0.03^{b}
FL4	34.20±4.60^{a}	14.88±0.74^{a}	115.23±17.35^{d}	21.40±1.20^{c}	81.04±0.68^{d}	100±0.0	2.76±0.01^{d}	0.74±0.01^{b}	2.50±0.02^{b}
FL5	34.28±6.01^{a}	14.59±1.06^{a}	123.10±23.01^{e}	21.75±1.43^{c}	88.82±1.38^{e}	100±0.0	2.91±0.03^{e}	0.76±0.01^{b}	2.43±0.05^{b}
FL6	34.00±6.41^{a}	14.58±1.14^{a}	124.33±23.11^{e}	21.67±1.48^{c}	90.33±0.39^{e}	100±0.0	2.95±0.01^{e}	0.82±0.02^{c}	2.25±0.04^{c}

Mean value of 2 replicates ± SD; on the same row mean values with the same superscript are not significantly different ($p>0.05$)

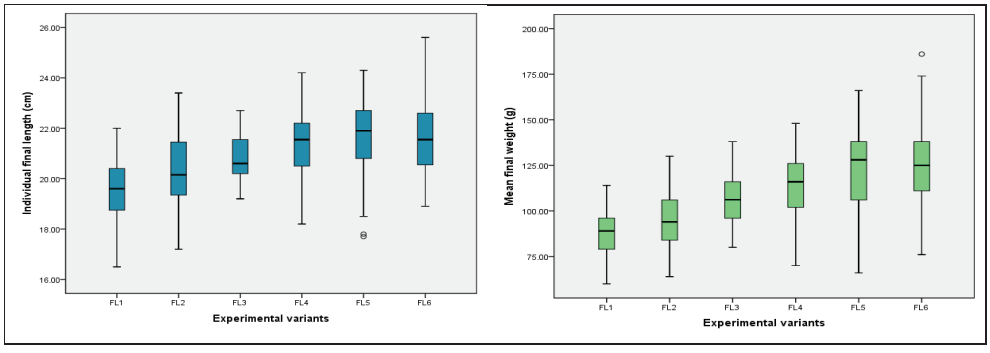

Figure 1. Individual final weight and the final length of juvenile's rainbow trout

Significant differences ($p<0.05$) were observed in weight gain, SGR, FCR, and PER of juvenile rainbow trout fed various feeding levels.

In order to have a precise image of the relationship between the feeding level and weight gain and feeding level and SGR,

second-degree polynomial regression analysis was performed. So, mean body weight gain = -0.6737 x^2 + 12.521x + 39.78; (r^2=0.984) and SGR = -0.0176 x^2 + 0.297x + 1.8265; (r^2=0.991), (where x= the feeding level) (Figure 2). Increasing of feeding level resulted in increased weight gain and SGR of juvenile rainbow trout, but at FL5 and FL6 (which was calculated at the end of the experiment at 5% BW day^{-1}), reached a plateau, fact which means that an increase of the feeding level does not necessarily lead to the somatic growth of the fish. The fish from the FL6 and FL5 had the highest weight gain, while the fish from the FL1 showed the lowest weight gain among all the experimental groups. However, there was significant ($p<0.05$) difference in weight gain between FL2, FL3, and FL4.

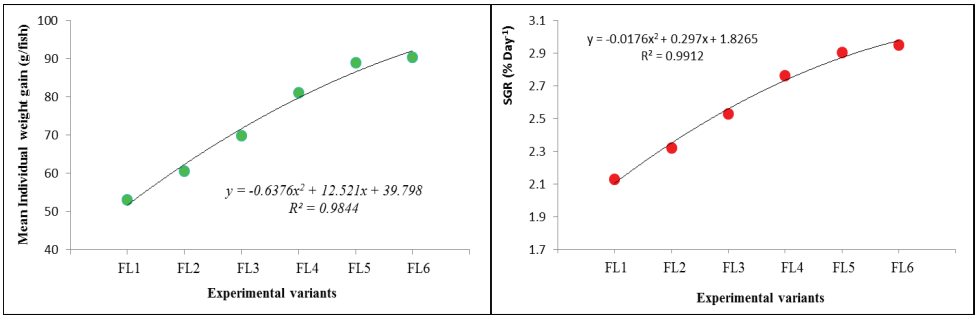

Figure 2. Changes in individual weight gain (g/fish) and specific growth rate (SGR) of juvenile rainbow trout fed with various feeding levels

Comparing the obtained values of SGR, the statistical analysis revealed no significant differences ($p>0.05$) between the experimental variants, FL5 and FL6, respectively significant differences ($p<0.05$) between FL1, FL2, FL3, and FL4.

Regarding the feed conversion ratio (FCR) and protein efficiency ratio (PER) ($p<0.05$), the best values was found in the case of FL1. No significant differences were reported between the FL2, FL3, FL4, and FL5, while in the case of FL6, it was registered the highest FCR and the lowest PER. However, a further increase in the feeding level resulted in no improvement of FCR and PER. According to Tvenning and Giskegjerde (1997), a poor FCR at higher ration levels appears because fish took a long time to consume food in order to reach satiation, thus causing the loss of nutrients and wastage of food.

Effects of feeding levels on body composition and morphological indices are shown in Table 3.

Table 3. Proximate composition (percentage of wet weight) of juvenile rainbow trout fed with various feeding levels

Feedinglevels	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	HSI (%)	VSI (%)
FL1	84.180±0.148^a	12.673±0.358^a	2.304±0.119^a	1.455±0.019^a	7.68±0.026^a	0.985±0.06^a
FL2	83.925±0.148^a	12.790±0.057^a	2.398±0.048^a	1.431±0.042^a	7.909±0.235^a	1.085±0.158^a
FL3	83.165±0.502^b	13.650±0.297^b	2.765±0.163^b	1.381±0.019^b	8.464±1.705^a	1.093±0.077^a
FL4	82.323±0.527^b	13.865±0.064^b	3.153±0.126^b	1.369±0.035^c	8.651±0.390^a	1.135±0.093^b
FL5	81.555±0.573^c	14.395±0.502^c	3.392±0.057^c	1.270±0.034^d	8.821±0.699^a	1.165±0.131^b
FL6	80.625±0.460^c	14.745±0.078^c	3.615 ±0.035^c	1.238±0.023^d	10.502±0.132^a	1.254 ±0.131^b

Mean value of 3 replicates ± SD; on the same row mean values with the same superscript are not significantly different ($p>0.05$)

Moisture, protein, lipid, and ash was significantly affected by the feeding level ($p<0.05$). Body moisture content decreased significantly ($p<0.05$) with the increasing of ration levels. The proteinand lipid content was found to be significantly high ($p<0.05$) in fish from FL5 and FL6 and a significant fall ($p<0.05$) was evident in fish from FL1 and FL2. Regarding the ash and moisture content, it was observed a significantly decreased ($p<0.05$) with the increase of feeding levels.

The results obtained by us are comparable to those reported by other authors. Storebakken and Austreng (1987) reported in the case of

rainbow trout an increase in the lipid content, proportional to the level of feeding. Also, Palmegiano et al. (2008) obtained for rainbow trout with an individual weight of 180 g, a significant decrease in water content, while proteins and lipids significantly increased with feeding levels (1.2%, 1.4%, 1%, 8%, and *ad libitum*). In a recent study, Bureau et al. (2006) reported an increase of moisture content and a decreasing of the percentage of lipids in lower feeding levels (restricted by 25%, 50%, 75% or 100%).

Rasmussen and Ostenfeld (2000) reported that fat accumulation is prevalent during fast growth at high feeding levels, while fat deposition is subtle during slow growth due to the presence of adequate energy in excess feed deposition as fat. Also, according to Rasmussen and Ostenfeld (2000), the decline of the ash content at higher feeding levels is caused by relatively low skeletal growth compared with other tissue.

HSI and VSI of fish were gradually increased as the increasing feeding level. No significant ($p>0.05$) differences were found in HSI, while VSI from FL1, FL2, and FL3 was significantly lower than those from FL4, FL5,and FL6. Generally, HSI may provide information on growth, physical condition, energy reserves and the ability of fish to tolerate the environmental stresses. In a poor, stressful and unfavourable environment, fish usually have a smaller liver which means less energy reserved (Mihelakakis et al., 2002; MdMizanur et al., 2014). Increasing values of HSI and VSI at higher feeding levels were also confirmed by Storebakken and Austreng (1987), which after 6 weeks obtained a doubling of the HIS values from 0.71 ± 0.10% in the case of a feeding level of 0% to 1.40 ± 0.20% at a feeding level of 2%.

In aquaculture farms, feeding fish under the satiation level without affecting the growth is strongly recommended, mainly because of production costs. Thus, the determination of optimum feeding level is one of the key factors for better growth, feed conversion, nutrient retention efficiency and chemical composition of fish (Zhang et al., 2011). According to Jobling et al. (1994), the optimal feeding level corresponds to that feeding level at which the best FCR of the feed is obtained, while Cho et al. (2003) suggests that maximum growth occurs at the limit of voluntary food intake (satiation). In our study, increasing of the feeding level showed lower nutrient utilization and wastage of food, respectively a lower economic efficiency, since similar FCR is achieved between the FL2, FL3, FL4, and FL5 groups.

The results obtained by us are supported by the research of other authors (Van Ham et al., 2003), which, in the case of higher feeding levels, noticed a decrease of digestive efficiency, due to the increased metabolic consumption generated by the catching food processes, digesting and metabolizing the amount of feed that goes beyond the optimal nutritional level. Therefore, according to some authors (Van Ham et al., 2003; Ahmed, 2007) fish tend to optimize their digestion to extract nutrients more efficiently at lower feeding levels.

In the present study, it appears that the daily feed application feeding level of 2.5% BW day^{-1} was near to optimum when the fish grew from 30 to 130 g. Earlier studies have also reported different optimal feeding rate at rainbow trout. Storebakken and Austreng (1987) reported an optimum feeding level of 2% BW day^{-1} for rainbow trout weighing 0,5- 1,0 kg, while Imtiaz (2018) recommended for rainbow trout (5,65±0,45cm; 1,42±0,25g) a feeding level between 4,6 to 5,3% body weight. For rainbow trout with the weight of 300 g, Storebakken and Austreng (1987) reported that maximum feeding efficiency was obtained when the quantity of food was given 2% BW day^{-1}.

CONCLUSIONS

As a result of the present study it can be concluded that the increasing of the level of feeding, can lead to a qualitative production, from nutritional point of view, but the choice of the optimum feeding level should also take into account the efficiency of the feed utilization (FCR), and the fact that too much feed can lead to deterioration of water quality and the occurrence of mortality. Taking into account the higher cost of feeding for rainbow trout the results of this study suggest that the optimum feeding level for juvenile rainbow trout growing from 30 g to 130 g was 2.5% BW day^{-}

[1] if we take in consideration the main growth parameters FCR and SGR.

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THE EFFECT OF AFLATOXIN FOOD CONTAMINATION ON THE IMMUNE RESPONSE (LEUKOCYTE REACTION) OF THE EUROPEAN CATFISH (*SILURUS GLANIS,* L. 1758*)*

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Abstract

Silurus glanis are an important romanian aquaculture species raised for food and sport fishing, especially in polyculture tehnology but also in the intensive semi-closed and recirculation sistems. The aim of present study was to obtain a basic knowledge of the leukocyte reaction of European catfish under the action of toxic metabolites (aflatoxin B) secreted by the mold Aspergillus flavus. The sampling of blood from the healthy and affected Silurus glanis exemplars allowed determination of white blood cell count. In order to achieve the purpose of the experiment the blood samples were immediately used to make smears which were colored with May-Gründwald Giemsa panoptic method. By studying different types of leucocytes was determined leukograma and absolute number of leukocytes. Physiological stress induced by toxic metabolites secreted by the mold is reflected in the hematological parameters - white blood cell count (significant decrease, p=<0.05). In the infected fish with aflatoxin the total number of leukocytes has been registered statistically significant changes with lymphopenia, neutrocytosisand eosinophilia. The hematological changes of infected fish led to the affect the immune defense system of the Silurus glanis specie.

Keywords: *aflatoxin B, european catfish, immundefence, white blood cell count (WBCc).*

INTRODUCTION

European catfish is an economically important cultured species in the Romanian aquaculture, reared especially in polyculture technology with cyprinids in the systematic and semi-systematic farms, began to be reared lately in the intensive, semi-closed and recirculating production systems (Docan et al., 2011). In fish culture, nutrition obviously plays an important role in the maintenance of a healthy and marketable product. Most of the diseases of nutritional nature represent the consequence of nutrition errors, lack of balances or deficiencies in the fodders composition, lent also of their contamination with toxic substances (Munteanu and Bogatu, 2003). The knowledge of the hematological characteristics is an important tol that can be used as an effective and sensitive index to monitos physiological a pathological changes in fish species (Kori-Siakpere et al., 2005). The differential leukocyte count has been used as an biomarker of physiological stress in a number of teleosts fish (Ellsaesser and Clem, 1986; Khangarot et al., 1999). Interpretation of changes in leukocytes proportions must be carefully considered when dealing with relative and absolute ratios. The analysis of leukocyte distribution within the peripheral blood of a fish may provide valuable insight into the function of the immune system in response to stress conditions (Ainsworth et al., 1991). The leukocytic reactions of the blood may be correlated with the pathogeny/pathogenesis of various acute or chronic, infectious, parasitical or toxic diseases. The importance of the hematological examination in the fish diseases diagnosis and in the mycotoxins effect evaluation has been widely accepted. The aflatoxins are mycotoxins with hepatotoxic and carcinogenic action, metabolism products of the *Aspergillus flavus* and *A. parasiticus* stems (Carlson et al., 2001). Therefore, even in the cases when the exposure to small dosage of aflatoxin does not lead to mortality, this is responsible for a higher sensibility to infectious diseases due to the insufficiency of the immune function (Sahoo and Mukherjee, 2001).

The aim of the present study was to obtain a basic knowledge of the immune response (measured changes in the distribution of leukocytes in the peripheral blood) of European catfish under the action of toxic metabolites

(aflatoxin B) secreted by the mold *A. flavus*, but also the assessment of the physiological changes.

MATERIALS AND METHODS

Fish biomass and the growing conditions. Fish biomass used in this study was represented by *Silurus glanis* specimens raised into a flow-through system of the pilot aquaculture station from the Aquaculture, Environment Science and Cadastre Department.

Fish were examined for any sign of infection or disease condition and only those fishes considered to be healthy were used for the study. The two experimental fish groups had individual mean weights of 682 ± 64.49 g/ex. in the first tank (C1), respectively 743 ± 41.79 g/ex. in second tank (C2). The stocking density was 74.7 kg/m³ for C1, respectively 74.3 kg/m³ for C2. The fishes from C1 were fed with fodder with 46% protein, and those from C2 with fodder with 30% protein. For both experimental types, the settled ration was of 1% BW per day. The fodder that was given to the fish from C1 was contaminated with *Aspergilus flavus*.

Blood sampling and analysis. The blood was sampled from 10 fish of each tank by caudal venous puncture using lithium heparin as anticoagulant, at the healthy and for the infected catfish. Blood was analysed with routin methods used in fish haematology (Blaxhall and Daisley, 1973). For each exemplar two blood smears were immediately dried, fixed and then colored with May-Grünwald Giemsa panoptic method (MGG). The relative proportion (percentage) of each type of white blood cells was obtained by microscopic examination of 200 leukocytes on blood smears. The type of leukocytes were determined based on identification characters listed by Svobodova (Svobodova et al., 1991). Absolute number of circulating blood leukocytes and thrombocytes were determined in relation to 1000 erythrocytes in haemograms stained with panoptic method MGG and converted to unit blood volume.

Statistical analysis. The different types of white blood cells (expressed as a percentage and absolute number) of the two experimental groups were expressed by mean and standard deviation and differences between the values were statistic analyzed with t-Student test.

RESULTS AND DISCUSSIONS

Following this experiment were also analyzed the reactions of the leukocyte's system, in order to determine the effect of the influence of aflatoxin in feed on the immune system defenses and for a fair assessment of physiological changes in *Silurus glanis*. To assess the leukocytemic changes were performed both qualitative analysis by observing the morphological particularities of the leukocytes and quantitative analysis to evaluate the relative changes (leukogram) and absolute changes (cells/µl blood) of different types of leukocytes. Microscopic examination of blood smears colored by MGG, did not show morphologic changes in leukocytes.

The results obtained after examining the blood smears are capable of supplying important information about the physiological state of the fish. In order to establish the effect of mycotoxin has upon the European Catfish's immune defence system, special attention was paid to the study of the leukocytic response.

The relative (the leukogram) and the absolute modifications of the various types of cells that make the leukocytic complex are given in Table 1.

Table 1. The relative and absolute modification of healthy and infected catfish

WBCc	SI units	Healthy catfish	Infected catfish
Leukocytes	$x10^3 \mu l^{-1}$	95.89±26.34	*42.46±8.08*^{a}
Lymphocytes	$x10^3 \mu l^{-1}$	76.67±27.88	*22.35±8.17*^{a}
	%	78.53±7.14	*51.48±9,12*^{a}
Monocytes	$x10^3 \mu l^{-1}$	2.89±1.08	*0.90±0.52*^{b}
	%	3.18±1.32	*2.21±1.3*^{b}
Neutrophiles	$x10^3 \mu l^{-1}$	16.10±5.49	*26.40±2.72*^{a}
	%	18.00±6.48	*45.15±8.28*^{a}
Eosinophiles	$x10^3 \mu l^{-1}$	0.24±0.05	*0.48±0.13*^{a}
	%	0.28±0.09	*1.17±1.11*^{a}

The morphological study of the blood smears both for the healthy catfish and for the infected one, clearly show a predominance of the lymphocytes, followed by the neutrophils (promyelocytes, metamyelocytesand neutrophils with kernel unsegmented or with

two or four lobs), monocytes and eosinophils (Figure 1). Basophiles were not found.

The analysis of the variation of the absolute number of ***leukocytes*** shows that these are severely reduced in the circulating blood of the catfish affected by mycotoxin. Thus, if in the case of the catfish in good physiological status, the blood smears presented approximately 95.89 $x10^3$ μl^{-1}, in the case of the diseased catfish, leucopoenia (a decrease of the number of leukocytes) was noticed. The leukocytes are significantly reduced by 55.7 % going down to 42.46 $x10^3$ μl^{-1}.

For fish, as for the other higher vertebrates, a general decrease in the number of leukocytes, as a consequence of repressing the natural defence immune system, is considered to be the result of acute stress (Ellis, 1977).

In order to emphasise the stressing effect of mycotoxin upon the physiological state of the catfish as well as its modus operandi we had to establish the leukocytic line responsible for the variation of the total number of leukocytes.

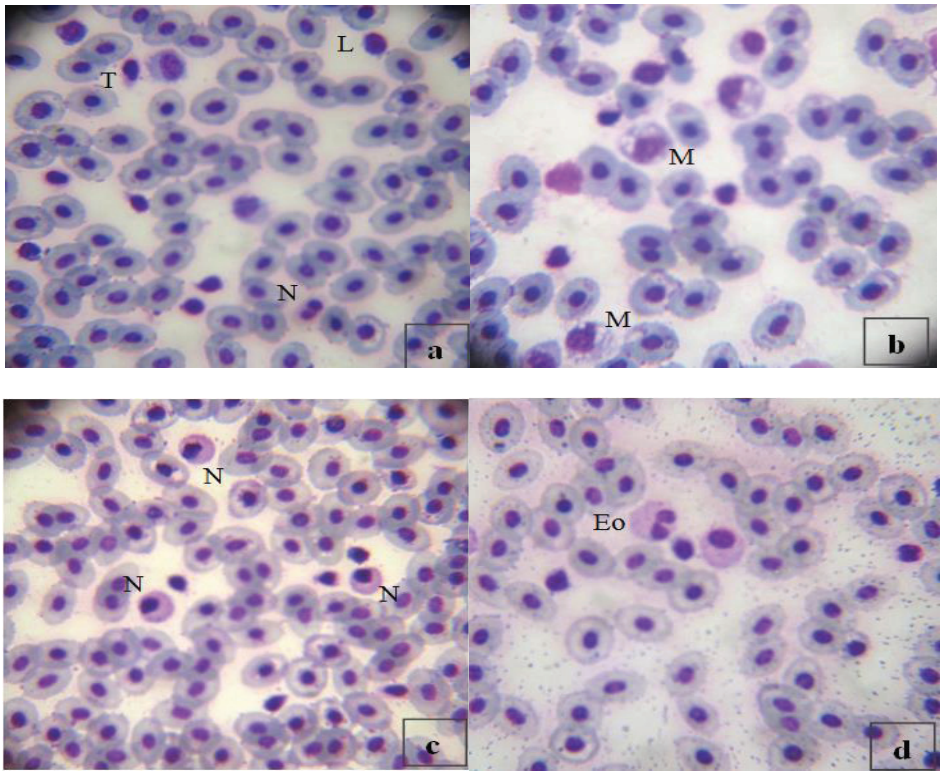

Figure 1. Light microscopic micrographs of peripheral blood of *Silurus glanis*:
a) L - lymphocyte, N - neutrophile;
b) M - monocytes with vacuolated cytoplasm;
c) N - young neutrophil, T - thrombocyte; d) Eo–eosinophile.

The monocytic reaction of the blood of the catfish affected by aflatoxicos is is different from the one of the healthy catfish. The number of the monocytes in the blood of the infected catfish decreases by 32.5 % as compared to the healthy catfish. As far as the absolute number of monocytes is concerned, we also notice a decrease by 69 % as compared to the healthy catfish. The monocytes transit only for 1-2 days through circulation, after which they reach the extravascular tissues and continue their maturation becoming functional as macrophages (Bârză, 1985).

The macrophages regulate the intensity of the antigenic stimulus but also interfere with the activity of the lymphocytes (Patriche, 2008). The study of the blood smears shows that, as compared to the total number of granulocytic

leukocytes, the neutrophils are the most numerous ones.

The neutrophilic reaction in the case of the catfish affected by aflatoxicosis is different from that of the healthy catfish.

We notice the statistically significant increase of the relative number of neutrophils by 150% as compared to the healthy catfish (from 18 to 45.15 %). The same ascending trend is preserved in the case of the absolute number of neutrophils as well (no. cel/µl blood) but only with 68 %.

In general, stress in fish induces granulocytosis (Ellis, 1977). Moreover, in the case of the species *Ictalurus punctatus*, a neutrophils level higher than 4% represents an indicator of the disease stress (Ellsaesser and Clem, 1986), and the relative neutrophilia is typical of the stress induced by manipulation, transportation, the percentage of neutrophils increasing from 4.2 ±1.5 to 20.9 ±1.5 %.

The microscopic examination of the blood smears in the catfish affected by aflatoxicosis showed the following: the increase of the absolute number of neutrophils is accompanied by the appearance in the blood flow of an increased number of myelocytes, metamyelocytes and young neutrophils. This increase in the number of young neutrophils characterises the initial stage of neutrophil fight. By means of phagocytosis, their main function, the neutrophils can neutralize bacteria, toxins or other alien substances in the organism (Bârză, 1985), leading to an increase in their number.

Eosinophilic granulocytes are generally characterised by motility and phagocytosis and have been correlated with the immune defence as well. The number of eosinophils in the blood of the catfish affected by aflatoxin was significantly higher, increasing approximately three times, counting for 1.17%. Expressed in absolute number of cells, eosinophils preserve the same ascending tendency reaching 0.48 $x10^3$ μl^{-1} in the infected catfish, respectively 2 times more as compared to the healthy catfish, which had only 0.24 $x10^3 \mu l^{-1}$.

Characteristic for eosinophilic granulocytes is the presence within the cytoplasm of certain secretory vacuoles whose product has the property of neutralising the toxic substances produced by some parasites. For these reasons the number of eosinophils may have increased in the blood of the catfish affected by aflatoxin precisely with the purpose of neutralising the toxin secreted by the *Aspergillius* strain. The increase in the absolute number of the circulating eosinophils suggests, more often than not from a pathogenic perspective, a reaction of late sensitivity (Bârză, 1985).

The answer of the ***thrombocytary system****,* under the stressful action of mycotoxin, is prompt and intense, manifesting through a thrombocytosis phenomenon (increase in the number of thrombocytes) in the circulating blood of the infected catfish. Thus, the absolute number of thrombocytes found in the blood smears increased, statistically significant ($p=0.002<0.05$) reaching 15.67 x 10^3 μl^{-1} in the case of the infected catfish, 112% higher than that in the blood of the healthy specimens, where the number of the thrombocytes did not exceed 7.38 $x10^3$ μl^{-1}.

The biotic factors (age, season, sex), the abiotic ones (water temperature, pH, content in the dissolved oxygen) as well as the stress may induce modifications in the number of thrombocytes (Tavares-Dias and Oliveira, 2009). The haemostatic mechanisms have been activated according to the stressing agent and its action, and this led to a rapid decline of the blood's coagulation time accompanied by a corresponding increase of the circulating thrombocytes in the blood. Practically, due to the increase of the corticosteroid hormones (catecholamine, cortisol) level that appears during stress the number of blood thrombocytes increases and the coagulation time decreases.

Results from this study support findings by others authors, which demonstrated that the immune response in fish can by affected by mycotoxins. The exposure of the rainbow trout fry to the action of B1 aflatoxin proved that this can generate long-term dysfunctions of the cellular immunoregulation, inducing a significant reduction of memory B cells (cells responsible for mediated humoral immunity, with rapid activation and long life). Moreover, we noticed the deletion of the immunoglobulin production but also of the proliferation of lymphocytes (Ottinger and Kaattari, 2000). In the case of Nile tilapia the toxin also affected other immunologic parameters with influence upon the degree of immunosuppression,

manifested through a reduction of the phagocytic activity of the macrophages.
Generally, in fish, the hepatoxic effect of the B1 aflatoxin generates the reduction of the total quantity of proteins due to an inhibition of the protein synthesis induced by the aflatoxin's binding to the cellular macromolecules, thus leading to the alteration of the production of humoral factors. On the other hand, the toxicity of the hematopoietic organs (the anterior kidney and the spleen) generate lymphocytolysis (attributed to the deterioration of the kidney tissue) as well as the reduction of the immunoglobulin production (Sahoo and Mukherjee, 2001). In fish, under the action of aflatoxin, a reduction of the agglutination bacterial titer along with an increase in the number of bacteria was noticed, which indicates in fact that chronic exposure to aflatoxin leads to the inhibition of the antimicrobial factors release (lysozyme and antiprotease) thus contributing to an increased susceptibility of fish to infectious diseases.

CONCLUSIONS

Since there are no real means to fight the negative effects produced by mycotoxins, the prevention of the fungal invasion by prophylactic measures is a necessity. Due to their resistance to heat, humidity and steam, it is recommended to check the ingredients necessary for the production of extruded feeds, so as to detect the presence of mycotoxins.
In the case of the catfish, the leukocytic and thrombocytic lines were the most affected ones, whose modifications materialised in a leukopenia and an accentuated thrombocytosis. Thus, the leukocytes significantly decreased accompanied by lymphopenia, accentuated neutrophilia, and eosinophilia (an increase in the number of eosinophils, suggesting a delayed sensitivity reaction). Neutrophilia was accompanied, in the blood flow of the catfish affected by aflatoxin, by an increased number of young neutrophils, which proves a "regenerative reaction" specific to the initial stage of neutrophilic fight. The extremely active nature of this type of leukocytes as well as their intense phagocytic capacity are well known. The stressful effect of mycotoxin (produced by the metabolism of the strains of *Aspergillus flavus)* was felt by the catfish population leading to weakened and disordered metabolic processes by affecting the immunologic response cellular reaction which becomes obvious by the reduction in the number lymphocytes.
Lymphocytopenia accompanying the stress determined the reduction of the immunologic reactivity, leading to the appearance of certain agonal states which denotes a severe prognostic. Finally, the mortality among the biomass affected by aflatoxin was accentuated, reaching 83%.

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STUDY REGARDING CERVIDAE EVOLUTION IN CALARASI COUNTY

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Abstract

This is another just part for an ample study regarding evolution of species from Cervidae family in Romania. The programme is developed in collaboration with Romanian Hunter's Federation. The main purpose of this study is to reveal the reality, to find causes and to elaborate long term strategies in direction of biodiversity conservation, especially for wild game. In the last years the Romanian hunters indicate that the number of roe deer population decreasing, because of intensive agriculture and also because of high number of predators (bear, wolf and lynx population). We analyze the official data from national evaluation of sedentary game in Calarasi County. Hunting territories in this area are managed by National Forest Authority, county associations of hunters and other associations for conservation of biodiversity and management of hunting territories. We analyse cervidae real livestock between by counties, by sexes, and in comparison with optimal livestock (maximal number of individuals who can leave in a hunting area, without causing damage to the agricultural fields or in the forest). Considering the new agricultural technics and technologies it is relatively normal to find a numerical depreciation of wild game. But for cervidae populations, in analysed period in Calarasi County, we find a good and representative population. The differences between what can be saw in hunting territories and what is reported was analyzed. We can say that the evaluation, the official evaluation, is not perfect and we must work together to know exactly the livestocks and to develop a long term strategy for conservation of biodiversity.

Key words*: cervidae, evaluation, game, Calarasi.*

INTRODUCTION

All over the world, scientific organizations, hunter's associations and organizations involved in environmental protection collaborate in the direction of conservation of the environment and biodiversity, implicitly in the protection of wildlife. The subjects of these researches are mainly the members of cervidae species. A lot of researches have as principal subject the red deer, especially in North America and in North – Western European countries. The themes aim are deep, detailed topics, mainly focused on the influences of the special and general environment on behavior, growth rate, etc., as well as pathological aspects. So, in Scotland, Albon et al. (1983) studies the influence of climatic variation on the birth weights of Red deer. In Slovakia, Trdan et al. (2003) shows that, at the forest border, because of red deer grazing, the herbal production is damaged with 50%. In this case, probably they have a big density or it is a temporary agglomeration. In 2000, Slate et al., analyzing a red deer population in the Islands of Rum (Scotland), demonstrate that inbreeding depression influences lifetime breeding success in wild population of red deer.

It is a certitude that in the hunting areas the number of game species has decreasing. This situation was detected by hunters, no matter the hunting territories that they used for hunting.

In Europe, a big project was "Big carnivores in Carpathians" (1995-2003) developed by WWF in Romania. The aim of this project was to analyze the wild livestock of brown bears, wolves, lynx and wild cat and to determinate the status of this species. The conclusions were that all this four species of predators are endangered and must be protected. It is interesting that in the middle of '90's, some Romanian researchers show that the Romanian brown bear was the biggest livestock from Europe (Cotta et al., 2008). More than that, the brown bear real number was almost three times bigger than the optimal number (optimal population – maximum number of individuals who can live in an area without depreciating

forest and agricultural crops. Protection of these predators led to decreasing of prey species, especially of that species that cohabitate in the same area with the brown bear and wolf. We refer here especially to red deer and roe deer. In almost the same time, from South, a new predator arrives in Romania: the jackal (*Canis aureus*). In the past, some individual of *Canis aureus* was observed in South-East of Romania, more exactly in Dobrogea area, and especially in Danube Delta. But this time, jackals were hunted in Alba County, at more than 400 km from the South border. In comparison with foxes, jackals prefer small game and roe deer and red deer kids. In the absence of a predator, the number of jackals has increased numerically and has expanded vertiginously. It is a fox competitor and, due to superior physiological and morphological characteristics, he became the predominant predator of 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. In this situation, when in the field the red deer has became a rarity, and the roe deer it is obvious at a lower level, it is a must to know the real livestock and the real evolution of species, in order to developing medium and long-term strategies for the conservation of cervidae species. We can not leave aside the economic aspects, the deer representing the second species of hunting interest in Romania, after the rabbit (Comșia, 1961), by the species characteristics and hunting fees practiced.

Regarding the fallow deer, it is not a autochthonous species. In Romania the fallow deer was imported, for the first time, in centuries I-II, by Romans, being bred in fence area. After barbarians invasion, the fallow deer escape from this fence areas and became wild. In 1830 fallow deer were colonized in a forest with an area of 4,000 ha, situated along Crisul Negru, (today's territory of Hungary), on the border with Romania. Due to the existence of the wolves, entire stock grew hard. Because of this, in 1900 the forest and a part of the agricultural land have closed. Due to the favorable conditions the fallow deer stock has grown so much that it has created important forest damage. So, after about 15 years (roughly in 1915) the fence area has disbanded and it is supposed that some fallow deer has moved to the forest of Socodor, located at 9-12 km (Cazacu, 1983). In 1918 the fallow deer in Romania numbered 500 individuals grouped in nine cores. The only individuals who lived in freedom were at Savarsin and Socodor, Arad County (Geacu, 2009).

In 2007, according to the "Report on Romania's state of forests in 2007" the fallow deer livestock from freedom was evaluated at 5,700 specimens. Unfortunately, the economical value and the interest for hunting this species is low. More than that, due to physiological, ethological and morphological characteristics, the fallow deer is a food competitor for roe deer and red deer.

MATERIALS AND METHODS

Analyzed material was represented by Cervidae population from Calarasi County: Red deer, roe deer and fallow deer. It was analyzed the official data from national evaluation of sedentary game in Calarasi County area, more exactly for roe deer, fallow deer and red deer and it was calculated statistics, in order to have a better view of situation. The hunting territories in this county are managed by National Forest Authority, county associations of hunters and other associations for conservation of biodiversity and management of hunting territories.

It was analyzed the livestock of Cervidae between 2014 and 2018 by sexes, and in comparison with optimal livestock, in accordance with the rating keys for hunting territories.

We also use some statistics like average, standard deviation, error of average, and variability coefficient in order to have a better overview of the population evolution. In other way, our study is based on the official raports of hunting areas administrators, centralized at ministerial level, due to the fact that the evaluation of cervidae species, on such a big area, involve a huge number of observers and a lot of time (in according with the methodological norms for national game evaluation.

More than that, a correct evaluation must be done in the same time for all 42 hunting areas from Calarasi County (over 500000 ha).

RESULTS AND DISCUSSIONS

Analyzing the data from Table 1 and Figure 1, we can easily observe that the livestock is relatively stable until 2012, when the fallow deer population increasing from 84 to 136 individuals.

Table 1. Real livestock of cervidae in Calarasi County

Year	Roe deer (heads)	Red deer (heads)	Fallow deer (heads)
2014	4859	671	28
2015	4977	765	32
2016	4949	843	32
2017	5100	848	34
2018	5065	908	34

As we expected, the roe deer is dominating, from numerical point of view, the other two species, being the most important species of big game in south east, after the wild boar. We must say that the fallow deer is presented only in one hunting area administrated by A.V.P.S. Bucuresti – hunting area no. 20 – Frumusani.

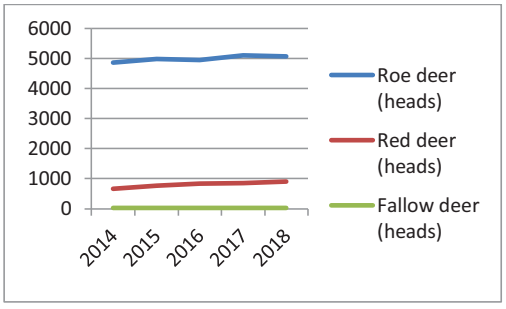

Figure 1. Cervidae evolution

For red deer, the individuals are mainly located on the hunting areas administrated by AVPS Natural Hunting. Analyzing by species we find, in red deer population (Figure 2), an increasing number of males starting from 2014 till 2018, at 23.23% in 2015, 0.96% in 2016, only 0.32% in 2017, and 5.68% in 2018. Females have a different evolution.

In females case, annual increasing was 8.39% in 2015, 16.59% in 2016, only 0.76% in 2017, and 7.91 in 2018. For the entire period, analyzed in this case, the differences between sexes, regarding the population evolution are not significant: 31.89% in males and 37.41% in females.

The natural increasing rate for red deer is normally 15%.

This situation, revealed above, it seems to be real, in comparation with other data who reveal an artificial way of increasing the real size of red deer population in other counties (Maftei et al., 2017).

More than that, an average yearly increasing that represents over 50% from natural increasing rate of species it's a healthy fact (it is considered that a normal hunting rate must be till 50% from increasing natural rate).

The statistics calculated for red deer is presented in Table 2

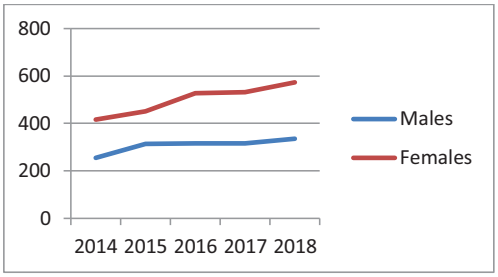

Figure 2. Red deer evolution

Table 2. Calculated statistics for red deer

Year	Optim	Males	Females	Total
2014	60	254	417	671
2015	60	313	452	765
2016	60	316	527	843
2017	60	317	531	848
2018	60	335	573	908
X	**51.43**	**307.00**	**500.00**	**807.00**
STDEV	**22.68**	**30.86**	**63.66**	**91.43**
Sx	**7.56**	**15.43**	**31.83**	**45.72**
CV%	**44.10**	**10.05**	**12.73**	**11.33**

In Figure 3 it is represented graphically the evolution of fallow deer in Calarasi County.

As we already say, the fallow deer in Calarasi County was reported only on Frumusani hunting area.

From this point of view, this species have a limited importance at county level.

Even if the importance of species is insignificant, at county level, we calculated statistics for this species. The data are presented in Table 3.

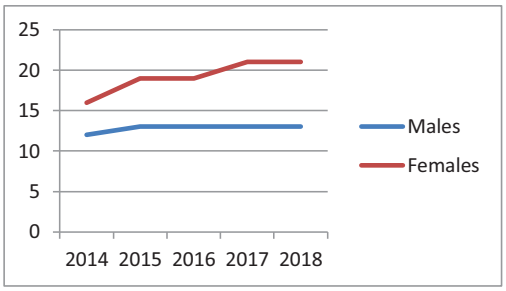

Figure 3. Fallow deer evolution

Table 3. Calculated statistics for fallow deer

Year	Optim	Males	Females	Total
2014	16	12	16	28
2015	16	13	19	32
2016	16	13	19	32
2017	16	13	21	34
2018	16	13	21	34
X	**16.00**	**12.80**	**19.20**	**32.00**
STDEV	**0.00**	**0.45**	**2.05**	**2.45**
Sx	**0.00**	**0.45**	**2.05**	**2.45**
CV%	**0.00**	**3.49**	**10.67**	**7.65**

In the male case it is obvious a small numerical evolution, one head, for the entire analyzed period (8.33%). For females we record an increasing of 5 heads in analyzed period which means 31.25%. The smaller increase of males can be explained by the fact that the hunting demands have as principal subject the fallow deer males. We must not forget that also the hunters interest for this type of cervidae is low, and the hunting and economic value is also low. In comparison with the others two species of cervidae that was analyzed, the fallow deer is cheap, being lower than red deer and near the roe deer (Maftei et al, 2017), as we can observe in Figure 3.

In roe deer population we observe a constant trend, with low fluctuation. In male case we remark an evolution, from numerical point of view, between 2014 – 2015 of 42 heads, more exactly 2.18%. Between 2015 – 2016 it was recorded a small decreasing, -0.86%, more exactly 17 heads. Between 2016 – 2017 the increasing of roe deer population was only 0.77, and between 2017 – 2018 the increasing of population was insignificant (0.05%). On the entire analyzed period the roe deer evolution was only 2.12%.

Graphic representation of roe deer evolution is presented in Figure 4 and statistics in Table 4.

The roe deer females record an increasing between 2014 – 2015 (2.59%) and an insignificant decreasing from 2015 to 2016 (0.47%). The situation became better in 2017 when the entire livestock of roe deer increase with 4.54%. In 2018 we record, again, a small decreasing, but insignificant, at only 1.15%. It is obvious that this entire situation, with small increasing and decreasing, is due to the fact that the real effective represents 292.27% from optimal effective. In this condition we talk about a reproductive natural inhibition of species. The sex ratio, in analyzed period was 1:5, except last two years, 2017 and 2018, when have a small tendency to became 1:6 (1:5.8, respectively 1:5.7.

Attention! Maintaining a sex ratio, in roe deer population, 1 female for 1.1 or maximum 1.5 males it is a good measure to maintain a good and strong population.

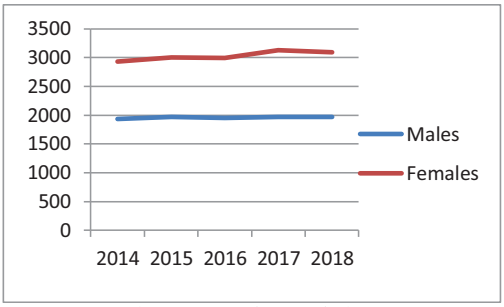

Figure 4. Roe deer evolution

Table 4. Calculated statistics for roe deer

Year	Optim	Males	Females	Total
2014	1733	1930	2929	4859
2015	1733	1972	3005	4977
2016	1733	1955	2994	4949
2017	1733	1970	3130	5100
2018	1733	1971	3094	5065
X	**1733.00**	**1959.60**	**3030.40**	**4990.00**
STDEV	**0.00**	**17.95**	**80.96**	**95.83**
Sx	**0.00**	**8.98**	**40.48**	**47.92**
CV%	**0.00**	**0.92**	**2.67**	**1.92**

CONCLUSIONS

The evaluation of game population it's seems to be ok in this county, in comparation with other counties from the south part of Romania. Unfortunately, some species, like fallow deer, are present only in a small part of the counties' hunting field. It is a must to have a support

from state authorities in order to populate some hunting areas with this species. This action is not necessarily only from hunting point of view. It is a necessity for conservation of the wild games species. Exaggerate extraction of roe deer males, and an unbalanced sex ratio can lead to decreasing of population from numerical and qualitative point of view.

We strongly recommend:

- Compulsory, for hunting areas administrators, to maintain a population with an ascendant trend till to the optimal population;
- Implication of hunters in surveillance of obligatory action of administrators (evaluation, feeding, etc.);
- Active implication of national hunting area administration in game evaluation;
- Compulsory, for hunting areas administrators, to maintain the sex ratio and all technical parameters in order to conserve and preserve biodiversity;
- Realization of some areas reports regarding principal factors that influenced the diagnosis keys;
- It is a MUST to update the diagnosis keys for hunting areas;
- Respect the term:"selection hunting";
- Diversification of fence hunting areas activity in direction of repopulation in free hunting areas.

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SOME ASPECTS ON ORNAMENTAL JAPANESE CARP REARING IN AQUARIUMS

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Abstract

Koi carp are descendants of the common carp, Cyprinus carpio. The production of the colored carp – the Japanese "nishikigoi"- presently exceeds in monetary value the production carp as human food. The researches were performed in Aquaculture and Fishing Department laboratory of Food Science and Engineering Faculty from January 8 to June 24, 2014. The experiments were conducted during five experimental periods. During the mentioned periods it was experimented two variants in aquariums with capacity of 150 l provided with independent aeration and filtration units. In V1 variant, an aquarium was populated with 14 koi carp fingerlings with mean initial weight of 5, 35 g/ex. and a total biomass of 75 g. In V2 variant, another aquarium was populated with koi carp fingerlings with mean initial weight of 29, 28 g/ex., respectively a total biomass of 205 g. Water temperature, dissolved oxygen and pH were measured every day with portable instruments. For feeding fish it used Nutra T (V1 variant) and Nutra 2.0 (V2 variant) pellets. At the end of experiments, mean final weight reached 1570 g for V1 variant and 1446 g for V2 variant.

Key words*: aquarium, koi carp, pellets, rearing.*

INTRODUCTION

The common carp (*Cyprinus carpio*, L.) belongs to the *Cyprinidae*, the largest freshwater teleost family, and is probably the oldest and most extensively cultured fish species in the world (Mondol et al., 2006). This fish has been acclimatized to a wide range of habitats and environmental conditions and therefore enjoys a world-wide distribution.

The colorful koi, or fancy, carp was developed in Japan for ornamental purposes, probably from color mutant types arising in the wild carp (Magoi) of Central Asia (McDowall, 1989). Other commercial stocks (e.g. gold, red and white, tricolour etc.) have been established by selective breeding and cross breeding of colour mutants (Purdom, 1993).

This fish is most famous for its beautiful colours that have been developed via selective breeding programmes. There are over 20 different varieties of koi that differ in colour, patterns and type of scales. Successful koi rearing it depends on maintaining of an artificial environment that simulates as closely as possible the natural environment in which koi are to be found. This is no different from traditional tropical fish keeping with which many aspirant koi keepers may be familiar already. Ornamental carp value increases with intensity of skin colour, which is an important quality criterion. The ornamental carp colors are determinate by specifically cells names chromatophores that are situated in the epidermis and in the dermal superior tissue. The feeding pattern, and thus the growth rate of koi depend on many factors, such as water temperature, water quality, stocking density and genetic background. Koi feed most actively at temperatures in excess of 15^{o}C (59F), thus sexually immature fish can grow rapidly during the summer months when the temperature is warmer (http://www.lagunakoi.com/Koi-Food/Nijikawa-Koi-Food-p-758.html).

Once koi are mature, their growth rate slows considerably; in sexually mature fish, most of the food eaten is utilized in producing eggs or sperm in preparation for breeding. However, unlike many other vertebrates, fish continue to grow throughout their lives and it is easy for koi to reproduce and continue to grow because of their artificially high feeding rates.

The average life span of a koi fish is 15 to 20 years. Some can live up to 30 years or more.

MATERIALS AND METHODS

The study was conducted in two aquariums with a capacity of 150 litres each, provided with independent aeration and filtration units.
In V1 variant an aquarium was populated with 14 koi carp fingerlings with mean initial weight of 5, 35 g/ex., and a total biomass of 75 g. They were brought from Natural Science Museum of Galati where hatched in September 2013.
The fish were fed with Nutra T pellets with crude protein of 52%. Daily feeding rates decreased from initially 5% to 1.5 % of the total fish biomass.
In V2 variant another aquarium was populated with 7 koi carp fingerlings with mean initial weight of 29, 28 g /ex. and a total biomass of 205 g. In this variant the fish were fed with Nutra 2.00 pellets with crude protein of 54%. Daily feeding rates decreased from initially 3% to 1% of the total biomass.
The fish were fed four times / day. Dry feed was dispensed on the surface manually and it was completely consumed by the fish. The pellets contain fish meal, cereal and cereal by-products, oils, antioxidants (BHT). The biochemical composition is presented in the Table 1.

Table 1. Biochemical composition of pellets

Biochemical composition	UM	V1 (Nutra T)	V2 (Nutra 2.0)
Crude protein	%	52	54
Crude fat	%	20	18
Crude cellulose	%	1	0.6
Ash	%	10	10
Phosphorus	%	1.4	1.45
A vitamin	U.I./kg	18000	16000
D3 vitamin	U.I./kg	1800	2300
E vitamin	mg	500	500
Copper ($CuSO_4$)	mg	4.5	5

For the water filtration it was used pomp with capacity of 500 l/h, made in Italy."Aquaclear" pumps have been chosen because they are suitable for low storage density and provide superior filtration, biological, mechanical and chemical. The size of the sponge and the carbon particles provide a large surface that increases the filtering capacity.
Sponges can be easily washed and reused, allowing for the conservation of colonies of biological bacteria. Effective carbon removes dissolved organic compounds, drug substrates and colorants.
Ceramic components provide an environment conducive to the growth of colonies of bacteria that convert ammonia and nitrites into nitrates. We mention that washing the sponge was done with the dechlorinated water, in order not to affect the colonies of bacteria.
In order to maintain good water quality, faeces were siphoned every day. Every three days 20% of total volume of water was changed.
In both experiments, the survival rate was 100%. The temperature, *p*H and dissolved oxygen were monitored every day.
The following equipment was used to measure the water quality parameters: oxygen concentration and temperature were measured with the WTW Oxi 315 I, *p*H was measured with the *p*H meter WTW, model *p*H 340.
Data on fish growth were recorded every month. Growth was determined in term of net weight gain and specific growth rate.
At the end of the experiment the fish were weighed, based on which the following parameters were calculated:

- Weight Gain (W) = Final Weight (Wt) – Initial Weight (W0) (g)
- Feed intake = amounts of feed supplied – uneaten feed.
- Food Conversion Ratio (FCR) = Total feed (F) / Total weight gain (W) (g/g)
- Specific Growth Rate (SGR) = 100 x (lnWt– lnWo) /t (%BW/d)
- Protein efficiency ratio (PER) = Total weight gain (W) / amount of protein fed (g)

RESULTS AND DISCUSSIONS

A high biological feed efficiency can only be guaranteed by an appropriate ratio between the quality and quantity of the feed on the one hand and the physiological requirements of the fish body in nutrients on the other.
In carp, crude protein from fodder ranges within fairly wide ranges, between 25 and 45% depending on age.
These values are also influenced by the growth system and financial resources.
For intensive and superintensive systems, where water is a simple physical substrate,

without natural food, it is necessary to choose a diet rich in protein (Oprea and Georgescu, 2000). So, in this experiment crude protein was 52% in V1 variant and 54% in V2 variant, respectively.

The experiments were running over 167 days between 8th January and 24th June 2014. The water quality parameters monitored were within the tolerable limits for koi carp.

Water temperature ranged from 18.5°C to 25°C for V_1 variant and from 18.5°C to 24°C for V_2 variant (Figure 1); pH from 7.3 to 8.2 for V_1 variant and 7.2 to 8.2 for V_2 variant (Figure 2); dissolved oxygen, from 2.3 to 6.8 ppm from V_1 variant and 2.5 to 5.7 ppm for V_2 variant (Figure 3).

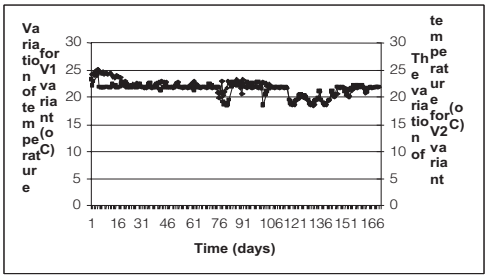

Figure 1. Variation of temperature for both experimental variants

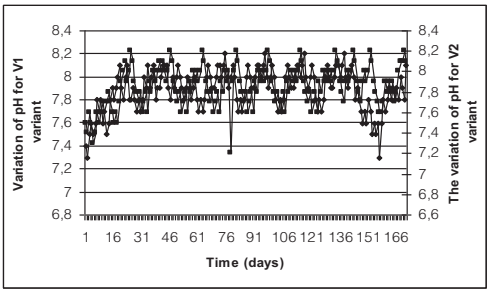

Figure 2. Variation of pH for both experimental variants

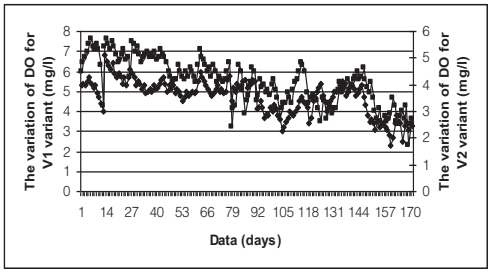

Figure 3. Variation of dissolved oxygen for both experimental variants

In Figure 4 is presented growth rate evolution for both experimental variants during this five experimental periods.

All experimental data regarding fish growth performance are resumed in the Table 2.

Regarding FCR, the best value (0.73) was registered in V1 variant during the second period, when the feeding level was 3% BW g/day.

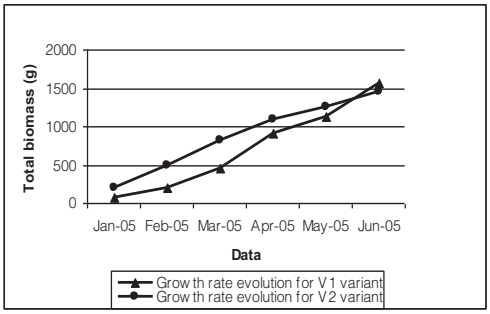

Figure 4. Growth rate evolution for both experimental variants

The feed conversion ratio is an indicator that is commonly used in all types of farming, as well as in the field of research. It can provide a good indication of how efficient a feed or a feeding strategy can be.

The maximum value of FCR (2.7) was register in V2 variant during the fourth period, when the feeding level was 1.5.

In terms of PER (protein efficiency ratio) the best result (2.63) was obtained for V1 variant in the second period.

Regarding individual gain, it can be observed that in the second period the fish from V2 variant showed the best growth of 45.29 g.

Different environmental factors play an important role in the growth and survival of fish. Temperature is probably the most important abiotic factor affecting fish life.

Feeding, digestion and food conversion activities are strongly influenced by ambient temperature, which ultimately reflects the variation in growth rate.

Temperature affects the rate of food digestion by influencing digestive enzyme activity.

The optimal temperature required for growth and other physiological activities varies greatly depending on the species.

Each species has an optimal growth temperature that is probably determined by the

optimum temperature for specific enzyme activity. Ornamental carp tolerates a fairly high temperature range between 3-32^{0}C.

At optimal temperatures, fish grow faster, efficiently convert food, and are relatively more resistant to disease (MasserM., 1999).

The variations in fish growth and survival could be due to rearing densities and not the water quality.

However, the mechanisms linking rearing density and growth are not fully understood, but it is generally accepted that when water quality is not affected by the increased number of fish per cubic meter and sufficient food is provided, differences in growth performances could be attributed to the onset of hierarchies and dominant relationship (Usandi et al., 2019).

Specific stocking density can have positive and negative effects on fish growth and survival, knowing the optimal stocking density is one of the basic factors influencing intensive fish culture. Fish stocking density is the most sensitive factor determining the productivity of a culture system as it affects growth rate, size variation and mortality (Kaiser et al., 1997).

An important factor to ensure good fish production is water pH.

The optimum pH range differs among species; however, the pH 6.5-9.0 range is generally accepted for fish culture (Heydarnejad, 2010).

Table 2. The growth parameters for both variants during five experimental periods

Parameters	First period		Second period		Third period		Fourth period		Fifth period	
	V_1	V_2	V_1	V_2	V_1	V_2	V_1	V_2	V_1	V_2
Biomass stocked (g)	75	205	198	488	461	810	912	1091	1135	1263
No. fish stocked	14	7	14	7	14	7	14	7	14	7
Mean initial fish weight (g/fish)	5.35	29.28	14.14	69.71	35.46	115	65.14	155.9	81	180.5
Biomass harvested (g)	198	488	461	810	912	1091	1135	1263	1570	1446
No. fish harvested	14	7	14	7	14	7	14	7	14	7
Survival (%)	100	100	100	100	100	100	100	100	100	100
Mean final fish weight (g/fish)	14.14	69.71	35.46	115	65.14	155.6	81	180.5	112.1	206.6
Individual fish gained (g)	8.79	40.43	21.32	45.29	29.68	40.85	15.86	24.65	31.14	26.07
Total biomass gained (g)	123	283	263	322	451	281	223	172	435	183
Feeding level (%)	5	3	3	2	3	2	2	1.5	1.5	1
Total feed given per aquarium (g)	168	320	192	320	476	612	522	464	510	360
FCR (g/g)	1.37	1.13	0.73	0.99	1.06	2.18	2.34	2.70	1.17	1.97
Growth rate (g/kg/d)	0.2	0.96	0.66	1.41	0.87	1.2	0.54	0.85	1.03	0.86
SGR (% BW/d)	2.3	2.07	2.65	1.56	2	0.88	0.75	0.51	1.06	0.43
Protein efficiency ratio (PER)	1.4	1.63	2.63	1.86	1.5	0.85	0.82	0.68	1.64	0.94

According to Al-Hafedh (1999) growth rate of fish increases with increase in the level of dietary protein till the optimum level is reached. Jana and Chakrabarti (1993) suggest that growth, reproductive potentials, and survival of each species are affected by the nutrient conditions of the culture media.

In the present study the experiment was conducted in closed condition in the aquaria that were different than any natural environment. Hashem et al. (1997) conducted an experiment on *Cyprinus carpio* using different food ingredient having a protein level of about 25% for all feed in floating pellets.

They concluded that optimum weight and length of 24.52 g and 8.07 cm, respectively for six months rearing against the initial weight and length were 5.94 g and 3.76 cm, respectively.

The variation may be due to experimental period because they conducted the experiment for six months and the present study was limited within 167 days (Mahfuj et al., 2012).

The level of dissolved oxygen in water is one of the most important parameters in determining its quality, because it indirectly indicates whether there is some kind of pollution.
The dissolved oxygen depends on water temperature, dissolved salts, atmospheric pressure (and therefore of altitude), the presence of reducing compounds, suspended matter, and living species.
In this experiment, at each aquarium level, air pressure was introduced through Elite 402 pumps to provide an adequate level of dissolved oxygen in the process water.

CONCLUSIONS

The experiments indicated that feeding fingerlings of koi carp with diets containing over 50% protein result in a higher growth response.
Utilizing a ratio which ranges between 2-3% BWg/d it was obtained 26-39% weight gain and FCR of 0.7-1.13.
During the period of experiment, it wasn't registered mortalities; the fish were adapted very well to the aquariums conditions.
In the ornamental carp nutrition it is recommended the colors mention through permanently utilization of natural sources or pellets richer with colors additives, which have a precise and predictable effect.

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ADVANCING SHELLFISH AQUACULTURE AS A SUSTAINABLE FOOD PROCUREMENT OPTION IN EMERGING BLACK SEA RIPARIAN COUNTRIES: ROMANIA COUNTRY REPORT

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Abstract

Aquaculture offers great potential for providing sustainable sources of food, thus playing a key role towards achieving food security and nutrition, employment and economic development. Shellfish (mussel) aquaculture offers a great development opportunity or Black Sea riparian countries, however, significant focus should be put on zoo-sanitary conditions and public health.In some countries bordering the Black Sea, mussel culture is relatively well represented, having an obvious increasing development over the last two decades. However, given that mussel culture is little developed in Romania, the promotion of scientific, technical and technological bases for this activity is absolutely necessary. In the frame of the NIMRD - GFCM collaboration, in 2017 the Shellfish Aquaculture Demonstrative Center in Constanta (S-ADC) was established at NIMRD's headquarters. The demonstration module for mussel production form the basis of training activities in the field of mitiliculture and covers all aspects of the production cycle: biology and ecology of Mytilus gallo provincialis; providing brood and collecting larvae from the natural environment; design and construction of the long-line system; mussel growth and handling technologies; mussel processing and purification technologies; production management systems (production costs, market analysis); training in methodological and practical aspects of the sanitary-veterinary classification of mollusks for domestic consumption/export.

Key words *:mariculture, mussels, long-line, food safety, market.*

INTRODUCTION

Aquaculture, the farming of aquatic organisms including fish, mollusks, crustaceans and aquatic plants, offers great potential for providing sustainable sources of food, thus playing a key role towards achieving food security and nutrition, employment and economic development at regional Black Sea level. So far, mariculture concerns around the Black Sea have been focusing mainly on fin fish species of high economic value, such as turbot and sturgeons (Niță and Nenciu, 2017; Niță et al., 2018c).

In recent years, however, other living resources have started to be targeted for human consumption, such as mussels and the rapa whelk (*Rapana venosa*).

Mytilus gallo provincialis (Lamark, 1819) is the marine mollusk (mussel) with the highest ecological and economic importance in the Black Sea ecosystem (Rosioru et al., 2012).

Due to their organoleptic qualities and the high content of biochemical compounds with nutritional value (amino acids, vitamins, enzymes, proteins, carbohydrates),many species of mollusks are industrially harvested or grown in specialized aquaculture farms (Niță et al., 2018b).

The world production of bivalves has increased over the last 50 years, from 0.9 million tons in 1950 to over 22 million tons in 2010. The increase is largely due to the share of aquaculture, which grew rapidly in the 1990s. World production of farmed bivalves increased from 3.3 million tons in 1990 to nearly 20 million tons in 2010, with an annual average increase of 11% (Zaharia et al., 2017).

Mussel culture has been known since the last century (Ursache et al., 2013). However, the

development of technologies based on scientific observations was possible only a short time after advancing the knowledge of the physiology and ecology of mollusks (Ursache, 2014).
Currently, however, shellfish aquaculture is not developed to its full potential in the Black Sea region (except for Bulgaria) due to, on the one hand, environmental constraints, and, on the other hand, an unclear legislative framework (Niță et al., 2018).
In some countries bordering the Black Sea, mussel culture is relatively well represented, having an obvious increasing development over the last two decades; for example, Ukraine produces about 400 tons per year, while Bulgaria is approaching 4,000 t/year (Zaharia et al., 2017). However, given that mussel culture is little developed in Romania, the promotion of scientific, technical and technological bases for this activity is absolutely necessary.
In Romania, bivalves are not considered a common food, but in the last decade there has been a slight increase in the consumption of mussels and oysters in public nutrition.
The increase in the demand for bivalves for food consumption in recent years has encouraged the harvesting of mussels from natural populations, growing mussels on floating installations (long-line systems) and acclimatization of high-value bivalves - the Japanese oyster, for instance (Zaharia et al., 2017).
The annual quantity of mussels harvested in the Romanian Black Sea coast area amounts to approx. 15 tons (estimated value), and the only existing mariculture farm (with interrupted activity), SC MARICULTURA SRL, can produce annually approx. 5 tons of cultured mussels (Zaharia et al., 2017).
Significant focus should be put on zoo-sanitary conditions and public health of mussel consumption (Nicolae et al., 2015).The European sanitary control of live bivalve mollusks is historically based on the Council Directive (EC) No. 492/91, which had previously set the hygiene rules for the production and marketing of live bivalve mollusks. Currently, food safety monitoring of shellfish production areas in the European Community is regulated by the "Hygiene Package" which entered into force on 1 January 2006, repealing the Directive (EC) No. 492/91. This legislative package includes the Regulations (EC) No. 852/2004 and 853/2004, which are addressed to industry professionals, and Regulations No. 854/2004 and 882/2004, which are addressed to competent authorities, responsible of carrying out official sanitary controls. End product standards required for bivalve mollusks are regulated by Regulations (EC) No. 854/2004 and 2073/2005.
Thus, there is an extensive legislative framework at EU level, however it is poorly implemented (except for Bulgaria, where sanitary classification of shellfish waters was made by private operators, in order to be able to export their products on the Community markets) (Nicolae et al., 2018).

MATERIALS AND METHODS

The methodology used in order to draw-up an accurate overview of the mussel culture in Romania was applying modern analysis tools during the *"Demonstrative Training on Mussel Farming"*, carried-out during 17-28 September 2018 in Constanta, Romania. This training was organized in the frame of the Shellfish Aquaculture Demonstrative Center, operating within NIMRD "Grigore Antipa" under coordination of the General Fisheries Commission for the Mediterranean/Working Group for the Black Sea (Niță et al., 2018b).

Figure 1. S.W.O.T. and P.E.S.T.E.L. analyses during the "Demonstrative Training on Mussel Farming", 17-28 September 2018, Constanta (Photo by M.I. Nenciu)

The course involved trainees from Bulgaria, Georgia, Turkey, Ukraine and Romania, from research organizations, authorities and the

business sector. Representatives from the sanitary-veterinary authorities also attended and were engaged in discussions regarding certification aspects of shellfish waters. The aim of the training was to enhance the theoretical and practical knowledge, focusing on legal and administrative issues.

During this training, S.W.O.T. and P.E.S.T.E.L. analyses were performed, aiming at obtaining the inputs and feedback of trainees (Figure 1).

S.W.O.T. (Strengths, Weakness, Opportunities and Threats) analysis is a methodological approach to problem formulation and the mapping of possible management strategy solutions (Rauch, 2007). As a knowledge-based tool, it has been recommended for strategic planning in small and medium-sized enterprises (Houben et al., 1999), as well as for a whole-industry sectors such as fisheries (Stead, 2005) and aquaculture (Theodorou et al., 2015; Theodorou and Tsovenis, 2018). Strengths and weakness are frequently internally-related, while opportunities and threats commonly focus on the external environment.

A P.E.S.T.E.L. analysis is an acronym for a tool used to identify the macro (external) forces acting on an organization/activity. The letters stand for Political, Economic, Social, Technological, Environmental and Legal. The P.E.S.T.E.L. framework is an analytical tool used to identify key drivers of change in the strategic environment (Narayan and Fahey, 2001).

RESULTS AND DISCUSSIONS

The results collected were organized in the following tables (Table 1 for S.W.O.T. and Table 2 for P.E.S.T.E.L.), thus summarizing the issues Romanian mussel aquaculture is facing.

Table 1. S.W.O.T. analysis for Romanian mussel aquaculture

STRENGTHS (Internal)	OPPORTUNITIES (External)
- Extensive scientific knowledge on mariculture: NIMRD "Grigore Antipa"'s expertise (qualified staff, existing infrastructure); - Existing and future partnerships between fishery/aquaculture stakeholders and other sectors of the public and private sector (Fisheries Local Action Groups - FLAGs, fisheries associations/organizations); - Previous small-scale experience (private operator who already implemented the long-line culture system); - Mussels have a short life cycle and require no added food; - Closeness to the EU markets and possibility for exports.	- Improving and equipping NIMRD's Laboratory for aquaculture microbiology and its subsequent accreditation, in order to the classification of mussel harvesting areas (Class A, Class B, Class C); - Support in the implementation of Allocated Zones for Aquaculture(AZA) in Romania with direct implications in mussel culture; - Develop - European funding opportunities (developing) projects in the field of shellfish mariculture.
WEAKNESSESS (Internal)	**THREATS (External)**
- Constraints related to environmental factors (climate, salinity, exposed coastline, no sheltered areas), which cannot be controlled; - Major coordination problems between institutions (Sanitary Veterinary Directorate, Public Health Directorate, Romanian Waters Administration).	- Difficulties in integrating mariculture with other uses of the marine and coastal environment (transport, tourism, etc.) = conflicts on maritime space use; - Potential harmful algal blooms (HAB) and bacterial outbreaks (food safety).

Table 2. P.E.S.T.E.L. analysis for Romanian mussel aquaculture

POLITICAL	ECONOMIC	SOCIAL	TECHNOLOGICAL	ENVIRONMENTAL	LEGAL
- there is willingness for the development of aquaculture/ mariculture.	- there is demand/ market for mussels.	- this activity is well accepted by the community.	- there is theoretical documentation for the development of the sector; - lack of practical expertise.	- marine shellfish aquaculture is beneficial for the environment (biofilters).	- major coordination problems between institutions (Sanitary Veterinary Directorate, Public Health Directorate, Romanian Waters Administration).

The S.W.O.T. analysis revealed that the main strengths for developing shellfish aquaculture in Romania are represented by the extensive scientific knowledge in this field, through the expertise and existing infrastructure of NIMRD ”Grigore Antipa”, as well as the practical experience of a private operator who has already implemented the long-line system (Figure2). Moreover, the willingness and cooperation between the public and private sector are favourable factors, fostering the development of this activity in the area. From the economic point of view, the fact that mussels have a short life cycle and require no added food is very attractive for potential investors, as well as the closeness to the EU markets and possibility for exports.

The weaknesses and constraints limiting marine aquaculture in Romania are mainly related to environmental factors. Water temperature has wide variations from one season to another and, in shallow areas, negative temperatures are recorded in winter time, while in summer water temperature can overcome 28°C for quite long periods of time. Salinity is variable, in many areas, mainly in those subjected to river input, salinity may drop considerably during certain periods of the year, which can be fatal for mussels (Figure 2).

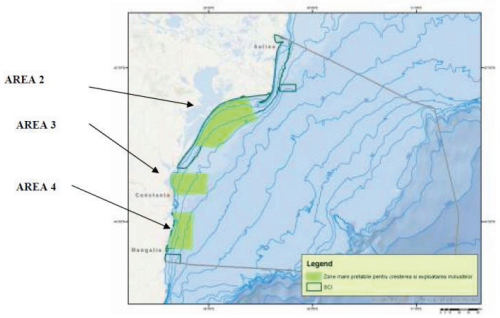

Figure 2.Map with the designated marine areasfor growth and economic exploitation of marine mollusks in Romania

This is the reason why, in 2015, by ministerial order, one of the four initially designated areas for exploitation of shellfish at the Romanian coast (according to Directive 2006/113 EC), was removed due to its closeness to the Danube mouths (Sulina-Sf. Gheorghe, with salinity values below the minimum recommended threshold of 12‰) (M.O. no. 983/2015). Severe storms must also be considered (high waves and strong currents), as well as seabed topography, when setting-up long-line installation (Figure 3). The absence in many areas of the Black Sea coast of the sheltered areas, suitable for aquaculture, is another limiting factor.

Concerning biotic factors (plankton, fouling, predators etc.), they are rather favorable to the development of aquaculture in the region; fouling, however, may cause problems if regular maintenance works on installations are not performed.

One key aspect concerning mussel aquaculture is the zoo-sanitary and food safety issue. The concept of food hygiene, according to FAO/WHO(Niță et al., 2018a), includes precautions and measures which, if adopted in the right way, during production, handling, storage and distribution of food, lead as a result to a salubrious and marketable product.

The production chain of bivalve molluscs begins with breeding or collection of different species of mollusks in the production areas.

Figure 3. Long-line system suitable for Black Sea mussel culture (Photo by M. Crivăț, MARICULTURA S.R.L.)

EU regulations exist to control the public health risks associated with consumption of microbiologically contaminated shellfish. The risk of contamination of shellfish with bacterial and viral pathogens is evaluated by reference to

(i) the sources and types of fecal contamination (human and animal) in the vicinity of the shellfish production areas and (ii) the results obtained, based on the indicator bacteria *Escherichia coli*, from samples taken in these areas. Areas are classified following a full assessment of this risk and the classification given to an area determines whether shellfish harvested in that area require post-processing treatment and, where appropriate, the level of such treatment (Nițǎ et al., 2018a).

As previously mentioned, there is an extensive legislative framework at EU level, however in Romania it is poorly implemented due to major coordination problems between various institutions, concerning both zoo-sanitary classification and water concession.

In this context, there is urgent need to provide an updated scientific based legal and procedural framework for developing shellfish aquaculture in Romania, as an opportunity for development. This can be done by equipping the laboratories in compliance with the required framework for shellfish quality and food safety (Regulation (EC) No 854/2004),skill-development and capacity building in order to perform high accuracy analyses, and developing a shellfish water and meat quality monitoring protocol for the implementation of classification and monitoring programmes of bivalve harvesting and culture areas.

Moreover, it must be taken into account that aquaculture mostly takes place in common spaces, where different uses and users co-exist, if not always amicably, this being one of the threats identified. Further effort is required to enhance multiple use, in order to better understand co-location and co-existence perceptions with tourism and other marine uses. There is, therefore, a need for active aquaculture spatial planning and area management that accounts for the range of uses of marine space, and human interactions in general, focused on a core requirement to increase space for aquaculture. In the Black Sea area there has been an expansion of aquaculture in recent years, but comprehensive regulation has been slower to develop. The site selection process, as well as the allocation of zones for aquaculture is a relatively recent focus of the General Fisheries Commission for the Mediterranean (GFCM), which in 2012 adopted a resolution (i.e. Res. GFCM/36/2012/1) that provides guidelines on allocated zones for aquaculture (AZA). It is not a mandatory regulation, but the resolution acts as a basic framework to guide GFCM contracting parties (Romania included) in the establishment of a spatial management of aquaculture, to avoid any potential contamination and/or conflicts with other uses of the maritime space (Nițǎ et al., 2018a).

Potential harmful algal blooms (HAB) and bacterial outbreaks, with serious consequences for food safety, are also to be considered as a significant external threat to developing mussel aquaculture.

The P.E.S.T.E.L. model of analysis showed that, from the political point of view, in Romania there is willingness to support and promote the development of aquaculture, in general, and mariculture in particular, as an alternative for providing nutritious food. Moreover, economically, in Romania the market demand for mussels has increased in recent years, as many restaurants/catering facilities have included them in their menu and they are promoted as a healthy dish. This leads to a rather good social acceptance of the product (Figure 4).

Figure 4. Mussels are becoming more and more socially accepted by Romanian consumers (Photo by M.I. Nenciu)

From the technological perspective, it was acknowledged that there is theoretical documentation for the development of the sector, yet a lack of practical expertise, with only one active operator, cannot guarantee its success of implementation.

The environmental benefits of bivalve cultures are scientifically demonstrated: by their filter-

feeding technique, mussels contribute to the natural depuration of marine areas, acting as bio-filters and clearly improving water quality (Ursache et al., 2013).
Legally, the most significant aspect identified is the lack of coordination between institutions (Sanitary Veterinary Directorate, Public Health Directorate, Romanian Waters Administration).

CONCLUSIONS

At a first glance, the Romanian Black Sea coast does not seem suitable for developing shellfish aquaculture activities, mostly due to the environmental constraints (variable salinity, storms, absence of sheltered areas etc.) and legislative/administrative gaps, which may prevent investors from marketing their production. However, the establishment of the Shellfish Aquaculture Demonstrative Center in Constanta (S-ADC), in the frame of the NIMRD - GFCM collaboration, will lead to the promotion of scientific, technical and technological bases for this activity, by providing focused training for targeted beneficiaries: on the one hand, national and local management authorities/administration involved in aquaculture planning, management, sanitary control, and, on the other hand, representatives of the private sector, especially the small-scale producers with limited investment capacity, as well as potential and existing investors.

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AGE AT FIRST SEXUAL MATURITY OF *TRACHURUS MEDITERRANEUS* (STEINDACHNER, 1868) FROM ROMANIAN BLACK SEA WATERS, INDICATOR OF GOOD STATUS OF THE POPULATION

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Abstract

The horse mackerel is a pelagic marine species of commercial interest at the Romanian Black Sea coast. The present study presents the analysis of the age of horse mackerels at the first sexual maturity. For the determination of age, the method used was the interpretation of the otoliths, these are bone structures located in the inner ear. Age of sexual maturity of horse mackerel is 1-2 years. As a general rule, a fish population is considered to be in a good condition when the age at first sexual maturation exceeds 30% of the individuals present in the catch. The analyzed periods were 2012-2015 and 2016-2017. In the period 2012-2015, the percentage of the age of the first sexual maturation exceeded the 30% threshold compared to the 2016-2017 period when a decrease was recorded. The reduction is mostly due to fishing pressure on these species. In conclusion, it can be said that the horse mackerel population on the Romanian Black Sea coast is not in good condition status, and measures are needed to conserve the population.

Key words*: age, biodiversity, conservation, otoliths, sexual maturation.*

INTRODUCTION

The Black Sea ichtyofauna has undergone major changes over the last decades, both in the qualitative and quantitative structure that's why the population parameters are very important to be analyzed (Nicolae et al., 2018).

Trachurus mediterraneus - horse mackerel is a pelagic, migratory species living in schools. It is spread in the Black Sea, the Azov Sea (except its fresh water parts), and the Marmara Sea (especially in winter) and also in the Eastern Atlantic. In the Black Sea it is spread mostly in the northern part and wintering in the eastern and southern areas at depths of 80-100 m (Radu and Radu, 2008).

The good environmental status (GES) of the Marine Strategy Framework Directive (MSFD) is achieved when the number, demographic characteristics (fertility, mortality) and the state of health of naturally occurring populations allow their maintenance and survivor on a long term, depending on the existing natural environmental conditions (Borges et al., 2010).

Within the ichtyofauna, the demographic characteristics of populations of fish species (eg. size and age structure, sex ratio, fecundity and survival rates) are healthy population indicators that are not adversely affected by anthropogenic pressures (Simeanu et al., 2015).

Thus, the aim of the study is to highlight by the analysis of the age at the first sexual maturity, the state of the horse mackerel population at the Romanian Black Sea coast.

Reproduction of horse mackerel takes place during the summer, from May to August, with maximum intensity in July when approaching the shore.

They spawn gradually and sexual maturity is reached in males at 1 year old and in females at 1 or 2 years old (Radu and Radu, 2008).

Age determination in fish is an important element contributing to the study of dynamics population of a species.

Despite the enormous importance and value, fisheries resources suffer the combined effect of overexploitation and environmental degradation (Nicolae et al., 2011).

MATERIALS AND METHODS

For the purpose of this study, samples were collected from the stationary (pound net) installed at fishing points along the Black Sea coastal area between Midia and VamaVeche, in the period 2012-2017 (Figure 1).

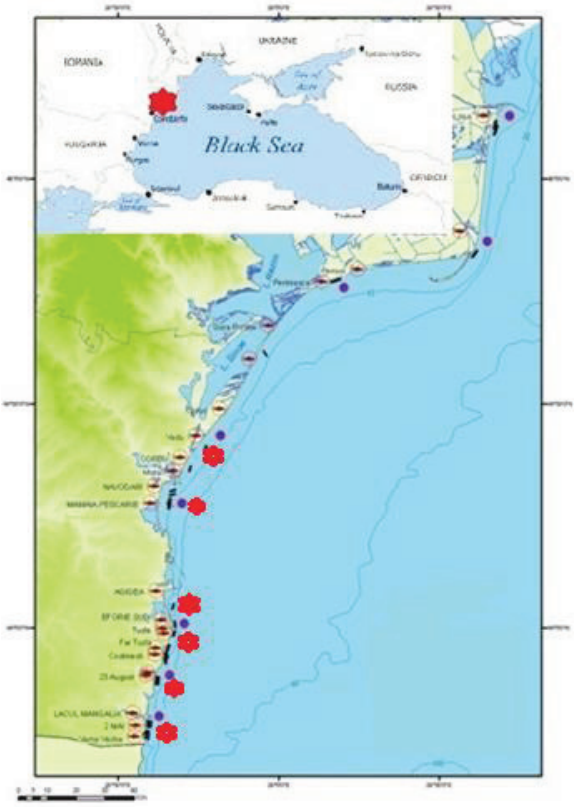

Figure 1. Map with sampling stations

After collecting, the samples were analyzed in the laboratory where measurements of length, weight, sexual maturity and age determination by otoliths analyses were performed (Figures 2, 3, 4).

Figure 2. Horse mackerel capture (Original photo)

Figure 3. Horse mackerel analyzed in the laboratory (Original photo)

Sexual maturity in fish has a great practical importance in the analysis of many population parameters. In general, fish are considered to be

mature when they reach the middle of their maximum size (Holden and Raitt, 1974).
Also, determining fish age is one of the most important elements in the study of population dynamics, being the basis for the study of growth, mortality, recruitment and other basic population parameters (NIMRD, 2018).

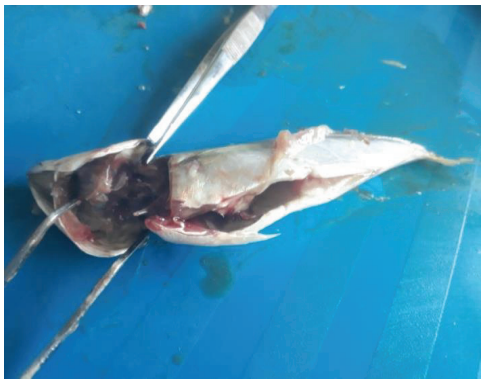

Figure 4. Otholithsprelevation of Horse mackerel analyzed in the laboratory (Original photo)

Determination of age in horse mackerel was done by identifying the growth rings on the otoliths surface using a binocular microscope (Figure 5).

Figure 5. Sampling and analysis of otoliths in horse mackerel (Original photo)

The otoliths are three-dimensional structures, concentric areas appear on their surface and depending on the organic matter deposited on each area, and the circles can be opaque or transparent.
Thus, an annual growth ring is considered to consist of an opaque area followed by a transparent one (Galatchi et al., 2017).

RESULTS AND DISCUSSIONS

During the analyzed period, were identified horse mackerel individuals with a length between 8 - 15.5 cm and with an average of 12.6 ± 0.5 cm (Figure 6).

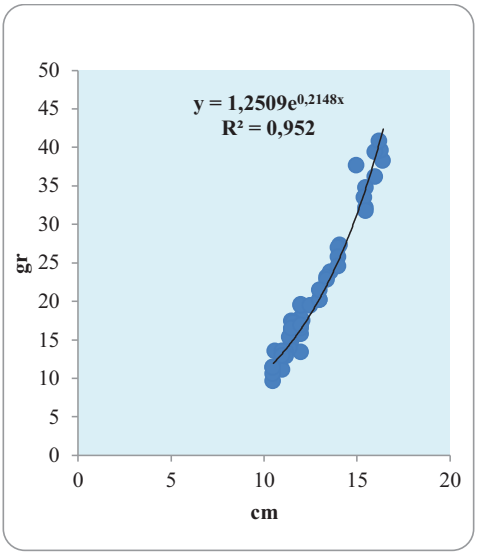

Figure 6. Length - weight relation of horse mackerel, analyzed in 2017

The length - weight correlation is positive, so the individuals analyzed have evolved both in length and in weight, with a higher increase in length in the first part of their life.
However, individuals were smaller than those identified on the Bulgarian Black Sea coast, up to 19 cm (Yankova, 2013).
In terms of weight, specimens from 9.62 g to 39.45 g have been identified with an average value of 19.12 g ± 0.5; a situation similar to that identified by weight.
Also, studies conducted for horse mackerel taken from the Turkish Black Sea region revealed a spectrum of length between 6.9 cm and 19.2 cm, weighing between 3.32 g and 59.98 g (Aydin and Karadurmuş, 2012).
Thus, horse mackerel specimens from the Romanian Black Sea coast have a growth rate different from those in other areas of the sea, most likely due to different living conditions and availability of food.
In May-September 2012-2017, fresh material was also analyzed in the ichthyology laboratory for determination of maturation rates.

In most samples analyzed, the females are predominated (Figure 7).

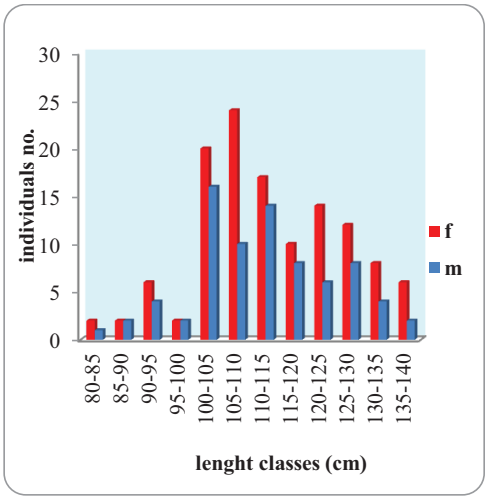

Figure 7. Distribution by length and sex classes for horse mackerel, 2017

Regarding maturation rates, young specimens predominated in the first part (May), followed in June by the predominance of specimens with maturation classes III and IV, which are in full reproductive process (Figure 8).

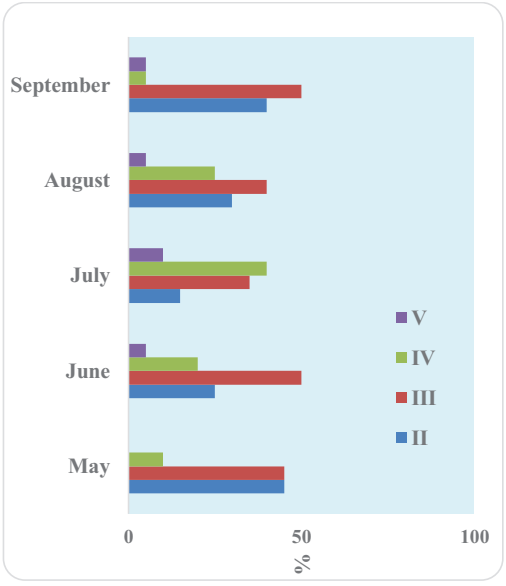

Figure 8. The distribution of degrees of maturation on months at the horse mackerel

Horse mackerel reproduction takes place during the summer and is located mainly in the area of shoreline more likely due to abundant food resources (Radu and Radu, 2008).

Regarding the age of horse mackerel, prevailed the individuals of 2^+ and 3^+ years in the first part of the analyzed period and then it was observed an increase in the percentage of specimens of 1^+ (Figure 9).

Specimens of 4^+ and 5^+ years have been underrepresented where we conclude a high pressure from fishing activities.

In addition, studies on the age of the horse mackerel caught at Bulgarian Black Sea coast have revealed a predominance of over 20% of individuals at the first sexual maturity stage (Yankova et. al, 2010).

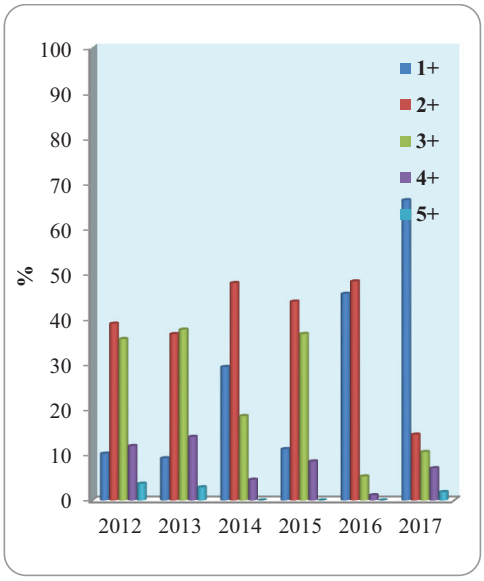

Figure 9. Distribution of age groups

Regarding the individuals analyzed in present study, has been observed in the period 2012-2015 that the percentage of the horse mackerel age at first sexual maturity stage has exceeded the threshold value of 30% but in the period 2016-2017 was registered a decrease; most likely as a result of pressure by fishing (Figure 10).

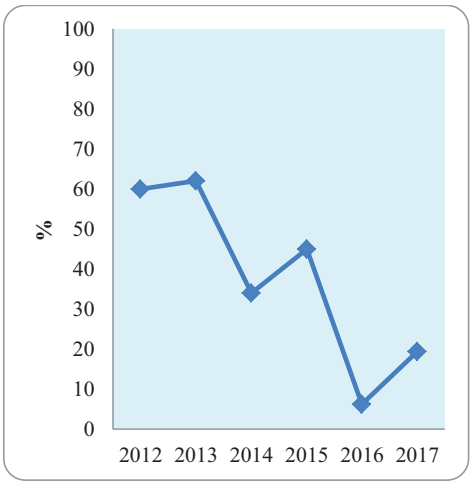

Figure 10. The proportion of the catch over the age at first maturity stage for horse mackerel

CONCLUSIONS

In the analyzed period were identified individuals of horse mackerel with a total length between 8-15.5 cm with an average of 12.6 cm ± 0.5 cm and in terms of weight, have been identified specimens between 9.62 to 39.45 g, with a mean value of 19.12 g ± 0.5. Length-weight relationship was a positive one.

Concerning maturation rates, the young specimens predominated in the first part (May) for the analyzed period, with individuals of classes III and IV, which are in full reproductive process, prevailing in June.

Analysis of the age of horse mackerel, it's revealed a dominance of specimens of 2^+ and 3^+ years old in the early part of studied period and then we observed an increase in the percentage of specimens of 1^+ years old, this situation corresponds to a rejuvenation of population.

Specimens of 4^+ and 5^+ years have been underrepresented where we conclude a high pressure made by fishing activities.

Regarding the age at first sexual maturity, it was observed that during the period 2012-2015 the percentage of the age of the female at the first sexual maturity exceeded the 30% threshold, but there was a decrease between 2016-2017; most likely as a result of fishing pressure on the species. It is therefore necessary to continue the research to highlight the state of health of the horse mackerel population at the Romanian Black Sea coast.

ACKNOWLEDGEMENTS

This research work was a part from the PhD thesis elaboration „The characterization of horse mackerel from the Black Sea coastal area" and was carried out with the support of Faculty of Engineering and Management of Animal Production, University of Agronomic Sciences and Veterinary Medicine of Bucharest.

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DETERMINATION OF TOTAL ORGANIC CARBON, TOTAL NITROGEN, AND TOTAL PHOSPHORUS FROM SOIL SEDIMENTS AND MACROPHYTES FROM HORIA LAKE, TULCEA

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Abstract

Nutrients play a significant role for the environmental state of lakes, mainly because the primary production of lakes is strongly influenced by nutrient availability. From all these nutrients special attention is accorded to the total organic carbon (TOC), total nitrogen (TN) and the total phosphorus (TP). In this context, the aim of this paper was to determine the TOC, TN,and TP from the sediment and macrophytes samples from Horia Lake, Tulcea County, Romania. The lake is located in the area of Horia, being limited to the north, west, and south by the agricultural field. The slope of the ground allows the accumulation of the waters from the versants adjacent to the lake, with suspension contributions, fertilizers,and herbicides used for the adjacent corn crop. All the samples were collected in the autumn season (November 2016), from six stations of the lake. The results revealed significant differences ($p<0.05$) in the concentration of the TOC, TN, and the TP. Comparing the means values of these nutrients from macrophytes and sediments, it can be concluded that the higher values from macrophytes are the result of the assimilation capacity of these nutrients.

Key words*: Horia Lake, macrophytes, sediments, TN, TOC, TP.*

INTRODUCTION

Determination of total organic carbon (TOC) total nitrogen (TN), and total phosphorus content (TP), from sediments, have big importance for the environmental status and estimation of terrestrial and aquatic ecosystems. Mainly these nutrients derived by decomposition of the plants and animals, plankton or anthropogenic sources such as chemical contaminants, fertilizers or organic-rich waste (Pavlos et al., 2015).

Aquatic macrophytes are important component ecological systemsin lakes and can be involved in biogeochemical cycling of nutrients, such as carbon (C), nitrogen (N) and phosphorus (P) (Sasha et al., 2016).

They can offer housing for zooplankton and young fish, reduce nutrient levels by assimilation and re-synthesis of N, P, serve as a habitat for macro-invertebrates (Jeppesen, 1998; Scheffer, 1998).

There are various environmental variables which influence the growth, distribution, and abundance of aquatic macrophytes, such as water transparency, nutrient concentrations (Akasaka, 2010; Alahuhta, 2012), physical and chemical characteristics of sediments, sediment organic matter, particle size (Lougheed et al., 2001), and sediment texture (Mikulyuk et al., 2011).

The aim of this study was to investigate nutrients (TOC, TN, and TP) and macrophyte interactions for Horia Lake, from Tulcea County, Romania.

MATERIALS AND METHODS

Horia Lake is located at 2 km northwest of Horia village, a 150-meter-long dike stops the waters of Taița River in a reservoir lake (Horia Lake).

The lake is limited to north-west and south by agricultural field, and the eastern limit is represented by the inter county road 222 A. The lake has a surface around 230 ha.

Station location. For this study, the sediments and macrophytes samples were collected from six stations (established in the symmetry axle of the lake) using Marinescu grab (Figure 1).

Figure 1. Study area location and sampling station distribution

Methodology. From each station were collected three sediments samples. A total number of 18 sediments samples were analyzed for their TOC, TN and TP content and all the analysis were performed in duplicate.

Sediments samples were pre-treated according to the ISO 11464 and 11465. Its principle is drying soil samples to constant mass at 105°C and using the difference in mass of an amount of soil before and after the drying procedure to calculate the dry matter and water contents on a mass basis. After that, a representative sub-sample has to be milled until it passes a 250 µm aperture sieve.

From these stations, also were collected macrophytes samples. In order to establish the qualitative and quantitative structure of the macrophytes, the whole plant is harvested by pulling out and then insert into a plastic bag filled with water to be transferred to the laboratory. The plants were washed with water in the laboratory to remove adhered periphyton and organic and inorganic particulate matter. After that, each plants species was systematically ranked by gender and family respecting the current phylogenetic classification of plants and then dried at 105°C till constant weight.

Then, the plants were ground and sieved through a 0.5 mm mesh net and stored in plastic flasks in order to determine the TN, TP and TOC content.

All the samples were collected in the autumn season (November, 2016).

Determination of the TN, TOC, and TP was made in the Nutrition Laboratory of Romanian Centre for Modelling Recirculating Aquaculture Systems (MoRAS), University Dunărea de Jos, Galați, Romania.

The principle for the TOC method. The total nitrogen determination was made using PrimacsSLCAnalyzer from Skalar Company. First, it was determinate the Total Carbon (TC) by combustion at 105°C. In the presence of the catalyst cobalt oxide, all organically and inorganically bound carbon is oxidized or decomposed in the flow of pure oxygen into the gaseous carbon dioxide.

The flow of oxygen transports the carbon dioxide to the IR detector and the carbon dioxide is measured at 4.2 µm by IR detector and recalculated to the total carbon content according to the calibration by the standards. Then it was determinate the Inorganic Carbon (IC) at 150°C. The sample is added in a test tube in which oxygen will be purged before analysis begins, in order to remove CO_2. Then orthophosphoric acid is added to the sample in order to decompose the inorganically bound carbon to the gaseous carbon dioxide. The flow of oxygen purges the carbon dioxide from the liquid into the IR detector to be measured again. The concentration of TOC is determined: TOC=TC-IC.

The principle for the TN: The total nitrogen determination was made using Primacs SNC Analyzer from Skalar Company. The equipment uses the Dumas principle, which is dependent on the quantitative conversion of the sample into distinct gaseous species at 1100 °C in the presence of Oxygen.
In the combustion phase, the total quantity of nitrogen from the sample is converted to nitrogen oxides, which are subsequently condensed to nitrogen gas and measured by a Thermal Conductivity Detection (TCD) and to remove interferences a background correction is performed by measuring the He gas.
Total phosphorus. For phosphorus determination, it was use the spectrophotometric molybdenum blue method which is a well-established method (Murphy and Riley, 1962). Shortly, this method involves the formation of molybdophosphoric acid from orthophosphate and an excess of molybdate in acidic solution followed by reduction to give molybdenum blue. The absorbance of thus produced molybdenum blue was measured spectrophotometrically at a 660 nm wavelength at SPECORD 210 from Analytikjena.
Data analysis. Data were analyzed using ANOVA test and if significant differences were found, a post hoc Duncan test was applied. The differencewas found significant at $p<0.05$. All the statistical analysis was performed using SPSS 21 for Windows.

RESULTS AND DISCUSSIONS

In the study period, (November, 2016), the presence of macrophytes was reduced. Five plant species were identified in the shore area of Horia Lake. The present species in the studied stations are presented in Table 1.
All identified species by macrophytes belong to the same classes - Liliopsida, but different families: Poaceae, Typhaceae, Butomaceae, Polygonaceae, and Potamogetonaceae and different genres: Phragmites, Sparganium, Butomus, Polygonum, and Potamogeton (Gurău, 2007; Antonescu, 1951). Macrophytes are poorly represented in all analyzed stations, with few exceptions.
In station S1, S2, the density of the species *Phragmites communis* is high (Figure 2), compared with the S3 station was the density of the species was lower. In the case of other species, the density of square meter is one-two species.

Figure 2. *Phragmites communis* from Horia Lake

In Table 2 are presented the results of TN, TOC, and TP from macrophytes samples, and in Figures 3, 4 and 5 are presented the minimum, average and maximum values obtained for all the macrophytes found in the lake.

Tabelul 1. The presence of macrophyte species on the studied stations

Stations / Taxons	*Phragmites communis*	*Sparganium sp.*	*Butomus umbrellatus*	*Polygonum amphibium*	*Potamogeton pectinatus*
S1	+++	-	-	-	++
S2	+++	-	+	-	-
S3	++	-	-	-	-
S4	-	+	-	-	-
S5	+	-	-	+	-
S6	-	-	-	+	-

+++ = frequent form; + + = sporadic form; + rare form; absence form

Table 2. TOC, TN, and TP from macrophytes samples from all the six stations

Para-meter	*Phragmitescommunis (Mean values of S1,S2and S5)*			*Butomus umbrellatus*			*Polygonum amphibium*		*Potamogeton pectinatus*	*Sparganium sp.*
	Leaves	Stem	Root	Leaves	Stem	Root	Leaves	Stem	Leaves+stem	Leaves
TP (%)	71.62±0.10	45.58±0.05	5.11±0.05	34.67±0.04	6.67±0.03	1.10±0.04	2±0.03	35.48±0.05	11.63±0.08	12.63±0.05
TOC %)	16.20±0.06	11.09±0.04	8.59±0.10	9.07±0.05	7.34±0.04	8.37±0.03	7.48±0.04	8.20±0.04	2.06±0.04	6.20±0.04
TN (%)	1.53±0.04	0.31±0.03	0.28±0.04	2.28±0.03	1.13±0.12	1.97±0.04	3.65±0.22	0.94±0.04	1.82±0.05	0.87±0.04

*The values are expressed as mean and standard deviation for the three samples/station (each sample was made in duplicate)

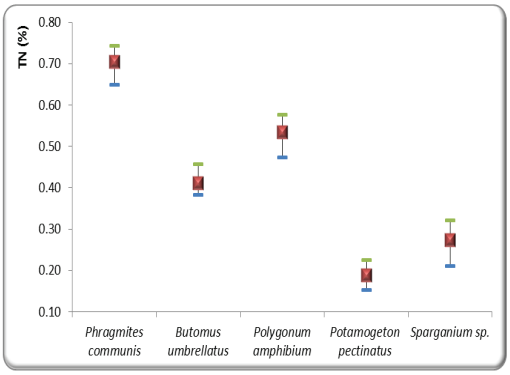

Figure 3. The minimum, average and maximum values of TN for macrophytes found in the Horia Lake

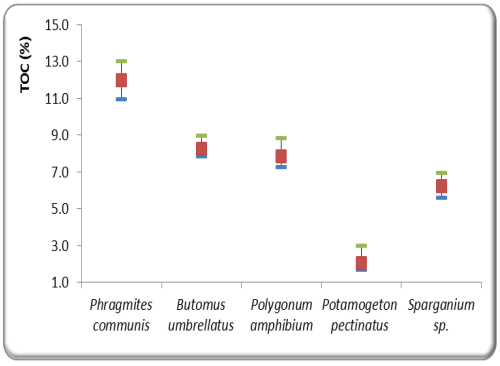

Figure 4. The minimum, average and maximum values of TOC for macrophytes found in the Horia Lake

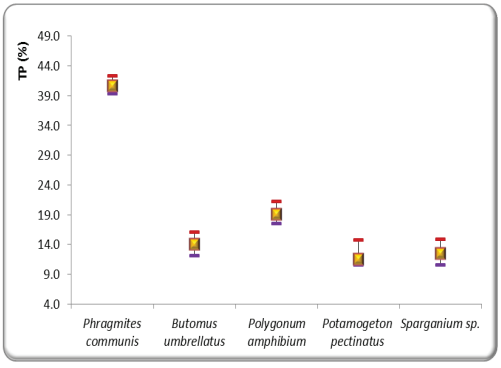

Figure 5. The minimum, average and maximum values of TP for macrophytes found in the Horia Lake

From Table 2, it can be observed that in the case of *Phragmites communis* and *Butomus umbrellatus* the highest nutrients retention was found in leaves, followed by stem and roots.

In the case of *Polygonum amphibium* the highest nutrients retention was found in the stem, an exception was in the case of TN were the highest retention was registered in leaves.

Potamogeton pectinatus is a long plant (over 1 m) and branched with narrow leaves (2-3 mm), (Sârbu et al., 2005) and for this reason,it was only possible to harvest the leaves and steam. Comparing the nutrients assimilation in *Potamogeton pectinatus* and *Sparganium sp.* with the aquatic plants from the lake, it was observed the lowest retention.

Comparing the minimum, means and maximum values of TN, TOC and TP contents in macrophytes found in Horia Lake, the values were higher in *Phragmites communis,* followed by *Polygonum amphibium*, *Butomus umbrellatus,* and *Sparganium sp.* The lowest contents of TN, TOC, and TP was found in *Potamogeton pectinatus*, which is an emersedaquatic plant (Figures 3, 4 and 5).

In Table 3 are presented the results of TP, TOC and TN, sediments samples. TP values showed significant differences (ANOVA, $p<0.05$) between the six stations. The TP values from S1 were significantly ($p>0.05$) higher than those from S6, followed by S5 and S2, while the lowest values were registered in S3 and S4.

Regarding the TOC values, the post hoc analysis Duncan divided the obtained values into three sets of data: the lowest values were registered in the S1 and S2, followed by the S4, while the highest values were recorded in the stations 3, 5 and 6.

Also, the TN content registered significant differences (ANOVA, $p<0.05$). The highest TN value was recorded, in S3, followed by the S4 and S1. No significant differences were found between the S2 and S5 stations. The lowest TN content was recorded in S6 station.

Table 3. The TP, TOC and TN content in sediments from the six stations of Horia Lake, Tulcea County

Parameter	S1	S2	S3	S4	S5	S6
TP (%)	1.58 ± 0.03^{a}	0.93 ± 0.05^{b}	0.53 ± 0.03^{c}	0.51 ± 0.03^{c}	0.96 ± 0.05^{b}	1.24 ± 0.04^{d}
TOC (%)	1.91 ± 0.03^{a}	1.82 ± 0.04^{a}	2.51 ± 0.18^{b}	2.06 ± 0.02^{c}	2.36 ± 0.09^{b}	2.41 ± 0.05^{b}
TN(%)	0.44 ± 0.04^{a}	0.36 ± 0.01^{b}	0.67 ± 0.02^{c}	0.51 ± 0.02^{a}	0.30 ± 0.04^{b}	0.28 ± 0.06^{d}

*The values are expressed as mean and standard deviation for the three samples/station (each sample was made in duplicate)

CONCLUSIONS

The results of study demonstrated the higher retention capacity of nutrients from sediment by aquatic plants. The capacity of macrophytes for reducing N, TOC, and P has a big importance in reducing phytoplankton blooms potential. Regarding the highest values of TOC and TN values from sediments samples from the S3a possible explanation would be corn culture from the adjacent lake area.
Further, a longer-term study is needed (minimum one year) to assess long-term nutrient dynamics.

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WATER QUALITY IN DIFFERENT AREAS OF TIMIS RIVER COURSE IN ROMANIA

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Abstract

Researchers have been conducted in the Timis River, which springs from the Semenic Mountains, in the western part of Romania. On its course on Romania's territory were established 6 sampling stations (Accumulation Three waters-Feneşti Confluence, Teregova, Lugoj-CFR Bridge, Lugoj-Timişana Confluence, Şag and Grăniceri) where analyzes were made of the chemical composition of the water. Analysis of the results showed an increase from upstream to downstream of 5 days-biochemical oxygen demand (B.O.D.5) (from 1.75 to 3.66 mg/l), also the accumulation of nitrates (from 1.53 to 3.08 mg/l), nitrites (from 0.01 to 0.10 mg/l), chloride (from 2.50 to 8.51 mg/l) and water hardness (from 1.12 to 6.02 dH°).

Key words*: river course areas, Timis River, water chemical composition..*

INTRODUCTION

The Timiş River, which springs from the Semenic Mountains, is the largest drainage river in the Banat hydrographic area. It collects the waters from a basin area of 5673 km, taking the water from rivers that drain the Ţarcu-Godeanu Mountains, as well as the Semenic and Poiana Rusca Mountains. From the Ţarcu-Godeanu Mountains receives the Rece River (Hidişelul), with a narrow and very deep valley and from the Semenic Mountains receives the Bistra River, with well-defined valleys and steep slopes. The middle course of Timiş River crosses the depressed area between Lipova and Buziaş Hills, having a large major riverbed and an average slope of 0.7-0.8 m/km. In the lower course, it has a wide meandering and rambling riverbed, with a particularly low slope, generating floods. Through a double interconnection of Timiş-Bega Rivers, the natural hydrological regime of the two rivers is regularized. In the lower course, the Timiş River receives as the most important tributary of Pogăniş.

MATERIALS AND METHODS

During the entire course of the Timiş River, 6 fishing stations were established, in which determinations of the chemical composition of the water were made.

The fishing stations were set at Accumulation Three waters-Feneşti Confluence (Figure 1), Teregova (Figure 2), Lugoj-CFR Bridge (Figure 3), Lugoj-Timişana Confluence (Figure 4), Şag (Figure 5) and Grăniceri (Figure 6).

The first two fishing stations (Accumulation Three waters-Feneşti Confluence and Teregova) were included in the upper part of Timiş, in the mountain area, the two from Lugoj (Lugoj-CFR Bridge and Lugoj-Timişana Confluence) in the hill area and the last two in the plain area (Şag and Grăniceri).

For the study of water chemical composition of each fishing station, the 5 days-biochemical oxygen demand (B.O.D.5), dissolved oxygen, pH, nitrates (NO_3^-), nitrites (NO_2^-), chlorides and hardness was determined.

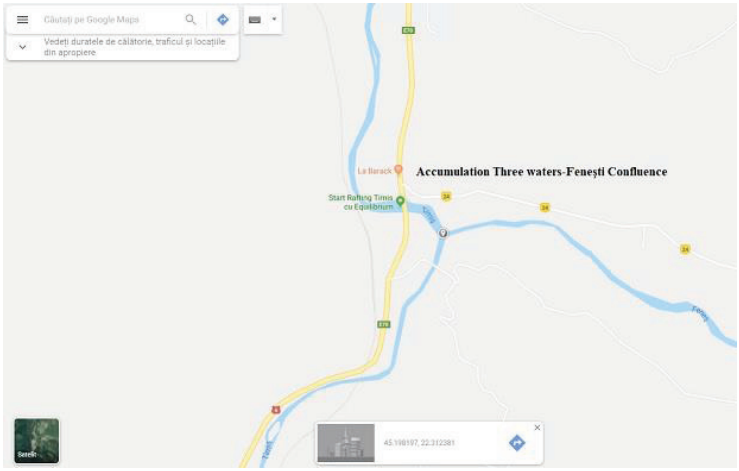

Figure 1. Timiș River at Accumulation Three waters-Fenești Confluence on Google Maps

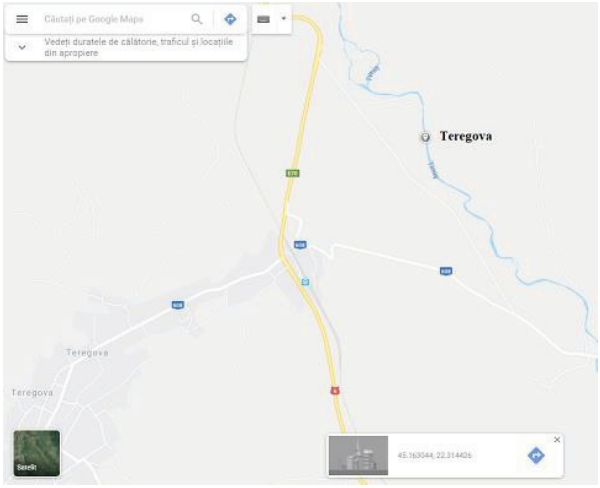

Figure 2. Timiș River at Teregova on Google Maps

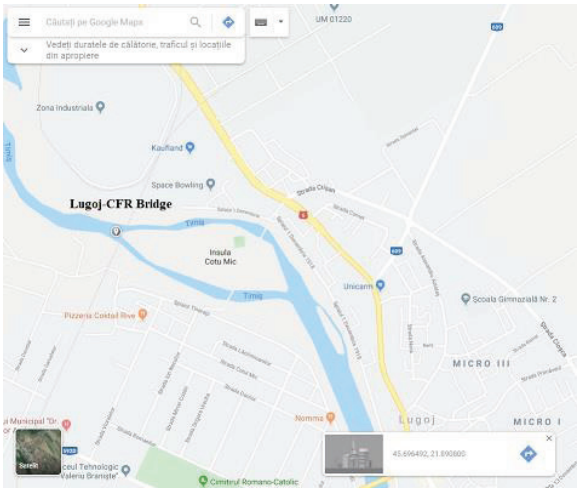

Figure 3. Timiș River at Lugoj-CFR Bridge on Google Maps

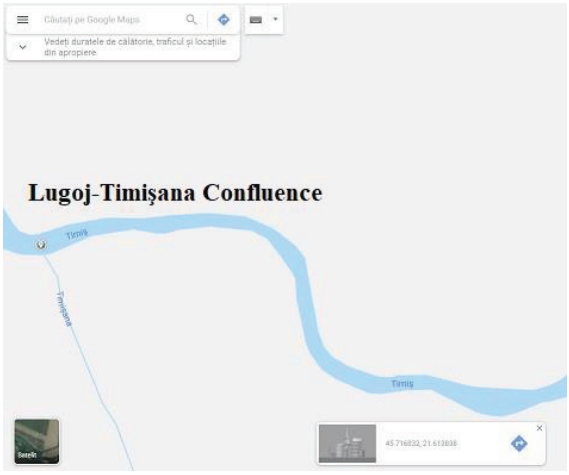

Figure 4. Timiș River at Lugoj-Timișana Confluence on Google Maps

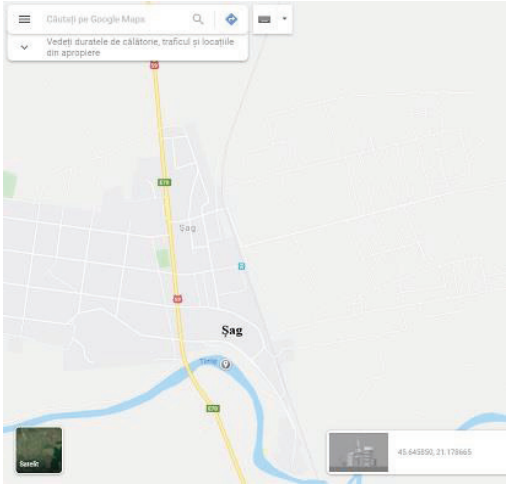

Figure 5. Timiș River at Șag on Google Maps

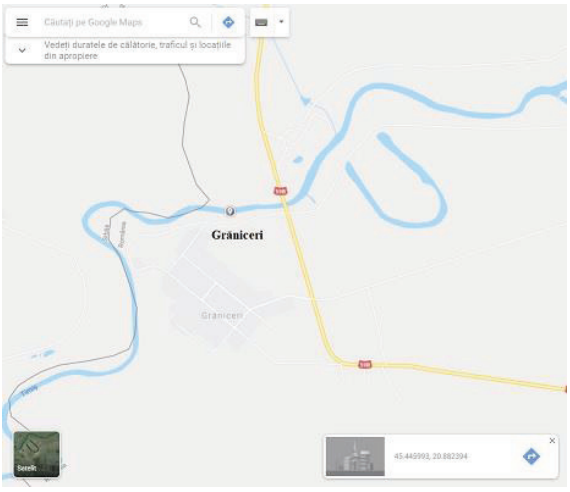

Figure 6. Timiș River at Grăniceri on Google Maps

RESULTS AND DISCUSSIONS

Table 1 shows the chemical composition of the water from the 6 fishing stations along the Timiş River.

The 5 days-biochemical oxygen demand (B.O.D.5) showed the lowest average value of the two years (1.80 mg O_2/l) to the station from reservoir lake Three Waters-Feneş and the junction with the highest average value of the two years (3.58 mg O_2/l) to the Grăniceri station. The extreme values of 5 days-biochemical oxygen demand were between 1.75 mg O_2/l at Feneş station and 3.66 mg O_2/l at Grăniceri station, both determined in 2016.

Table 1. Chemical composition of waters in different fishing stations of Timis River

Fishing stations	B.O.D.5 (mg O_2/l)		Dissolved oxygen (mg O_2/l)		pH		Nitrates (mg/l)		Nitrites (mg/l)		Chlorides (mg/l)		Hardness (dH°)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Three Waters Lake Accumulation-Feneş confluence	1.85	1.75	11.05	10.75	7.05	7.40	2.45	1.53	0.02	0.02	2.50	2.50	1.15	1.37
Teregova	2.00	1.80	10.90	10.80	7.10	7.40	1.87	1.77	0.02	0.01	2.50	2.50	1.44	1.12
Lugoj-CFR Bridge	2.49	3.00	10.29	9.73	7.25	7.50	1.70	2.16	0.04	0.06	2.50	2.50	3.57	3.99
Lugoj-Timişana Confluence	2.57	3.04	8.61	8.33	7.13	7.05	1.53	2.21	0.07	0.10	4.80	7.09	5.14	4.57
Şag	2.20	2.63	9.09	9.13	7.25	7.25	2.68	1.98	0.07	0.05	8.51	5.67	6.02	4.47
Grăniceri	3.50	3.66	10.54	8.24	7.55	7.50	3.08	2.46	0.05	0.04	8.51	8.51	5.99	5.61

The highest average level of dissolved oxygen of the two years - DO (oxygen dissolved in water) was 10.90 mg O_2/l water in Feneş Station and the lowest average level of the two years in Lugoj Station-confluent with Timişana 8.47 mg O_2/l water. In Teregova station the average dissolved oxygen level of the two years was close (10.85 mg O_2/l) to that of the Feneş station. As extreme values, the dissolved oxygen was between a minimum of 8.33 mg O_2/l at the Lugoj Station-confluent with Timişana and a maximum of 11.05 mg O_2/l at the Fenes Station.

Depending on the relationship between the acidic and basic components of the aquatic environment, according to the opinion of Bud et al. (2001, 2016), Bura et al. (2008) and Man (1989) water can have a neutral (pH=7), acid (pH<7) or alkaline (pH>7) reaction. The authors note that the flowing waters had an alkaline pH, given by the contained bicarbonates, while the stagnant fresh waters gradually became acid, due to the accumulation of humic substances, mineral acids, and carbon dioxide. Bogdan et al. (2002), and Grozea and Bura (2002), are of the opinion that both high acidity and strong alkalinity have a negative effect on aquatic organisms.

In all waters of the fishing stations, the pH was alkaline. On average of the two years, the pH ranged between 7.09 in the Lugoj Station-confluence with Timişana and 7.52 in the Grăniceri Station. The individual values were between a minimum of 7.05 in Feneş Station (the year 2015) and Timişana confluence Station (the year 2016), as well as a maximum of 7.55 in Grăniceri Station.

According to Bud et al. (2016), the water favorable for the development of fish must have a pH between 6 and 8. In the presented situation, the pH ranges from 7.05 to 7.55, shows favorable values for the development of aquatic fauna.

If the nitrates and the nitrites in the deep water have a mineral origin and do not have a harmful effect on the fish organism, those in the groundwater have an organic origin and are the consequence of water contamination, according to Bates et al. (2003), Ionescu et al. (1986) and Păcală et al. (2006).

On average of the two years, nitrates recorded values between 1.82 mg NO_3^-/l in the water of the Teregova Station and 2.77 mg NO_3^-/l in the Grănicieri. The highest values of nitrates were recorded at the Şag Stations (2.68 mg NO_3^-/l) and Grănicieri (3.08 mg NO_3^-/l).

The nitrites were reported in extremely small quantities, their average values being between 0.015 mg NO_2^-/l at Teregova Station and 0.085 mg NO_2^-/l at Lugoj Station-confluence with Timişana.

According to the determinations of Ionescu et al. (1986) the salts of Ca^{2+}, Mg^{2+}, Na^{2+}, HCO_3-, SO_4^{2-}, Cl^- ions predominate in natural waters. In the fresh waters, calcium carbonates and bicarbonates dominate.

In relation to the abundance of precipitations (when they decrease) or evaporation (when they grow), the concentration of chlorides, sulphates, and carbonates makes different evolutions. Freshwater contains between 0-0.5 g salts/l water. For most fish species the optimum salinity is between 0.10-0.50 g/l water.

Chlorides are commonly seen in terrestrial waters and may have an inorganic or organic origin. Chlorines from deep layers have an only inorganic origin and can be clearly tolerated when they exceed permissible level (20 mg/l). The chlorides in groundwater are chemically organic, being the consequence of contamination with waste-water.

In the six fishing stations on the Timiş River, the level of chlorides was between 2.50 mg/l in Fenes, Teregova and Lugoj-CFR Bridge stations, and the maximum value of 8.51 mg/l was reached in the Grăniceri station. The first decreasing value, of 7.09 mg chlorides/l, was reached at Şag station.

The hardness of the water is generated by the concentration of calcium and magnesium salts in the water. Bănărescu (1964) notes that calcium and magnesium bicarbonates give the water a temporary hardness, while calcium and magnesium chlorides and sulphates give the permanent hardness. By summing the two harnesses of water the total hardness is obtained, which, in our system, is expressed in German degrees of hardness (dH°) and represents the amount of calcium and magnesium salts dissolved in one liter of water, equivalent to 1 dH° = 10 mg CaO. In the case of flowing waters the total hardness is generally below 15 dH°. Moderate hardness is between 12-18 dH°.

Depending on the degree of hardness achieved, Man (1989) classifies the waters in: very soft (0-4 dH°), soft (4-8 dH°), medium (8-12 dH°), moderately harsh (12 -18 dH°), hard (18-20 dH°) and very hard waters (>30 dH°). For fish farming, the best waters are those with medium to moderate hardness (Bura et al., 2008).

On average, the water hardness recorded the lowest value (1.26 dH°) at the Feneş fishing station and the highest value at the Grăniceri fishing station (5.80 dH°). The lowest water hardness was recorded at Teregova station (1.12 dH°) in 2016, and the highest hardness at Şag station (6.02 dH°) in 2015.

Table 2 presents the chemical composition of the Timiş River in different areas.

Table 2. Chemical composition of water in different areas of Timis River

Area	B.O.D.5 (mg O_2/l)		Dissolved oxygen (mg O_2/l)		pH		Nitrates (mg/l)		Nitrites (mg/l)		Chlorides (mg/l)		Hardness (dH°)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Upper course	1.93	1.78	10.98	10.79	7.08	7.40	2.16	1.65	0.02	0.01	2.50	2.00	1.29	1.24
Middle course	2.52	3.02	9.45	9.03	7.19	7.28	1.62	2.19	0.05	0.08	3.65	4.79	4.35	4.28
Lower course	2.75	3.15	9.82	8.69	7.40	7.38	2.88	2.22	0.06	0.05	8.51	7.09	6.00	5.04

The six fishing stations on the Timiş River were divided by two on each area of the river course, constituting for analysis three zones: upper (Feneş and Teregova stations), middle (the two Lugoj stations) and lower (Şag and Grăniceri)

The 5th days-biochemical oxygen demand showed on average of the two years an increased evolution from the upper (1.80 mg O_2/l) to the lower water area (3.58 mg O_2/l). In relation to this consumption, the level of oxygen dissolved in water on average of the two years was highest in the upper river of the Timiş River (10.90 mg O_2/l) and much lower in the middle river (10.01 mg O_2/l) and lower (9.39 mg O_2/l). The water of all investigated

areas had a high level of oxygen dissolved in water, which ensures their existential comfort.
In all three areas, the average of the two years pH level was weakly alkaline, being between 7.22 and 7.52. This level places the water along the entire Timiş River at a level favorable to the development of many species of fish caught.
In the water of the Timiş River, the average of nitrates from the two years (between 1.82 and 2.77 mg/l) and the average of nitrites from the two years (between 0.015 and 0.06 mg/l) are found in small quantities, which do not endanger the life of the fish.
The average of chloride level from the two years in the Timiş River water was the lowest in the upper area (2.25 mg/l), almost doubled in the middle area (4.22 mg/l) and tripled in the lower area (7.80 mg/l).
The average of water hardness recorded from the two years increasing values from the upper area of the Timiş River (1.26 dH°), towards the middle area (4.31 dH°) and reached the maximum hardness in the lower area of this river (5.52 dH°).

CONCLUSIONS

During the passage from the springs to the water in the Timiş River the level of 5 days-biochemical oxygen consumption increased (from 1.75 to 3.66 mg O_2/l), the accumulation of nitrates (from 1.53 to 3.08 mg/l), of nitrites (from 0.01 to 0.10 mg/l) and of chlorides (from 2.50 to 8.51 mg/l). Compared to these parameters, the level of oxygen dissolved in water decreased (from 11.05 to 8.24 mg O_2/l) and the pH of the water recorded relatively uniform alkaline values (between 7.05 and 7.55).
The Timiş River water recorded the average lowest hardness from the two years in the upper area (1.26 dH°), which increased in the middle area to (4.31 dH°) and reached the maximum value in the lower area (5.52 dH°).

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WATER QUALITY AND STRUCTURE OF FISH POPULATIONS IN DIFFERENT AREAS OF THE TIMIS RIVER

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Abstract

Researchers have been conducted on the Timis River, during which 6 fishing stations were established where 6 determinations of the chemical composition of water were made and the existing fish populations were evaluated. In the water of Timis River, from springs to the mouth, increased the 5 days-biochemical oxygen demand (BOD5 from 1.80 to 3.58 mg O_2/l), also the accumulation of nitrates (from 1.82 to 2.77 mg/l), nitrites accumulation (from 0.015 to 0.045 mg/l), chlorides (from 2.50 to 8.51 mg/l) and hardness (from 1.27 to 5.52 dH°). In the Timiş river 36 fish species have been identified, which proves a good ecological status. Depending on the physic-chemical properties of the water, salmonids fish population was spread only in the mountain area, and cyprinids in hills and low land area. A large distribution was expressed in Barbel, Chub, Chased the Umber and Spirlin, which were found between the Teregova fishing station (mountain area), Lugoj stations (hilly area) and stations from Sag and Graniceri (lowlands area). In the warm waters of low lands were found: Bream, ide, pike-perk, sterlet, Verner, eels and Wheatear.

Key words*: Timis River, chemical composition of water, species of fish, areas of river course.*

INTRODUCTION

The Timis River, the largest drainage river in the Banat hydrographic area, collects the waters from a basin area of 5673 km. It takes water from rivers that drain the Tarcului-Godeanu Mountains, the Semenic Mountains and Poiana Ruscă Mountains. From the Ţarcu-Godeanu mountains receives the Cold River (Hidişelul), and from the Semenic Mountains receives the Bistra River. The middle course of Timiş River crosses the depressed area of Lipova and Buziaş Hills. In the lower course it has a wide riverbed, with low slope, generating floods. Through a double interconnection Timiş-Bega, the natural hydrological regime of the two rivers is regularized. In the lower course, Timiş River receives as the most important affluent, Pogăniş River (Catalogul habitatelor, speciilor şi siturilor. Info-natura 2000 în România, 2013).

MATERIALS AND METHODS

Along the Timiş River 6 fishing stations were established, where were made determinations of the chemical composition of the water and the existing fish populations were evaluated. The fishing stations were set at: Accumulation Three waters – Fenesti Confluence (Figure 1), Teregova (Figure 2), Lugoj-CFR Bridge (Figure 3), Lugoj-Timişana Confluence (Figure 4), Şag (Figure 5) and Grăniceri (Figure 6).

The first two fishing stations were included in the mountain area of Timiş, the two from Lugoj in the hilly area and the last two (Şag and Grăniceri) in the area of lowland.

For the study of the chemical composition of the water of each fishing station, the 5 days-biochemical oxygen demand was determined (BOD5), dissolved oxygen, pH, nitrates (NO_3^-), nitrites (NO_2^-), chlorides and hardness.

RESULTS AND DISCUSSIONS

Table 1 presents the average, the dispersion indices and the significance of the differences between the chemical compositions of the water in different fishing stations of the Timiş River.

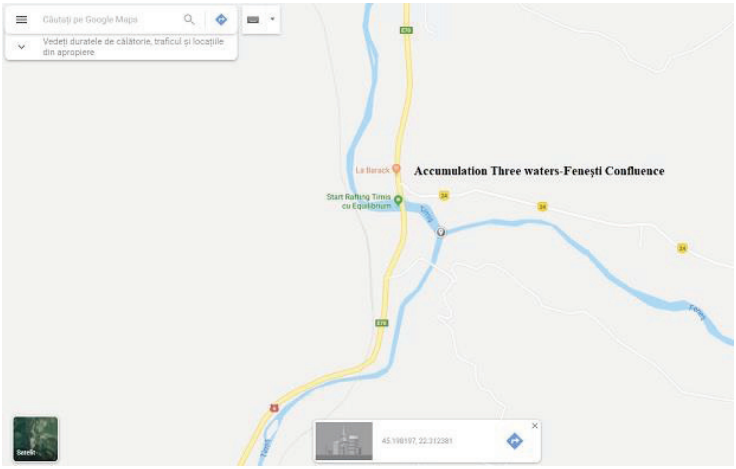

Figure 1. Timiș River at Accumulation Three waters-Fenești Confluence on Google Maps

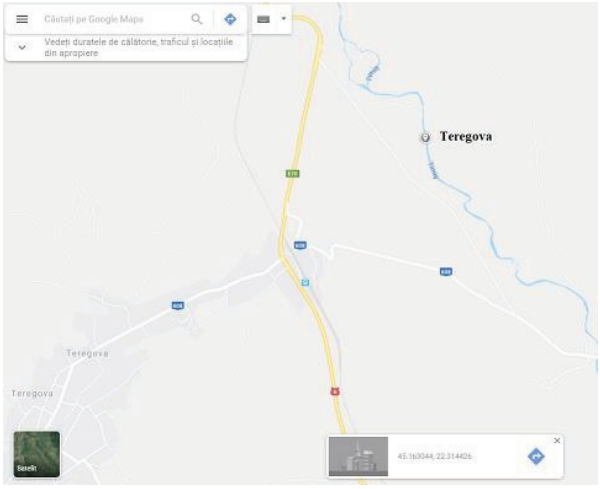

Figure 2. Timiș River at Teregova on Google Maps

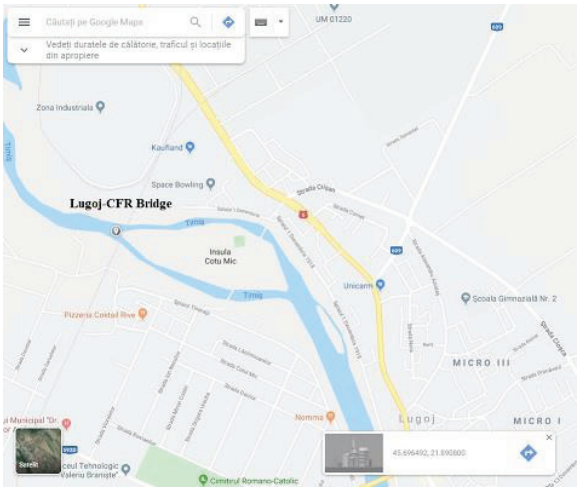

Figure 3. Timiș River at Lugoj-CFR Bridge on Google Maps

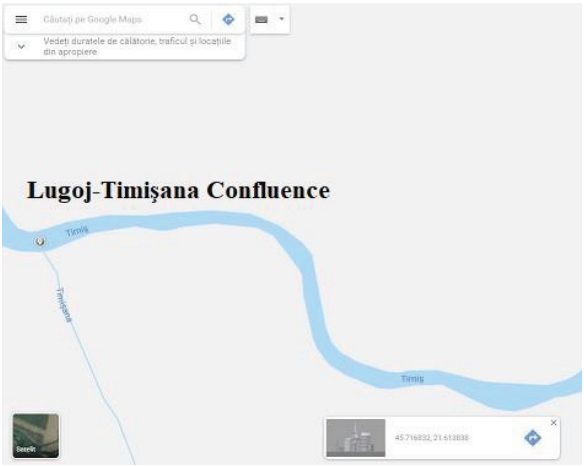

Figure 4. Timiș River at Lugoj-Timișana Confluence on Google Maps

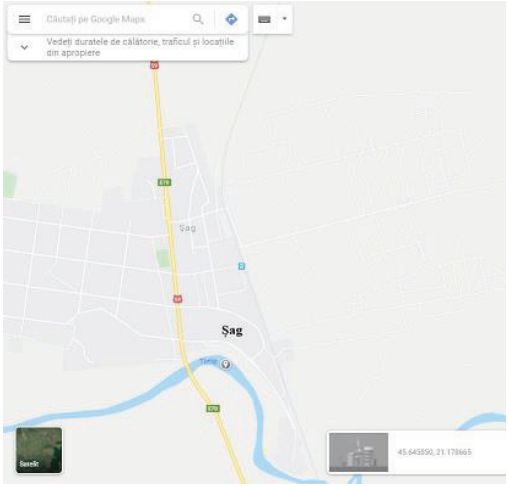

Figure 5. Timiș River at Șag on Google Maps

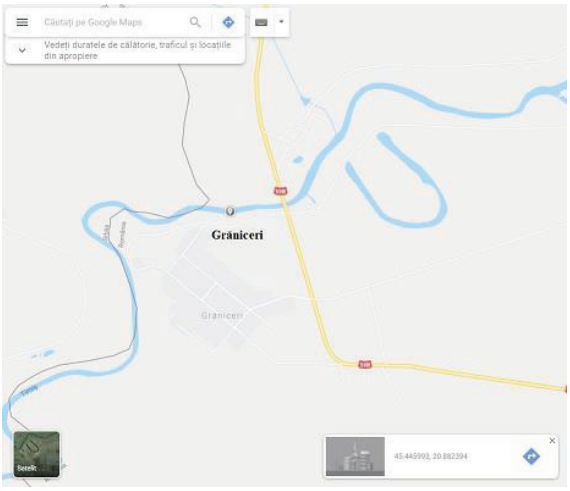

Figure 6. Timiș River at Grăniceri on Google Maps

During the passage from the springs to the discharge, in the water of the Timiş River, the level of 5 days-biochemical oxygen demand increased from 1.8 ± 0.05 to 3.58 ± 0.08mg O_2/l, the nitrates level increased from 1.82 ± 0.05 to 2.77 ± 0.31mg/l, and the chloride level increased from 2.50 to 8.51mg/l. BOD5 may be influenced by the discharge of dairy by-products, such as whey, into the river water, leading to a higher BOD5 with direct effect on fish species that do not tolerate low oxygen concentrations (Ahmadi et al., 2018, 2019).

Table 1. The mean and dispersion indices of chemical composition of water in different fishing station of Timiş River

Fishing station		Accumulation Three waters-Feneş Confluence	Teregova	Lugoj-CFR Bridge	Lugoj-Timişana Confluence	Şag	Grăniceri
5 days-biochemical oxygen demand (BOD5) (mg O_2/l)	x ± SE	$1.8^{a} \pm 0.05$	$1.9^{a,c} \pm 0.1$	$2.74^{a,d,e} \pm 0.255$	$2.8^{b,c,e} \pm 0.235$	$2.41^{a,c,d,f} \pm 0.215$	$3.58^{b,e} \pm 0.08$
	SD	0.07	0.414	0.36	0.332	0.304	0.113
Dissolved oxygen (mg O_2/l)	x ± SE	$10.90^{a} \pm 0.15$	$10.85^{a} \pm 0.05$	$10.01^{a} \pm 0.28$	$8.47^{a} \pm 0.14$	$9.11^{a} \pm 0.02$	$9.39^{a} \pm 1.15$
	SD	0.212	0.07	0.395	0.197	0.028	1.62
pH	x ± SE	$7.22^{a} \pm 0.175$	$7.25^{a} \pm 0.15$	$7.37^{a} \pm 0.125$	$7.09^{a} \pm 0.04$	$7.25^{a} \pm 0$	$7.52^{a} \pm 0.025$
	SD	0.247	0.212	0.176	0.056	0	0.035
Nitrates (NO_3^-) (mg/l)	x ± SE	$1.99^{a} \pm 0.46$	$1.82^{a} \pm 0.05$	$1.93^{a} \pm 0.23$	$2.33^{a} \pm 0.34$	$2.33^{a} \pm 0.35$	$2.77^{a} \pm 0.31$
	SD	0.65	0.07	0.325	0.48	0.494	0.438
Nitrites (NO_2^-) (mg/l)	x ± SE	$0.02^{a} \pm 0$	$0.015^{a} \pm 0.005$	$0.05^{a,b} \pm 0.01$	$0.085^{a} \pm 0.015$	$0.06^{a,b} \pm 0.01$	$0.045^{a,b} \pm 0.005$
	SD	0	0.007	0.014	0.021	0.014	0.007
Chlorides (mg/l)	x ± SE	$2.5^{a} \pm 0$	$2.5^{a} \pm 0$	$2.5^{a} \pm 0$	$5.945^{a,c,d} \pm 1.145$	$7.09^{b,c} \pm 1.42$	$8.51^{b,d} \pm 0$
	SD	0	0	0	1.619	2.008	0
Water hardness (dH°)	x ± SE	$1.26^{a} \pm 0.11$	$1.28^{a} \pm 0.16$	$3.78^{b} \pm 0.21$	$4.86^{b} \pm 0.285$	$5.24^{b} \pm 0.775$	$5.8^{b} \pm 0.19$
	SD	0.155	0.226	0.296	0.403	1.09	0.268

Note: ● between the means with the same letter there are insignificant differences ($p > 0.05$)
● between the means with different letter there are significant differences ($p < 0.05$)

Contrary to the upward evolution of these parameters, the level of dissolved oxygen in water decreased from 10.9 ± 0.15 to 9.39mg O_2/l, the level of nitrites decreased from 0.15 ± 0.005 to 0.045 ± 0.005mg/l, and the pH of the water recorded relatively uniform values, ranging from 7.22 ± 0.175 and 7.52 ± 0.025.

The water hardness of the Timiş River varied between 1.26 ± 0.11 and 5.8 ± 0.19 dH°.

The 5 days-biochemical oxygen demand was significantly lower ($p<0.05$) in the Feneş fishing station than in the Lugoj - Timişana Confluence stations and the Grăniceri one.

For the level of nitrites, significant differences ($p<0.05$) were recorded in the water from the fishing stations Feneş and Lugoj- Timişana Confluence, as well as between that of the station Teregova and Lugoj-Timişana Confluence.

Chlorides levels showed significant differences ($p<0.05$) between the waters of the fishing stations in Feneş and those of Şag and Grăniceri, between those of Teregova and those of Şag and Grăniceri, as well as between those of Lugoj station-CFR Bridge and those from Şag and Grăniceri.

The water hardness of the mountain stations (Feneş and Teregova) was significantly ($p<0.05$) lower than those of the stations of Lugoj-CFR Bridge, Lugoj- Timişana Confluence, and also Şag and Grăniceri.

Between the other components of the water chemical composition the differences were insignificant ($p>0.05$).

Table 2 shows the average, the dispersion indices and the significance of the differences ($p <0.05$) between the chemical composition of water in different areas of the Timiş River.

The 5 days-biochemical oxygen demand was lower (1.8 ± 0.054 mgO_2/l) in the upper area, recorded an average value (2.77 ± 0.142mg O_2/l) in the middle area and recorded the value higher (2.947±0.349mg O_2/l) in the lower area.

Table 2. The mean and dispersion indices of chemical composition of water in different areas of Timiș River

Area		Upper	Middle	Lower
5 days-biochemical oxygen demand (BOD5) (mg O_2/l)	x ± SE	1.8^{a} ± 0.054	$2,771^{b}$ ± 0.142	2.947^{b} ± 0.349
	SD	0.108	0.285	0.698
Dissolved oxygen (mg O_2/l)	x ± SE	10.881^{a} ± 0.066	$9,24^{b}$ ± 0.462	$9,25^{b}$ ± 0.476
	SD	0.132	0.925	0.952
pH	x ± SE	7.235^{a} ± 0.094	7.232^{a} ± 0.098	7.387^{a} ± 0.08
	SD	0.188	0.196	0.160
Nitrates (NO_3^-) (mg/l)	x ± SE	1.905^{a} ± 0.195	1.9^{a} ± 0.168	2.55^{a} ± 0.229
	SD	0.39	0.336	0.458
Nitrites (NO_2^-) (mg/l)	x ± SE	0.017^{a} ± 0.002	0.067^{b} ± 0.012	0.052^{b} ± 0.006
	SD	0.005	0.025	0.012
Chlorides (mg/l)	x ± SE	2.5^{a} ± 0	4.222^{a} ± 1,098	$7,8^{b}$ ± 0.71
	SD	0	2,197	1.42
Water hardness (dH°)	x ± SE	1.27^{a} ± 0.079	4.31^{b} ± 0.342	5.522^{c} ± 0.363
	SD	0.158	0.684	0.726

Note: ● between the means with the same letter there are insignificant differences ($p > 0.05$)
● between the means with different letter there are significant differences ($p<0.05$)

In the water of the Timiș River the dissolved oxygen content was highest in the upper zone (10.881 ± 0.066 mg O_2/l) and almost as small in the lower area (9.25 ± 0.476mg O_2/l) as in the middle one (9.24 ± 0.462mg O_2/l).

The chlorides level in the water of the Timiș River increased from the upper area (2.5 mg/l), to the middle one (4.222 ± 1.098 mg/l), to reach the maximum value in the lower area (7.8 ± 0.71 mg/l).

The water from the Timiș River recorded a nitrates content of 1.905 ± 0.195 mg/l in the upper area, 1.9 ± 0.168 mg/l in the middle area and the highest concentration 2.55 ± 0.229 mg/l, in the lower area.

Nitrites content was low, of 0.017 ± 0.002 mg/l in the upper area, 0.067 ± 0.012 mg/l in the middle area and 0.052 ± 0.006 mg/l in the lower area.

Significant differences ($p<0.05$) were registered for the 5 days-biochemical oxygen demand between the upper area and the middle and lower areas, for the oxygen dissolved in the water between the upper area and the middle and lower areas, as well as for the nitrites content between the upper area and the middle and lower areas, and for chlorides between the lower and upper and middle areas.

The water hardness increased from upstream to downstream, being 1.27 ± 0.079 dH° in the upper area, 4.31 ± 0.342 dH° in the middle area and 5.522 ± 0.363 dH° in the lower area.

For water hardness, significant differences ($p<0.05$) were reported between the middle and lower areas.

Among the other characteristics of the chemical composition of the waters in different areas of the Timiş River, the differences were insignificant ($p>0.05$).

In the researches reports it is noted that between the amount of dissolved oxygen in water and the fish species there is a conditionality ratio, oxygen being a limiting factor, of extension and of numerical regulation.

Depending on the oxygen requirement, fish species may be steno-oxibionts that require large amounts of oxygen dissolved in water (Vasiliu, 1959). Of this category belong: trout, grayling, Danube salmon, minnow, common chub, barbell, loach, European bullhead, and others, which require more than 6 mg O_2/l water. Euri-oxibiont species of fish require less oxygen in water (3-4 mg O_2/l water or even less). Most of the cyprinids (carp, goldfish, crucian carp, tench, asp, common rudd, common bream, ide and others), wels catfish, European weatherfish, Northern pike (Păcală et al., 2006) belong to this group.

In order to illustrate these characteristics, in table 3 we present the spreading areas of the fish species from the Timiş River.

From the analysis of the table, it appears that the salmonid species are widespread in the mountain area, and the cyprinids in the hilly and lowland areas (Bates et al., 2003; Bănărescu, 1964; Bud et al., 2001, 2016; Bura and Bănățean, 2017). A high biological plasticity we find at the barbell, common chub, riffle minnow, barley, clean and grassy, which

are presented starting from the area of the fishing station Teregova (mountain area), the stations of Lugoj (the area of hills) and the stations of Şag and Grăniceri (area of lowland).

Table 3. Spreading areas of fish species from Timiş River

Crt. no.	Species	Fishing stations					
		Feneş	Teregova	Lugoj-Timişeana Confluence	Lugoj-CFR Bridge	Şag	Grăniceri
1.	Danube lamprey (*Lampetra danfordi*)	+					
2.	Danube salmon (*Hucho hucho*)	+					
3.	River Indigenous Trout (*Salmo trutta fario*)	+	+				
4.	Minnow (*Phoxinus phoxinus*)	+	+	upstream			
5.	Grayling (*Thymallus thymallus*)		+				
6.	European bullhead (*Cottus gobio*)		+	+			
7.	Loach (*Nemacheilus barbatulus*)		+	+		lost	
8.	Romanian Barbel (*Barbus petenyi*)		+	+	+		
9.	Common nase (*Chondrostoma nasus*)		+	+	+		
10.	Danube streber (*Zingel streber*)		+	+	+		
11.	Ling or bubbot (*Lota lota*)		+	+	+	+	
12.	Barbel (*Barbus barbus*)		+	+	+	+	+
13.	Common chub (*Leuciscus cephalus*)		+	+	+	+	+
14.	Riffle minnow (*Alburnoides bipunctatus*)		+	+	+	+	+
15.	Romanian loach (*Cobitis romanica*)			+	+		
16.	Spined loach (*Cobitis taenia*)			+	+	+	+
17.	Asp (*Aspius aspius*)			+	+	+	+
18.	Tench (*Tinca tinca*)			+	+	+	+
19.	Northern (*Esox lucius*)			+	+	+	+
20.	Wels catfish (*Silurus glanis*)			+	+	+	+
21.	European pech (*Perca fluviatilis*)			+	+	+	+
22.	Common bleak (*Alburnus alburnus*)			+	+	+	+
23.	Vimba bream (*Vimba vimba*)			+	+	+	+
24.	Kessleri (*Gobio kessleri*)			+	+	+	+
25.	European carp (*Cyprinus carpio*)			+	+	+	+
26.	Roach (*Rutilus rutilus*)				+	+	+
27.	Common dace (*Leuciscus leuciscus*)				+	+	+
28.	Silver bream (*Blicca björkna*)				+	+	+
29.	Goldfish (*Carassius auratus gibellio*)				+	+	+
30.	Crucian carp (*Carassius carassis*)					+	+
31.	Sterlet (*Acipenser ruthenus*)					+	+
32.	Zander (*Stizostedion lucioperca*)					+	+
33.	Common bream (*Abramis brama*)					+	+
34.	European weatherfish (*Misgurnus fossilis*)					+	+
35.	Ide (*Leuciscus idus*)					+	+
36.	Common zingel (*Zingel zingel*)						+

Only in the warm waters of the lowlands have the following species: common bream, ide, crucian carp, starlet, common zingel, zander, and European weatherfish.

CONCLUSIONS

During the flow of springs to spills in the water of the Timiş River the 5 days-biochemical oxygen demand has increased (from 1.80 to 3.58 mg O_2/l), accumulating nitrates (from 1.82 to 2.77 mg/l), nitrites (from 0.015 to 0.045 mg/l) and chlorine (from 2.50 to 8.51 mg/l). Compared to these parameters, the dissolved oxygen in water decreased (from 10.90 to 9.39 mg O_2/l), and the pH was slightly uniform alkaline (between 7.22 and 7.52).

The Timiş River water recorded the lowest hardness in the upper zone (1.26 dH°), which increased in the middle zone (4.31 dH°) and

reached the maximum value in the lower area (5.52 dH°).

The good ecological status of the Timiş River is reflected by the diversity of the aquatic fauna, composed of 36 species of fish of community interest.

By correlating the physico-chemical properties of the water along the Timiş River with the habitats reached by the fish species, it has been found that salmonid species are widespread only in the mountain area, and the cyprinids are present in the hilly and lowland areas. High plasticity was proved by of the barbel, Romanian barbel, common chub, common nase, and riffle minnow that were encountered between the fishing station Teregova (mountain area), the stations of Lugoj (hilly area) and the stations of Şag and Grăniceri (the lowland area). Only in the warm waters of the plains were found; the common bream, ide, crucian carp, sterlit, zander, European weatherfish, and common zingel.

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