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GENETICS AND BREEDING

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ESTABLISH THE SELECTION OBJECTIVE USING A COMPETITIVENESS INDEX FOR ROMANIAN BUFFALO

Răzvan POPA, Dana POPA, Livia VIDU, Carmen NICOLAE, Marius MAFTEI

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: danasandulescu@yahoo.com

Abstract

This study was conducted in order to calculate the competitiveness index for milk yield, fat and protein percent in the population of Romanian Buffalo from Șercaia Research and Development Station. A total 609 milk yield and associated characters records, belonging to 87 females, which coming from 11 sire families, for 7 lactations were analysed. The method used was an index elaborated by Sandu (1983) which takes into account the genetic determinism of traits, genetic correlations and economic weight. The method used for genetic parameters estimates was REML, and for economic weights was linear multiple regression. The competitiveness index for milk yield, fat and protein percent in seven lactations were calculated as: 0.1158, -0.0040, -0.0279; 0.0683, 0.0024, -0.0087; 0.0817, 0.0141, -0.0249; 0.5265, 0.0013, -0.1134; 0.1441, 0.0479, -0.0567; 0.1806, 0.0005, -0.0478; 0.3170, -0.0300, -0.0921. Results indicated that the selection objective of Romanian Buffalo is milk yield genetic improvement. But, taking into account the genetic correlations between traits, an optimization of the female selection criterion becomes mandatory.

Key words*: selection objective, competitiveness index, milk yield, fat and protein percent, Romanian Buffalo.*

INTRODUCTION

In Romania, the buffalo entered with the invasion of the Huns and Avars in the Carpatho-Danubian area. It found the good pedo-climatic conditions and so, in our country, has developed a buffalo population which had its own evolutionary path as a result of reproductive isolation (Vidu et al., 2008). The Romanian Buffalo is one of the most important genetic resources for milk and meat production.
At present, the buffalo herds in Romania have fallen further, reaching about 14,000 heads (personal estimation from the National Institute of Statistics data). In our country, buffalo is predominantly grown in individual subsistence households, with a maximum of 5 heads. Romania has a tradition of growing this species, but with the aging and biological disappearance of the rural population, the species is vulnerable. Also, the vulnerability of the Romanian buffalo is generated by the lack of financial aid, the low milk price, the lack of strong associations of breeders that protect farmers' interests in recent years. However, Romania has the major advantage of the existence of a research station in the field of buffalo breeding, which has an extremely valuable breeding nucleus. Increasing the economic efficiency of buffalo production and developing a breeding program are keys to actively conserving of this genetic structure.
The buffalo is a species with remarkable quality, of which we can remember: high percentage of milk fat, meat with exceptional taste qualities, resistance to diseases and heavy environmental conditions, good valorization of poor quality feeds. On the international market, the main product obtained from buffalo milk is Mozzarella, a cheese specialty. The amount of Mozzarella is closely related to the quantity and quality of milk (Popa et al., 2014) and is a criterion for the selection of buffaloes.
Enormous advantage of exploitation of this species for characters associated with milk production, compared with cows and sheep, is the lower cholesterol content of milk and Mozzarella cheese type, despite higher values of the constituents (Zicarelli, 2004).
Compared with cows, buffalo milk has quality parameters with higher values. The fat percentage range between 6.87 to 8.59% (Rosati and Van Vleck, 2002; Tonhati et al., 2000), protein percentage between 4.13 to 4.55% (Macedo et al.2001; Rosati and Van Vleck, 2002). In Romania, Velea and

Mărginean (2004) specifies that buffalo's milk production falls in to the following parameters: average milk yield 1111.11 kg/lactation, average fat yield 82.10 kg (7.39%), and average protein yield 46.21 kg (4.23%).
So far, no breeding program related to this species has been developed in our country. As is well known, setting the selection objective is a first step in developing breeding programs. The inclusion of traits in the selection objective is based on the economic weight, degree of genetic determination, and genetic correlation with other traits (Grosu et al., 2005; Popa, 2009). Developing a breeding program for this species is an active conservation path that aims to improve the genetic structure of the population for useful economic characteristics, thus contributing to the economic efficiency of the species.
There are a number of studies showing estimates of genetic parameter values related to buffalo milk yield and quality (Aspilcueta-Borquisetal, 2010; Farhangfar et al., 2003; Rosati and Van Vlek, 2002; Sarubbi et al., 2012; Seno et al., 2006; Tonhati et al., 2000), but very few related to estimation of economic weight (Bahareh et al., 2011).
The objective of this study was to establish the traits which will be included in selection objective, according to a competitiveness index, using a methodology that gives the maximum accuracy in conditions of the existence an inconsistent data.

MATERIALS AND METHODS

A total 609 milk yield and associated characters records, belonging to 87 females, which coming from 11 sire families, for 7 lactations were analysed.
The traits studied were: milk yield per lactation, milk fat and protein percent.
In control milk production, records with length greater than 270 days were truncated at this point, as suggested by Tonhati et al. (2008) and Aspilcueta-Borquisetal (2010).
In order to estimate economic weights and genetic parameter values, were used the data resulting following control milk production in females belonging Şercaia Research and Development Station. To analyze parameters in dynamic were included in the analysis only animals presenting records to an equal number of lactations.
The method used to estimate economic weights is based on the multiple linear regression proposed by Hazel in 1943 (Grosu, 2003, 2005).
These economic weights have been presented in another paper by the authors („Economic weights of production traits for Romanian buffalo" - in process of publication) and will be used to calculate the competitiveness index.
The competitiveness index was estimated using a relationship proposed by Sandu (1983):

$$C_i = \left(a_i \cdot h_i^2\right) + \sum_{i=1}^{j} r_{G_{ij}} \cdot (1-s) \cdot \left(a_j \cdot h_j^2\right)$$

in which:
- $i, j = 1, 2, ..., n$ traits, but $i \neq j$;
- a_i = economic weight of trait for whom the competitiveness index is computed
- h_i^2 = heritability of trait for whom the competitiveness index is computed
- $r_{G_{ij}}$ = genetic correlation between *i* and *j*
- a_j = economic weight of trait with which it compares *i*
- h_j^2 = heritability of trait with which it compares *i*
- $s = 0$ when *i*şi*j* must be modified by selection in the same sense and $s = 2$ when *i*şi*j* must be modified by selection in different sense
- $\sum$ shows us that there will be so many assemblies, how many traits we haves.

The method used for genetic parameters estimates was REML developed by Fisher (1925) and perfected by Patterson and Thompson (1971).

RESULTS AND DISCUSSIONS

The setting of economic weights is of dual importance: on the one hand, only the economic important traits are included in the selection objective, and on the other hand it determines the weight to be given in the selection of the different traits that contribute to the complex character (Drăgănescu and Grosu, 2003).
The economic weights for milk yield, fat and protein percent are presented in Table 1.

According to Material and method section, these economic weights have been presented in another paper by the authors („Economic weights of production traits for Romanian buffalo" - in process of publication) and will be used to calculate the competitiveness index.

Table 1. The economic weights for milk yield, fat and protein percent

Specification	U.M.	Milk yield (kg)	Fat percent	Protein percent
Lactation 1	euro	0.9636	0.1367	-0.0974
Lactation 2	euro	0.9729	0.0912	-0.0661
Lactation 3	euro	0.9948	0.1978	-0.1935
Lactation 4	euro	0.9922	0.1506	-0.1452
Lactation 5	euro	0.9932	0.2691	-0.2645
Lactation 6	euro	0.9891	0.1454	-0.1352
Lactation 7	euro	0.9820	0.1708	-0.1597

The values presented in Table 1 show that an increase of average of milk production with one kilo in one lactation period, the income will be increase with approximatively 1 euro. Also, the unitary increase of fat percent (one percent per lactation) will be a positive effect to the income of farm. The negative values associated with protein percent indicate that, at least in the analyzed population, it is not desirable to increase it.

Heritability is defined as the rate of additive genetic variance in the phenotypic variance. Since the breeding program can act only on additive genetic variation within population, estimates of heritability coefficients are considered very important as indicators of effectiveness of these programs. Heritability coefficient values for the traits studied in dynamics are presented in Table 2.

Table 2. Heritability values for milk yield, fat and protein percent

Specification	Milk yield	Fat percent	Protein percent
	$h^2 \pm s_{h^2}$	$h^2 \pm s_{h^2}$	$h^2 \pm s_{h^2}$
Lactation 1	0.12±0.27	0.13±0.27	0.14±0.25
Lactation 2	0.07±0.25	0.10±0.26	0.06±0.24
Lactation 3	0.08±0.25	0.13±0.28	0.11±0.24
Lactation 4	0.52±0.44	0.48±0.43	0.49±0.39
Lactation 5	0.14±0.28	0.25±0.32	0.20±0.35
Lactation 6	0.18±0.29	0.21±0.29	0.21±0.30
Lactation 7	0.32±0.35	0.26±0.32	0.32±0.34

Heritability coefficient values for milk yield that we found are similar to those reported by other authors (Farhangfar et al., 2003; Rosati and Van Vlek, 2002; Sarubbi et al., 2012; Seno et al., 2006; Tonhati et al., 2000). The milk yield is a character that has a low to medium genetic determinism, along the 7 lactations analyzed (Table 2). The variation of heritability coefficient from one lactation to another can be explained by the variation of environmental variance (taking into account all elements which contribute to its determination), or existence of different polygenic complex that is involved in genetic determining of milk yield.

Regarding the milk fat and protein percent, data presented in Table 2 show that these traits can be placed in group of low to intermediate heritable traits. Same discussion for values variation from one age to another.

Heritability coefficient values for milk quality that we found are similar to those reported by other authors (Rosati and Van Vlek, 2002; Sarubbi et al., 2012; Seno et al., 2006; Tonhati et al., 2000).

The genetic correlation values between traits studied in dynamics are presented in Table 3.

Table 3. Genetic correlation between milk yield, fat and protein percent

Couple of traits	r_G
Lactation 1	
Milk yield x	
- fat percent	-0.16
- protein percent	-0.22
Fat percent x	
- protein percent	0.24
Lactation 2	
Milk yield x	
- fat percent	-0.09
- protein percent	-0.25
Fat percent x	
- protein percent	0.15
Lactation 3	
Milk yield x	
- fat percent	-0.10
- protein percent	-0.22
Fat percent x	
- protein percent	0.17
Lactation 4	
Milk yield x	
- fat percent	-0.11
- protein percent	-0.26
Fat percent x	
- protein percent	0.20
Lactation 5	
Milk yield x	
- fat percent	-0.09
- protein percent	-0.21
Fat percent x	
- protein percent	0.13
Lactation 6	
Milk yield x	
- fat percent	-0.14
- protein percent	-0.24
Fat percent x	
- protein percent	0.18
Lactation 7	
Milk yield x	
- fat percent	-0.18
- protein percent	-0.21
Fat percent x	
- protein percent	0.35

The results presented in Table 3 show that milk yield is negative correlated with fat and protein percent, which means that long-term selection of females for quantity of milk is made in detriment of its quality. Results are similar with those obtained by Popa et al. (2014).

The values of competitiveness index calculated according to the described model for milk yield, fat and protein percent are presented in Table 4.

Table 4. Competitiveness index values for milk yield, fat and protein percent

Specification	Milk yield	Fat percent	Protein percent
Lactation 1	0.1158	-0.0040	-0.0279
Lactation 2	0.0683	0.0024	-0.0087
Lactation 3	0.0817	0.0141	-0.0249
Lactation 4	0.5265	0.0013	-0.1134
Lactation 5	0.1441	0.0479	-0.0567
Lactation 6	0.1806	0.0005	-0.0478
Lactation 7	0.3170	-0.0300	-0.0921

The values presented in Table 5 show that on the basis of the competitiveness index, the objective of selection in the Romanian buffalo population is the genetic improvement of milk yield, in all the lactations analyzed. The results obtained are supported by the large economic weight of the milk quantity and the importance of it for the quantity of Mozzarella cheese. It is very clear that, once the desired level of milk yield is reached, it may eventually improve the quality of it, especially the quality of protein.

CONCLUSIONS

The results regarding competitiveness index for selection objective establish indicates that, in analyzed population and according to our available data, the objective of selection in the Romanian buffalo population is the genetic improvement of milk yield. But, according to the genetic determinism of traits, became mandatory an optimization of the female selection criteria.

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COMPARE THE PERFORMANCE AND EGG QUALITY OF TWO STRAINS OF LAYING HENS HOUSED IN FURNISHED CAGES

Muzaffer DENLI, Ramazan DEMIREL, Muhittin TUTKUN

Dicle University, Agricultural Faculty, Department of Animal Science, 21280, Diyarbakır, Turkey

Corresponding author email: muzaffer.denli@gmail.com

Abstract

This study was carried out to compare indices of production and egg quality of two strains of laying hens (Lohmann Brown and Atak-S) housed in furnished cages. Lohmann Brown (LB) and Atak-S (AS) hens were housed in furnished cages (n= 700; 14 cages; 25 hens per cage; floor space 600 cm2/hen) from18 to 50 wk. Furnished cages included nests, perches and sandbathes. The hen day egg production, feed intake and egg weight of LB hens were higher than AS hens at week 40 and 50 (P<0.05). The AS had a significantly higher body weight (2074.1 g and 1900.2 g respectively) and lower mortality rate (7.1% and 12.7 % respectively); than LB hen at week 50 (P<0.05). However, no significant differences on egg quality parameters were noticed between LB and AS hens throughout the experiment (P > 0.05). In conclusion, our results showed that strain selection is important for productivity of laying hens rearing in furnished cages. Furthermore it can be concluded that performance of LB hens was better than AS hens in furnished cages.

Key words*: egg production, laying hens, furnished cages, Lohmann Brown, Atak-S.*

INTRODUCTION

Egg production system is probably one of the most important challenges for the egg producing industry in the last decade. There are various factors including diseases, behavior, nutritional value, genetics and air conditions in house affecting the level of welfare laying hens (Denli et al., 2016).

Housing in the cage is the most common system for growing of laying hens. Conventional cages have some welfare advantages particularly that they keep hens in small groups and hygienic conditions (Appleby et al., 2002).

Because of some disadvantages of conventional cages system on animal welfare and other problems various alternative housing systems have been carried out to minimize these negative effects.

Aviaries, free-range and enriched cage systems are in the acceptable alternative systems with regard to alleviate the problems of conventional cage systems.

Recently furnished (enriched) cage rearing system has been received a great attention as an alternative housing systems for laying hens after the ban decision on conventional cages in the European Union by 2012 (CEC, 1999). Laying hen's performance and production parameters such as egg weight, feed efficiency, daily feed consumption, and mortality may be influenced by the different housing systems (Taylor and Hurnik, 1996; Batkowska et al., 2014), genotype and age (Zita et al., 2009) and environmental conditions (Hester et al., 2005). Moreover, egg quality may also be affected by the housing systems (Vits et al., 2005) as well as the age of the laying hens (Silversides et al., 2006).

Up to now, the production performance and egg quality characteristics of many laying hens strains in different housing systems have been compared (Abrahamsson and Tauson, 1997; Van Den Brand et al, 2004; Mallet et al, 2006). Atak-S (AS) is a Turkish domestic egg laying strain has been developed by Ankara Poultry Research Institute in 2004 (Goger et al, 2016) and because of many reasons AS strains is preferred by farmers.

However, there is no enough knowledge on the performance of Atak-S (AS) strain in different housing systems. Therefore, the aim of the current study was to determine and compare indices of production and egg quality parameters of two strains of laying hens (Lohmann Brown and Atak-S) housed in furnished cages.

MATERIALS AND METHODS

A totally seven hundred 18-wk-old Lohmann Brown and Atak-S hens were housed in furnished cages (n= 700; 14 cages; 25 hens per cage; floor space 600 cm^2/hen) to 50 week of age. The furnished cages (120 x 55 x 45; length × width × height; floor space 600 cm^2/hen) had wire floors and solid metal walls. Hens were fed the same diet formulated was based on National Research Council (NRC) (1994) containing 17.5 % CP, 2800 ME/kg, 3.6% Ca and 0.90% available P. Thought the experiment lights were on a 16L:8D schedule. Feeders were filled manually every day and egg collection was conducted daily during the morning hours. Body weight and feed intake and feed efficiency were determined weekly throughout the experiment period. Egg production per group, per-cage-hen-day production and quality parameters were performed at of 20, 30, 40 and 50 week of age on the random sample of 30 eggs per treatment. Totally 30 eggs were collected (in the morning) from each group for 2 consecutive days and stored at 4°C overnight and then broken onto a level surface. Percentage of cumulative mortality of laying hens were recorded during the rearing and laying periods. Egg height, width and shell thickness 8 mm were measured by using micrometer screw from Mitutoya. The height of the albumen and yolk were measured by using tripod micrometer. The width of the albumen and yolk were measured by using a standard caliper. Yolk color was measured with a Roche yolk color fan scale (Roche scale). Statistical analysis was performed using the mixed model and t-test procedure of SPSS 15.0. Tukey's test was used to separate group means. A significant difference was at $P < 0.05$.

RESULTS AND DISCUSSIONS

Housing system and the strains have an important influence on the productive performance (Moorthy et al., 2000; Singh et al., 2009) and egg quality parameters of laying hens (Vits et al., 2005). Results of hen-egg production, feed consumption, feed efficiency and mortality are presented in Table 1. LB had higher egg production and lower feed consumption than AS both at week 40 and 50 ($P<0.05$). The observation concerning egg production of LB hens made in this study was agree to those obtained by Küçükyılmaz et al, 2012. In addition, a significant effect of strain on feed efficiency was observed in all periods of trial ($P<0.05$). On the other hand, the AS hens had a significantly higher body weight (2074.1 g and 1900.2 g respectively) and lower mortality rate (7.1% and 12.7 % respectively); than LB hens at week 50 ($P<0.05$).

Shell and internal quality of egg is important for the economic success of a producer and also consumer demands (Singh et al., 2009). Egg quality may be influenced by several factors including housing regimen, hen strain and nutritional values. There are differences in egg quality parameters between different strains (Hocking et al., 2003). In this study, we no found significant difference between strains regarding the egg shape index, shell weight and shell thickness regarding appearance from 20 to 50 week of age (Table 2). However, the egg weight of LB hens was higher than that of AS hens at week 30, 40 and 50 ($P<0.05$). Similar results were reported by Basmacioglu and Ergul, 2005, but, our results of shell thickness of egg differ from Küçükyılmaz et al., 2012 who found the egg shell thickness of eggs from LB hens were higher than that of eggs from AS layer hens in conventional and organic rearing systems.

The strain has effects on yolk and albumen quality characteristics of eggs (Tumova et al., 1993). The effects of strains on albumen height, albumen width, and yolk height and yolk width are shown in Table 3. In our study, we no found the significant differences between strains housed in furnished cages at wk 20, 30, 40 and 50 ($P>0.05$). In contrast, Leyendecker et al. (2001) found significantly higher yolk weight in white egg chickens (Lohmann LSL) in comparison with the brown Lohmann Tradition.

The strain influenced cracked and dirty egg numbers markedly (Table 4). The cracked egg numbers from LB hens at 40 week and 50 were higher than those from AS hens but less dirty egg numbers ($P<0.05$). Eggs from LB and AS hens had similar yolk color.

Table 1. Production performance of Lohmann Brown and Atak-S laying hens housed in furnished cages from 20 to 50 weeks of age

Period	Hen-egg production (%)		Feed consumption (g/hen per d)		Feed efficiency (g of feed/g of egg)		Mortality (%)	
	LB	AS	LB	AS	LB	AS	LB	AS
Wk 20	24.1±1.11	26.0±1.12	98.6±0.44	100.7±0.44	2.23^{b}±0.04	2.34^{a}±0.04	0.4±0.001	0.5±0.001
Wk 30	91.9±1.17	89.0±1.06	106.7±0.46	115.4±0.45	2.14^{b}±0.02	2.26^{a}±0.03	2.8^{a}±0.05	1.8^{b}±0.01
Wk 40	96.1^{a}±1.18	84.9^{b}±1.15	108.1^{b}±0.47	120.6^{a}±0.47	2.05^{b}±0.01	2.24^{a}±0.03	8.0^{a}±0.07	3.0^{b}±0.05
Wk 50	88.9^{a}±1.16	82.1^{b}±0.05	112.9^{b}±0.48	120.4^{a}±0.48	2.01^{b}±0.01	2.18^{a}±0.02	12.7^{a}±0.10^{a}	7.1^{b}±0.09

^{a,b}Means± SE within each period with different superscript letters are significantly different ($P < 0.05$).
LB = Lohmann Brown; AS= Atak-S

Table 2. Weight, shape index, shell weight and shell thickness of eggs of Lohmann Brown and Atak-S laying hens housed in furnished cages from 20 to 50 weeks of age

Period	Egg weight (g)		Shape index		Shell weight (g)		Shell thickness (mm)	
	LB	AS	LB	AS	LB	AS	LB	AS
Wk 20	46.7±0.17	45.2±0.16	77.6±0.35	77.6±0.35	6.23±0.07	6.04±0.04	0.32±0.006	0.33±0.007
Wk 30	60.3^{a}±0.28	57.1^{b}±0.25	77.4±0.35	75.8±0.33	6.62±0.08	6.44±0.06	0.30±0.004	0.30±0.005
Wk 40	62.8^{a}±0.32	60.9^{b}±0.76	76.6±0.34	74.5±0.33	6.70±0.08	6.67±0.08	0.29±0.003	0.31±0.003
Wk 50	64.8^{a}±0.32	62.4^{b}±0.47	77.2±0.27	76.7±0.38	7.1±0.08	7.4±0.11	0.27±0.003	0.28±0.004

^{a,b}Means± SE within each period with different superscript letters are significantly different ($P < 0.05$).
LB = Lohmann Brown; AS= Atak-S

Table 3. Albumen height and width, yolk height and width of eggs of Lohmann Brown and Atak-S laying hens housed in furnished cages from 20 to 50 weeks of age

Period	Albumen height (mm)		Albumen width (cm)		Yolk height (mm)		Yolk width (mm)	
	LB	AS	LB	AS	LB	AS	LB	AS
Wk 20	9.6±0.11	9.8±0.12	6.5±0.12	6.5±0.12	18.8±0.11	18.9±0.11	39.2±0.24	39.0±0.23
Wk 30	9.5±0.10	9.2±0.10	6.5±0.15	6.4±0.14	18.5±0.10	18.8±0.10	39.8±0.13	39.9±0.22
Wk 40	9.5±0.09	9.8±0.10	6.9±0.21	7.3±0.11	18.8±0.11	18.5±0.12	40.1±0.13	41.5±0.16
Wk 50	9.5±0.09	9.3±0.09	7.5±0.22	7.4±0.16	18.7±0.12	19.2±0.11	40.8±0.21	42.4±0.19

^{a,b}Means± SE within each period with different superscript letters are significantly different ($P < 0.05$).
LB = Lohmann Brown; AS= Atak-S

Table 4. Cracked, dirty eggs and yolk color of Lohmann Brown and Atak-S laying hens housed in furnished cages from 20 to 50 weeks of age

Period	Cracked eggs (%)		Dirty eggs (%)		Yolk color	
	LB	AS	LB	AS	LB	AS
Wk 20	4.80±0.04	4.88±0.04	0.07±0.001	0.02±0.001	11.1±0.11	11.0±0.09
Wk 30	3.80±0.04	3.90±0.03	0.23^{b}±0.001	0.46^{a}±0.003	12.0±0.13	11.6±0.11
Wk 40	2.41^{a}±0.02	1.42^{b}±0.01	0.05^{b}±0.001	0.10^{a}±0.001	11.7±0.13	11.9±0.14
Wk 50	2.08^{a}±0.02	1.35^{b}±0.02	0.10^{b}±0.001	0.17^{a}±0.001	12.1±0.16	12.1±0.12

^{a,b}Means± SE within each period with different superscript letters are significantly different ($P < 0.05$).
LB = Lohmann Brown; AS= Atak-S

CONCLUSIONS

Our results showed that strain selection is important for productivity of laying hens rearing in furnished cages. Furthermore it can be concluded that performance of LB hens was better than AS hens in furnished cages.

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DETERMINATION OF MILK YIELD CHARACTERISTICS OF KARAKAS SHEEP, BIRTH AND WEANING WEIGHT OF LAMBS IN FARM CONDITIONS

H. Deniz ŞİRELİ

Dicle University, Faculty of Agricultural, Department of Animal Science,
21280 Diyarbakır, Turkey

Corresponding author e-mail: hdsireli@gmail.com

Abstract

The aim of this study is to determine birth and weaning weights and milk yield characteristics of Karakaş sheep. The animal material of the study was composed by 187 lambs and 93heads of ewes aged between 2-6 in Diyarbakır province. Birth weights and weaning weights were found to be 4.08 kg and 18.56 kg in the females and 4.35 kg respectively 19.68 kg in the males. Lactation milk yields, lactation length and mean milk yields in the 2, 3, 4, 5 and 6 year old ewes were: 86.86, 95.74, 95.00, 94.50, 94.85 liters; 166, 92, 167, 47, 168, 79, 169, 36, 165 days; 480.5, 516.3, 529.2, 501.9, 589.0 gr, respectively. According to the research, the yield characteristics of Karakaş sheep breeders in Diyarbakır province were found to be satisfactory for the region.

Key words: *Karakaş Sheep, milk yield, birth weight, weaning weight.*

INTRODUCTION

Akkaraman sheep constitutes of 40-45% of sheep population in Turkey and has some different varieties. Karakaş sheep is an important variety of Akkaraman sheep, It raises as an important indigenous breed, in Elazığ, Diyarbakır, Bitlis and Van Province as well (Gökdal et al. 2003).

Determining the performance, yield and morphological characteristics of the native breeds raised in Turkey will contribute for the breeding of breeds and benefit the development of country's livestock policies (Karaca et al., 1996).

The results obtained from many studies carried out in breeder conditions tend to a successful establishment of breeding program which can respond to the expectations and orientations of breeders conventional production.

As in all the species, one of the most important values in small ruminants breeding is the heigher reproductive efficiency.

Another important fertility feature is the high birth and weaning weights and their productive life (Özmen et al., 2015).

The average milk yield of Akkaraman and Karakaş sheep is around 50-60 kg and lactation length is 140 days approximetly under the different breeding conditions.

The average birth and weaning weights of the lambs vary between 4.5-4.7 and 18-24 kg respectively (Anonymous, 2009).

In this study, it was aimed to determine the milk yield features, birth and weaning weights of Karakaş sheep in breeder conditions in order to increase the yields of conventional breeding enterprises and to provide a basis for the protection of native gene resources and other studies to bedone.

MATERIALS AND METHOD

Material

This research was carried out total 187 head of lambs which is 89 female and 98 male of lambs belongs to 93 head of Karakas ewes at the 2, 3, 4, 5 of age raised in Diyarbakir Höyükdibi Village in 2016.

Method

The study was started with the determination of their ages by looking at the teeth of Karakaş ewes.Immediately after birth, ear tag and disinfectionof umbilical cord care were applied. The birth weights were recorded by precision scale at 10 gr sensitivity. At the same time, gender and birth types of the lambs were also identified and recorded. Colostrum was given to the lambs after birth at 3 days periods.

Lambs were weaned at 2.5-3 months of age and weaning weights were determined and recorded. Ewes are not milked until the pasture season and were milked twice a day after season.
Lactation milk yields wasestimated by using the Trapeze II method based on monthly controls.
In milk control, milking was performed by hand. Milk yielded was determined as morning and eveningmilk which was detected with a precision scale of 10 gr sensitivity.
Lambs were breastfeeding half an hours after milking of ewes in the early hours of the morning, and the sheep were brought to the pasture.
Pastures are returned to the pasture for 8-10 hours, then after half an hour of rest, they are passed to the evening milk. After the end of the milking, the lambs are separated from the lambs after a half hour of absorption.
Ewes grazing for 8-10 hours in the pasture, were milked half an hour after returning to the farm. Following the milking, ewes had breastfeeding the lambs for half an hours than seperated. Additional feeding was also provided depending on the conditions of the pasture and weather conditions.

Statistical Analysis

In the study, the Khi-square method was used to compare features mentioned above.
The effects of environmental factors on the milk yield such as age, genderand type of birth on the live weight were determined by "Least Squares Method".
The control of the significance of differences between the subgroup meanings was done by the Tukey test (Kesici and Kocabas, 2007). SPSS 22 package program was used for statistical analysis.

RESULTS AND DISCUSSIONS

Birth and weaning weights
In the study, birth weight and weaning weight of the Karakas lamb in terms of sex and type of birth was given in Table 1 and Table 2.
According to this, male lambs were found to be heavier than both female lambs in terms of In the study contucted for Karakaş lambs by Gökdal et al. (1999), they reported the birth weight of male lambs was 4.03 kg, and 3.85 kg for female lambs.
They found birth weights in single lambs as 4.19 kg and in twin lambs as 3.64 kg when considering the type of birth.
Demirel et al. (2000) found that the birth weights of Karakaş lambs were 3.84 kg in their studies.both birth weight and weaning weight as well.
Bingöl and Aygün (2014) found that the birth weights in male lambs were 3.01 kg and 3.01 kg in female lambs.

Table 1. Birth and weaning weight of Karakaş lambs according to the sex

Lamb	n	Birth Weight (kg)			General	n	Weaning Weight (kg)			General
		Mean	Min	Max			Mean	Min	Max	
Female	89	4.08±053	2.72	5.26	4.22±0.06	59	18.56±3.05	11.63	24.00	19.08±3.38
Male	98	4.35±0.67	2.52	5.82		72	19.68±3.71	11.51	24.98	

The values found (Karaca et al., 1993; Aygün and Karaca, 1999; Öter, 2000) were lower than the birth weight obtained in this study.
Özge et al. (2015) determined birth weights as 3.74 kg in Akkaraman lambs.
The values obtained by different researchers were found to be lower than obtained in this study.
Ülker et al. (2004) found that the birth weights of male lambs were 4.78 kg, and 4.43 kg of female, 5.05 kg in single births and 4.16 kg in twins in their studies with Karakaş and Norduz lambs.
Birth weights obtained in this study was found higher than the study conducted by Karakuş et al. (2008) in Norduz and Karakaş lambs as 4.90 and 4.65 kg, respectively.
The results of the researchers' results are higher than the results obtained in this study. The differences were interpreted as due to using different breeds and different nutrition and environmental conditions in the enterprises.

Table 2. The birth and weaning weight of the Karakaş Lambs by birth type

Lambs	n	Birth weight (kg)			n	Weaning Weight (kg)		
		Mean	Min	Max		Mean	Min	Max
Single	161	4.32±0.58	2.52	5.82	119	19.81±2.89	14.19	24.94
Twin	26	3.59±0.51	2.52	4.66	12	16.67±3.90	11.51	24.98

Weaning Weight

Gökdal et al. (1999) were found the average weaning weight of 118 day weaned Karakasmale Lambs as 26.32 kg, while in female lambs as 23.85 kg. They found weaning weight in single born lambs as 25.63 kg, for twin lambs as 24.54 kg when considering of birth type.

Demirel et al. (2000) weaned Karakaş lambs at 90 days of age and they have identified the weaning weight in kg 21.46 kg. These values were found higher than this study.

While the values obtained from the studies performed by different researchers (Bingöl, 1998; Akçapınar and Kadak, 1982) were found to be higher than the values obtained from this study.

Gökdal (1998), Gökdal et al. (1999) conducted a study with Karakaş lambs weaned on the 90th day, they found weaning weight as 18.93 kg and 20.43 kg respectively. Bingol (1998) reported weaning weight of Norduz lambs as 20.27 kg. Öter (2000) reported the weight of weaning weight for Karakaş lambs was similar to that of 19.72 kg.

Bingol and Aygun (2014) reported the Karakas lambs weaned on 90 days of age lamb male weaning weights as 14.99 kg, while in female lambs 14.82 kg.

According to the type of birth, They found the weaining weight as 15.20 kg in singleborn lambs while in 15.20 the twins born lambs as 14.61 kg.

They found that the weaning weights were lower than obtained from this study.

As a result, we should interpret the results obtained in different researches in terms of birth and weaning weights to be different, due to used different breeds and hybrid genotypes in their studies, and the studies were conducted under different climatic, geographical and growing conditions and durations as well.

Lactation Length, Milk Yield and Daily Average Milk Yields

The lactation milk yield, lactation length and daily average milk yield results beginning after one week of birth to dry period in Karakas ewes were given in Table 3.

Table 3. Daily Milk Yield, Lactation Length and Daily Average Milk Yields by age in Karakas Sheep

Features		Lactation Milk Yield (Liters)			Lactation Length (Day)			Daily Average Milk Yields (gr)		
Age	n	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
2	26	86.86±19.96	47.05	132.32	166.92±1.38	151.00	179.00	480.5±19.6	151.00	179.00
3	35	95.74±24.99	45.35	157.57	167.47±0.96	152.00	176.00	516.3±21.2	152.00	176.00
4	14	95.00±18.60	61.52	124.27	168.79±2.00	157.00	185.00	529.2±33.6	157.00	185.00
5	14	94.50±19.51	67.58	149.64	169.36±1.56	160.00	184.00	501.9±31.3	160.00	184.00
6	4	94.85±15.51	73.52	110.65	165.00±4.38	152.00	171.00	589.0±18.2	152.00	171.00
General	**93**	**92.91±21.55**	**45.35**	**157.57**	**167.50**	**154.40**	**179.00**	**523.38**	**154.40**	**179.00**

Lactation Milk Yield

Aydoğan and Gül (1992) found the average lactation milk yield as 49 liters in Karayaka ewes which was lower than the values obtained in Karakaş sheep in this study due to the effect of different breed characteristics.

Gökdal (1998) reported that the lactation milk yields in the 3, 4, 5 and 6 years old Karakaş sheep were 39.42, 56.77, 61.50, 59.97 liters, respectively.

Gökdal et al. (2000) reported the average milk yield of Karakas sheep raised in village as 59 liters.

Gökdal et al. (2000) reported that the lactation milk yields of 2,3,4 and 5 years old ages of Karakaş sheep as 44.4, 63.7, 62.9 and 64.8 liters, respectively.

Altın (2001) has reported that average milk yields in 2, 3, 4 and 5 year old Karakaş sheep were: 55.1, 55.6, 69.7 and 47.1 liters, respectively.
Lactation milk yield obtained in this study were significantly higher than any of the reported literature value.
The variation in the milk yields of Karakaş sheep can be explained by the fact that due to the regional conditions were different.
Karakaş sheeps which have high adaptability ability in poor maintenance and feeding conditions are indispensable due to this ability to adapt to the regional conditions.

Lactation Length

Aydogan and Gül (1992) found that the average lactation length as 131 days in Karayaka was lower than the Karakaş sheep due to the different race characteristics (Table 3).
Cengiz et al. (1998) reported the lactation length as 166.13, 169.54, 167.79 and 157.12 days in the Karakaş sheep weaned at 45, 60, 75 and 90 days of age respectively. These values was shown similarity to those obtained in this study.
Gökdal (1998) reported that the length of lactation in the Karakaş sheep of 3, 4, 5 and 6 years old was 169.6, 195.3, 213.2, 205.7 days, respectively.
The lactation length of 3 years older sheep was shown similarity while values for other age grup were found higher with the work which was done.

CONCLUSIONS

In this study, it was aimed to determine the birth weights, weaning weights, lactation milk yields, lactation length and daily avarage milk yields of Karakaş sheep in breeders conditions.
The milk yield characteristics, birth and weaning weights of the Karakaş sheep show similar characteristics when compared to the findings obtained from studies conducted on native breeds raised in Turkey.
In this regard, although Karakaş sheep are not at a satisfactory level in terms of the characteristics discussed, this breed are an important source of production when considering the conditions of the breeding enterprises. Together with the identification of the yield performances of the Karakaş sheep which is one of the native gene sources, studies on improving the yield characteristics of this sheep breed provide the necessary information to protect native gene resources of Turkey.
In terms of protection of gene resources of native sheep breeds have to be protected in their natural environment.It is important that Karakaş sheep breeders continue their breeding activities and do not leave their sheep breeding habits. In this sense, studies aiming to reveal the characteristics of Turkey' native sheep breeds gain importance.

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A PART OF GENETIC HISTORY IN HORSES: IMPORTANT ANCESTORS OF GIDRAN HORSE FROM TULUCESTI STUDFARM

Marius MAFTEI, Gheorghe MARGINEAN, Iulian VLAD,Razvan POPA, Dana POPA, Elena POGURSCHI, Lucica NISTOR, Daniela IANITCHI, Andra SULER, Paula POSAN

University of Agricultural Sciences and Veterinary Medicine of Bucharest, 59 Marasti, District 1, code 011464,Bucharest, Romania

Coresponding author: mariusmaftei@yahoo.com

Abstract

Study of important ancestors give us information about breeding methodic in analized population (origins, selection on ascendants) and also about possibilities of breed evolution (small number of ancestors, maintaining the genetic similarity with remarkable ancestors or the preference for descendants of certain individuals, may lead to inbreeding and genetic drift).In pedigrees of Gidran horse, from Tulucesti studfarm, was identified 104 male ancestors. It was ordered by three criteria: the kinship with sire stallions, the relationship with brood mares, and with the population, as a whole (reproductive nucleus, stallions and mares) at that time. The results highlight the fact that the kinship share of male ascendants in the population is between 0% and 19.46%. Eight stallions have a higher genetic resemblance to the population, ranging from 15.84% to 19.46%. The relatively small differences between sire stallions reveal concerns for maintaining a large number of males, necessary to avoid inbreeding and to ensure a normal population evolution under reproductive isolation.

Key words*: Gidran, Tulucesti, ancestors, relationship, stallion.*

INTRODUCTION

The Gidran horse is an anglo-arabian population, that has been formed in parallel in Mezohegyes and Radautistud farms (Marginean, 2012). Due to the small size of population, it became one of the preserved breeds. These kind of researches could facilitate conservation efforts for preserving the genetic diversity of the Gidran horse (Sziszkosz et al., 2016).

This study is just a part of an ample research regarding the genetic history of most important Romanian horse breeds. We must say that we had this opportunity because of a good collaboration with National Autthority for Forestry, with the support of specialists from Horse Breeding Department. This study of important ancestors, in Gidran horse, give us information about breeding methodic in analized population (origins, selection on ascendants) and also about possibilities of breed evolution (small number of ancestors, maintaining the genetic similarity with remarkable ancestors or the preference for descendants of certain individuals, may lead to inbreeding and genetic drift).

MATERIALS AND METHODS

In pedigrees of Gidran population, from Tulucesti studfarm, it was identified 104 sire stallions.They were odered through three chriterias: relatedness with sire stallions, with brood mares (mother mares), and with overall population at the time of research (Table 1a and 1b). Method used was „The numerator relationship matrix" elaborated by Henderson and Cuningham in 1976, presented for the first time in Romania by Grosu in 1997.

RESULTS AND DISCUSSIONS

The results highlight the fact that the share of male ascendants in the population is between 0% and 19.46%. Eight stallions have a higher genetic resemblance to the population, ranging from 15.84% to 19.46%. The relatively small differences between the sires reveal the preoccupations for maintaining a large number of males, necessary to avoid inbreeding and to ensure a normal evolution of the population, under conditions of reproductive isolation (Figure 1). Our desire was to identify the stallions that are relatedness at least 15% with

population. So, in the population's pedigree of the Gidran from Tuluceşti studfarm, we identified, with maximum certainty, 8 such ancestors.

Table 1a. Genetic relationship of male ascendants with sire stallions, brood mares and with overallpopulation

INDIVIDUALS	CODE	Ra	$S_{\bar{X}}$	Ri	$S_{\bar{X}}$	R pop	$S_{\bar{X}}$
G K	1	0.0273	0.0093	0.0273	0.0022	0.0273	0.0022
SACHMAT	4	0.0273	0.0093	0.0273	0.0022	0.0273	0.0022
SOUVENORDER	6	0.0137	0.0067	0.0113	0.0016	0.0116	0.0016
PERIS	8	0.0137	0.0067	0.0113	0.0016	0.0116	0.0016
G 46	10	0.0530	0.0063	0.0380	0.0031	0.0400	0.0029
G 60	12	0.1060	0.0126	0.0760	0.0062	0.0800	0.0059
G IX	15	0.1094	0.0106	0.0872	0.0071	0.0901	0.0064
G 53	18	0.0532	0.0050	0.0363	0.0028	0.0386	0.0027
TABOR	21	0.0176	0.0067	0.0117	0.0018	0.0125	0.0018
OLEANDER	23	0.0176	0.0067	0.0117	0.0018	0.012	0.0018
RAUFBOLDT	25	0.0352	0.0133	0.0234	0.0036	0.0250	0.0036
RAZBEG	27	0.0703	0.0267	0.0469	0.0073	0.0500	0.0072
G XI	30	0.0618	0.0117	0.0430	0.0044	0.0455	0.0042
G XIII	31	0.0901	0.0103	0.0713	0.0060	0.0738	0.0054
G XV	33	0.0773	0.0121	0.0567	0.0053	0.0595	0.0050
G XIX	34	0.1251	0.0233	0.0871	0.0084	0.0922	0.0081
K VI	37	0.0410	0.0128	0.0262	0.0024	0.0282	0.0027
K VIII	39	0.0674	0.0149	0.0485	0.0048	0.0510	0.0046
K XIX	40	0.0513	0.0102	0.0397	0.0049	0.0412	0.0044
G VIII	41	0.0117	0.0068	0.0103	0.0019	0.0105	0.0019
G VI	46	0.0547	0.0186	0.0545	0.0044	0.0546	0.0044
G XIV	48	0.1094	0.0372	0.1091	0.0088	0.1091	0.0087
G 54	52	0.0000	0.0000	0.0027	0.0011	0.0023	0.0010
G L	55	0.0234	0.0070	0.0139	0.0022	0.0152	0.0021
G X	61	0.0000	0.0000	0.0074	0.0026	0.0064	0.0023
G XII	64	0.1233	0.0269	0.0797	0.0092	0.0855	0.0090
G IV	68	0.0486	0.0168	0.0246	0.0024	0.0278	0.0032
G XVII	69	0.0989	0.0196	0.0884	0.0087	0.0898	0.0079
H VII	70	0.0000	0.0000	0.0313	0.0047	0.0271	0.0046
HADIK	77	0.0352	0.0133	0.0306	0.0051	0.0313	0.0047
G 52	82	0.0098	0.0074	0.0026	0.0001	0.0035	0.0013
G 53'	84	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SB I 5	85	0.0078	0.0078	0.0021	0.0001	0.0029	0.0013
G VII	92	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
WIZZER	93	0.0273	0.0133	0.0225	0.0032	0.0232	0.0032
H IV	95	0.0000	0.0000	0.0198	0.0025	0.0120	0.0023
G III	101	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
K G I	104	0.1193	0.0192	0.0843	0.0066	0.0890	0.0065
K X	111	0.0352	0.0174	0.0169	0.0020	0.0194	0.0029
M V	122	0.0000	0.0000	0.0138	0.0025	0.0120	0.0023
M VI	123	0.0000	0.0000	0.0138	0.0025	0.0120	0.0023
GAZAL V	125	0.0000	0.0000	0.0138	0.0025	0.0120	0.0023
N PERKUN	129	0.0000	0.0000	0.0138	0.0025	0.0120	0.0023
S XXVIII	131	0.0000	0.0000	0.0138	0.0025	0.0120	0.0023
N IV	134	0.0000	0.0000	0.0138	0.0025	0.0120	0.0023
S XVIII	140	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
G LIII	142	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
G 49	143	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

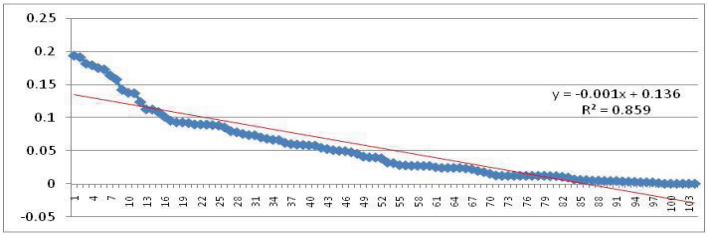

Figure 1. Genetic relationship of male ascendants with population

Table 1b. Genetic relationship of male ascendants with sire stallions, brood mares and with overallpopulation

INDIVIDUALS	CODE	Ra	$S_{\bar{x}}$	Ri	$S_{\bar{x}}$	R pop	$S_{\bar{x}}$
PRINCE ORANGE II	144	0.0000	0.0000	0.0024	0.0017	0.0021	0.0014
ARKINA	146	0.0000	0.0000	0.0048	0.0033	0.0042	0.0029
MANPAEOD	148	0.0000	0.0000	0.0024	0.0017	0.0021	0.0014
FIRDAUSSI	150	0.0000	0.0000	0.0054	0.0030	0.0047	0.0026
FANTASTIC	153	0.0000	0.0000	0.0063	0.0036	0.0055	0.0031
CORDON ROUGE	155	0.0000	0.0000	0.0036	0.0020	0.0031	0.0017
M IX	160	0.0000	0.0000	0.0276	0.0050	0.0240	0.0047
H IX	163	0.0000	0.0000	0.0276	0.0050	0.0240	0.0047
M VIII	166	0.0000	0.0000	0.0276	0.0050	0.0240	0.0047
PETRUS	173	0.0000	0.0000	0.0012	0.0012	0.0010	0.0010
G XVIII	177	0.0734	0.0220	0.0583	0.0082	0.0603	0.0076
G XXI	179	0.1227	0.0287	0.0887	0.0087	0.0932	0.0085
G XXII	180	0.1480	0.0505	0.0673	0.0094	0.0781	0.0113
G XXV	181	0.0612	0.0189	0.0514	0.0058	0.0527	0.0055
G XXVII	183	0.1383	0.0455	0.1088	0.0150	0.1128	0.0142
G XXIX	196	0.0948	0.0381	0.0704	0.0112	0.0737	0.0108
G XXIV	202	0.1367	0.0546	0.1221	0.0117	0.1241	0.0120
K XIII	203	0.0454	0.0189	0.0243	0.0024	0.0271	0.0033
RIVOL	207	0.0000	0.0000	0.0048	0.0033	0.0042	0.0029
MANTALORZ	209	0.0000	0.0000	0.0048	0.0033	0.0042	0.0029
EPIGOR	211	0.0000	0.0000	0.0054	0.0034	0.0047	0.0029
M XIII	213	0.0000	0.0000	0.0553	0.0100	0.0479	0.0093
H XVI	215	0.0000	0.0000	0.0571	0.0098	0.0495	0.0092
G XXIII	217	0.1167	0.0521	0.0610	0.0072	0.0684	0.0094
RG I	224	0.1424	0.0340	0.1086	0.0159	0.1131	0.0145
G XXX	226	0.0885	0.0291	0.0676	0.0069	0.0704	0.0070
G XXVIII	229	0.0905	0.0171	0.1037	0.0141	0.1019	0.0124
G XXXI	231	0.2201	0.0531	0.1563	0.0173	0.1648	0.0167
G XXXII	232	0.2120	0.1075	0.0751	0.0114	0.0933	0.0182
G XXXIV	235	0.0692	0.0228	0.0562	0.0082	0.0579	0.0076
G XXVI	248	0.0802	0.0138	0.0643	0.0076	0.0664	0.0068
M XI	251	0.0000	0.0000	0.0276	0.0050	0.0240	0.0047
GAL	252	0.0000	0.0000	0.0108	0.0075	0.0094	0.0065
SATELIT	254	0.0352	0.0133	0.0316	0.0071	0.0321	0.0063
M XVIII	257	0.0000	0.0000	0.1106	0.0200	0.0958	0.0186
G XXXV	260	0.0808	0.0232	0.0647	0.0113	0.0668	0.0102
COPIL	263	0.0000	0.0000	0.0068	0.0050	0.0059	0.0044
G K I	265	0.0596	0.0096	0.0589	0.0094	0.0590	0.0082
G XXXVI	272	0.0982	0.0226	0.0888	0.0088	0.0900	0.0081
G XXXIX	274	0.2400	0.1040	0.1636	0.0251	0.1737	0.0253
G XXV 38	276	0.0762	0.0079	0.0554	0.0061	0.0582	0.0055
G XXVIII	279	0.0905	0.0171	0.0881	0.0085	0.0884	0.0076
GR I	285	0.1784	0.0652	0.1310	0.0249	0.1374	0.0230
NANDOR	288	0.0000	0.0000	0.0204	0.0138	0.0177	0.0120
G XXXVII	290	0.0689	0.0066	0.0768	0.0137	0.0758	0.0119
M XXII	292	0.0000	0.0000	0.2216	0.0400	0.1921	0.0373
G XXXVIII	295	0.1997	0.1108	0.1800	0.0297	0.1826	0.0288
NAUM	297	0.0176	0.0067	0.0228	0.0096	0.0221	0.0083
G XL	303	0.1913	0.1120	0.1301	0.0196	0.1383	0.0218
GRUIA	318	0.0088	0.0033	0.0449	0.0270	0.0401	0.0234
G XLIV	322	0.1309	0.0254	0.2019	0.0295	0.1946	0.0268
G XLV	324	0.1678	0.0550	0.1772	0.0343	0.1759	0.0304
G XLII	326	0.1109	0.0350	0.1881	0.0361	0.1801	0.0328
G XLVI	330	0.2141	0.1061	0.1498	0.0237	0.1584	0.0243
G XLIII	340	0.0791	0.0214	0.0602	0.0062	0.0621	0.0059
G XLVII	341	0.1399	0.0136	0.1430	0.0148	0.1427	0.0133

Note
Ra = average relatedness with sire stallions;
Ri = average relatedness with broodmares;
R pop. = average relatedness with population.

Table 2. Important ancestors in Gidranpopulational pedigree

Ancestor's name	Code	Relatedness with:		
		Sire stallions	Broodmares	Population
G XLVI	330	0.2141	0.1498	0.1584
G XXXI	231	0.2201	0.1563	0.1648
G XXXIX	274	0.2400	0.1636	0.1737
G XLV	324	0.1678	0.1772	0.1759
G XLII*	326	0.1109	0.1881	0.1801
G XXXVIII	295	0.1997	0.1800	0.1826
M XXII	292	0.0000	0.2216	0.1921
G XLIV*	322	0.1309	0.2019	0.1946

* Active in the current reproductive nucleus

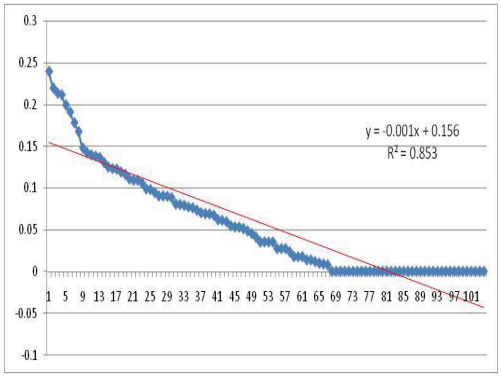

Figure 2. Genetic relationshipof males ascendant with sire stallions from current reproductive nucleus

In terms of reproductive isolation, homozygosity has the meaning of maintaining greater genetic similarity with valuable reproducers (Popa, 2009). In the Gidran population from Tulucești studfarm, it was considered the presence of the descendants from as many stallions as possible, thus maintaining genetic diversity and creating a field of action for selection. There are, however, 8 male ascendants, whose descendants were selected mainly for the basic flock.

Regarding the relationship of male ascendants with current sire stallions, were determined values ranging from 0% to 24%. 37 male offspring of the population have the relatedness coefficient equal with 0, at the time of analysis, of which more than 30 are immigrants (one part is in the ascendancy of the mares, the other is not genetically persistent in the population). At the other end of the series of relatedness coefficients, between the male ascendants and the actual sire stallions (ordered according to the coefficients values), there are 4 stallions with a strong accentuated relationship with the sire stallions: G XXXII (21.20%), G XLVI 21.41%), G XXXI (22.01%) and G XXXIX (24%) - Figure 2. These stallions have a greater participation in the establishment of the population's genetic found, being present in a significant proportion in the pedigrees of the current sire stallions.

Regarding the relatedness of the 104 male ascendants with the current stock of broodmares, from the graph shown in figure 3, it is noted the existence of coefficients of relatedness between 0% and 22.16%. Only 6 males from the population's ascendancy have a null value of the relatedness coefficient with the broodmares at the time of the analysis. 4 male ascendants have a stronger relationship with the mare herd, which also determines the high values of the population with the whole population: M XXII (22.16%), G XLIV (20.19%), G XLII (18.81%) and G XXXVIII (18%).

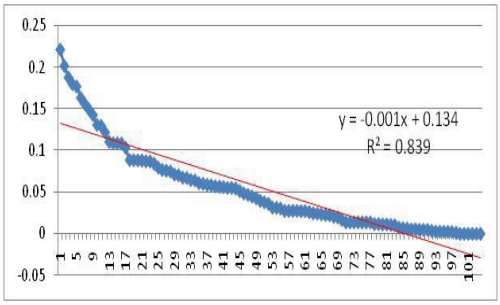

Figure 3. Genetic relationship of males ascendants with current broodmares

CONCLUSIONS

Great resemblance to the population of important ancestors is explained firstly by the adoption of a working model specific to the desire to maintain genetic resemblance with remarkable ancestors.

According to this model, the population is not maintained as a whole, but the mating plan allows the pairing of coupleswhich presents in the pedigrees common ascendants.

The values of the population of these important ancestors are determined by their presence in the pedigrees of both, stallions and mares, with some equilibrium (certainly undisturbed).

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PRODUCTIVITY AND EGG QUALITY OF TWO HEN GENOTYPES KEPT IN FREE-RANGE SYSTEM

Muhittin TUTKUN, Muzaffer DENLI*, Ramazan DEMIREL

Dicle University, Agricultural Faculty, Department of Animal Science, 21280
Diyarbakır, Turkey

*Corresponding author email: muzaffer.denli@gmail.com

Abstract

The purpose of this study was to compare the performance and egg quality parameters of two hybrids of laying hens (Lohmann Brown and Atak-S) reared in free-range system. The experiment was carried out with a total of 300 laying hens. From 18 to 50 weeks of age Lohmann Brown (LB) and Atak-S (AS) were housed in two groups of 150 hens in a poultry house with a stocking density of 7 hens/m^2. Feed intake and feed conversion rate during the all laying period were 111.2 g vs 124.3 g, and 2.46 vs 2.58 respectively for LB and AS genotypes ($P<0.05$). The AS had a significantly higher body weight (2200.5 g and 2022.2 g respectively) than LB hen at week 50 ($P<0.05$). There was no a significant difference concerning the egg production between two genotypes ($P>0.05$). However, egg weight was significantly higher in LB genotype than AS genotype ($P<0.05$). Similarly, no significant difference was found between two genotypes on egg quality characteristics throughout the experiment ($P>0.05$). In conclusion, our results showed that strain selection is important for productivity of laying hens rearing in free-range system.

Keywords: *productivity, egg quality, free-range system, Lohmann Brown, Atak-S.*

INTRODUCTION

Egg production system is probably one of the most important challenges for the egg producing industry in the last decade. There are various factors including diseases, behavior, nutritional value, genetics and air conditions in house affecting the level of welfare laying hens (Denli et al., 2016).

The great majority of egg chickens are grown in cages in the world. However, the results obtained from scientific studies in recent years have revealed that chickens raised in traditional cages may not meet the physiological and behavioral requirements (Bozkurt, 2009). After finding that the breeding conditions in conventional cages affected the animal welfare negatively. The European Parliament passed the decision "to ban the use of cages" in 1999 and it was decided to be implemented until 2012. After this directive (1999/74 / EC), it is permitted for the use of enriched cages (Lumvery, 1999). After the ban in 2012, the search for alternative breeding systems for laying hens has accelerated. Animal welfare is a definition that prescribes the quality of life of an animal by creating the conditions that animals can show their natural behavior.

The use of cages enriched instead of the traditional cage system has come to the forefront. Other alternative breeding systems that keep animal welfare in the forefront are free-range and aviary systems. In some countries such as Germany and the Netherlands, the poultry industry has a tendency towards fully alternative breeding systems while the enriched cage system in the UK, Belgium and Sweden has come to the forefront (Rodenburg et al., 2005).

In alternative breeding systems, chickens are able to exhibit many natural behaviors, walking and have enough exercises. Among the factors influencing the selection of breeding systems are epidemic diseases, behavior, nutritional value, genotype and environmental conditions (Denli et al., 2016). However, due to some disadvantages and other problems in the animal welfare of the conventional cage breeding system, various alternative systems have been carried out in order to minimize negative effects of conventional cage. Free-range and enriched cage systems are acceptable alternative breeding systems in terms of alleviating the problems of conventional cage systems. Laying hen's performance and production parameters such as egg weight, feed

efficiency, daily feed consumption, and mortality may be influenced by the different housing systems (Taylor and Hurnik, 1996; Batkowska et al., 2014), genotype and age (Zita et al., 2009) and environmental conditions (Hester et al., 2005). Moreover, egg quality may also be affected by the housing systems (Vits et al., 2005) as well as the age of the laying hens (Silversides et al., 2006)

Up to now, the production performance and egg quality characteristics of many laying hens strains in different housing systems have been compared (Abrahamsson and Tauson, 1997; Van Den Brand et al., 2004; Mallet et al., 2006). Atak-S (AS) is a Turkish domestic egg laying strain has been developed by Ankara Poultry Research Institute in 2004 (Goger et al., 2016) and because of many reasons AS strains is preferred by farmers. However, there is no enough knowledge on the performance of Atak-S (AS) strain in different housing systems. In this study, we aimed to determine and compare indices of production and egg quality parameters of native (Atak-S) and foreign (Lohmann Brown) laying hybrids reared in free-range systems.

MATERIALS AND METHODS

A totally three hundreds 18-wk-old Lohmann Brown and Atak-S hens were housed in free-range systems (n= 300; 10 houses; 15 hens per house; floor space 200 cm^2/hen) to 50 week of age. Hens were fed the same diet formulated was based on National Research Council (NRC) (1994) containing 17.5% CP, 2800 ME/kg, 3.6% Ca and 0.90% available P. Thought the experiment lights were on a 16L:8D schedule. Feeders were filled manually every day and egg collection was conducted daily during the morning hours. Egg weight, feed intake and feed efficiency were determined weekly throughout the experiment period. Egg production per group, per-house-hen-day production and quality parameters were performed at of 20, 30, 40 and 50 week of age on the random sample of 30 eggs per treatment. Totally 30 eggs were collected (in the morning) from each group for 2 consecutive days and stored at 4°C overnight and then broken onto a level surface. Percentage of cumulative mortality of laying hens were recorded during the rearing and laying periods. Egg height, width and shell thickness 8(mm) were measured by using micrometer screw from Mitutoya. The height of the albumen and yolk were measured by using tripod micrometer. The width of the albumen and yolk were measured by using a standard caliper. Yolk color was measured with a Roche yolk color fan scale (Roche scale). Statistical analysis was performed using the mixed model and t-test procedure of SPSS 15.0. Tukey's test was used to separate group means. A significant difference was at $P < 0.05$.

RESULTS AND DISCUSSIONS

Housing systems in layer have an important influence on the productive performance (Moorthy et al., 2000; Singh et al., 2009) and egg quality parameters of laying hens (Vits et al., 2005). Research results relating to hen-egg production, feed consumption, feed efficiency and mortality was presented in Table 1. LB had higher egg production than AS at week 20 and week 30, However, the egg production of AS hens was higher than that of LB hens at week 50 ($P<0.05$). Feed consumption of AS hens was found higher than LB at week 40 and 50 ($P<0.05$). The observation concerning egg production of LB hens made in this study was agree to those obtained by Küçükyılmaz et al. (2012). In addition, a significant effect of strain on feed efficiency was found in all periods of trial ($P<0.05$). On the other hand, the LB hens had a lower mortality rate (0.7% and 1.8% respectively) than AS hens at week 30 ($P<0.05$).

Shell and internal quality of egg is important for the economic success of a producer and also consumer demands (Singh et al., 2009). Egg quality may be influenced by several factors such as housing systems, hen strain and nutritional values. There are differences in egg quality parameters between different strains (Hocking et al., 2003). In this study, there was no significant difference between the egg shape index, shell weight and shell thickness regarding appearance from 20 to 50 week of age (Table 2). However, the egg weight of LB hens was higher than that of AS hens at week 30, 40 and 50 ($P<0.05$). Similar results were reported by Basmacıoğlu and Ergul (2005). However, results of shell thickness of egg was

shown difference from Küçükyılmaz et al. (2012) who found the egg shell thickness of eggs from LB hens were higher than that of eggs from AS layer hens in conventional and organic rearing systems.

The strain has effects on yolk and albumen quality characteristics of eggs (Tumova et al., 1993). The effects of strains on albumen height, albumen width, and yolk height and yolk width was shown in Table 3. In the study, no the significant differences was found between strains housed in furnished cages at week 20, 30, 40 and 50 (P>0.05). In contrast, Leyendecker et al. (2001) found significantly higher yolk weight in white egg chickens (Lohmann LSL) in comparison with the Brown Lohmann.

The strain influenced cracked and dirty egg numbers in a marked manner (Table 4). The cracked egg numbers from LB hens at 20 week was found higher than those from AS hens (P<0.05). Eggs from LB and AS hens have shown similar yolk color.

Table 1. Production performance of Lohmann Brown and Atak-S hens housed in free-range (20 to 50 week of age)

Period	Hen-egg production (%)		Feed consumption (g/hen per d)		Feed efficiency (g of feed/g of egg)		Mortality (%)	
	LB	AS	LB	AS	LB	AS	LB	AS
Wk 20	36.4^{a}±1.41	22.8^{b}±1.18	100.2±0.9	100.1±0.6	2.61^{b}±0.07	2.78^{a}±0.01	0.0±0.00	0.0±0.00
Wk 30	95.4±1.22	95.2±1.42	113.4±0.1	118.8±0.7	2.49^{b}±0.06	2.62^{a}±0.08	0.7^{b}±0.05	1.8^{a}±0.01
Wk 40	95.7^{a}±1.18	93.9^{b}±1.46	118.6^{b}±0.7	126.4^{a}±0.1	2.37^{b}±0.01	2.56^{a}±0.08	2.8±0.07	2.5±0.03
Wk 50	85.3^{b}±1.16	87.3^{a}±1.36	113.4^{b}±0.5	124.3^{a}±0.2	2.33^{b}±0.01	2.42^{a}±0.09	4.8±0.10	5.0±0.06

^{a,b}Means± SE within each period with different superscript letters are significantly different ($P < 0.05$).
LB = Lohmann Brown; AS= Atak-S

Table 2. Weight, shape index, shell weight and shell thickness of eggs of Lohmann Brown and Atak-S laying hens housed in free-range from 20 to 50 week of age

Period	Egg weight (g)		Shape index		Shell weight (g)		Shell thickness (mm)	
	LB	AS	LB	AS	LB	AS	LB	AS
Wk 20	46.3±0.12	44.1±0.12	78.2±0.31	77.0±0.29	5.10±0.04	4.85±0.02	0.34±0.007	0.33±0.007
Wk 30	58.4^{a}±0.22	54.8^{b}±0.33	78.1±0.42	75.8±0.29	6.38±0.04	5.90±0.07	0.33±0.008	0.34±0.006
Wk 40	64.9^{a}±0.28	60.1^{b}±0.52	76.1±0.38	74.9±0.46	7.17±0.07	6.38±0.05	0.34±0.005	0.36±0.004
Wk 50	65.4^{a}±0.42	61.8^{b}±0.38	76.2±0.62	76.3±0.42	7.16±0.12	6.71±0.15	0.32±0.004	0.31±0.006

^{a,b}Means± SE within each period with different superscript letters are significantly different ($P < 0.05$).
LB = Lohmann Brown; AS= Atak-S

Table 3. Albumen height and width, yolk height and width of eggs of Lohmann Brown and Atak-S laying hens housed in free-range from 20 to 50 week of age

Period	Albumen height (mm)		Albumen width (cm)		Yolk height (mm)		Yolk width (mm)	
	LB	AS	LB	AS	LB	AS	LB	AS
Wk 20	9.6±0.10	9.8±0.12	6.4±0.12	6.6±0.12	18.5±0.13	18.7±0.16	37.2±0.32	38.2±0.31
Wk 30	9.6±0.11	9.3±0.11	6.7±0.15	6.7±0.18	18.9±0.15	18.3±0.13	39.0±0.14	38.6±0.28
Wk 40	9.5±0.09	9.8±0.10	6.5±0.12	7.0±0.23	18.8±0.11	18.8±0.11	40.2±0.15	40.5±0.18
Wk 50	9.3±0.09	9.5±0.11	7.3±0.20	7.8±0.12	18.7±0.13	18.8±0.14	41.7±0.28	42.4±0.38

^{a,b}Means± SE within each period with different superscript letters are significantly different ($P < 0.05$).
LB = Lohmann Brown; AS= Atak-S

Table 4. Cracked, dirty eggs and yolk color of LB and AS laying hens housed in in free-range from 20 to 50 week of age

Period	Cracked eggs (%)		Dirty eggs (%)		Yolk color	
	LB	AS	LB	AS	LB	AS
Wk 20	2.44[a]±0.06	0.54[b]±0.01	0.00±0.00	0.00±0.00	11.0±0.14	11.2±0.08
Wk 30	1.04±0.05	1.14±0.01	0.12±0.001	0.19±0.001	11.4±0.16	11.8±0.13
Wk 40	0.10±0.01	0.78±0.01	0.00±0.001	0.00±0.000	12.1±0.12	12.0±0.13
Wk 50	0.85±0.02	0.89±0.01	0.0±0.00	0.0±0.00	12.0±0.14	12.2±0.11

[a,b]Means± SE within each period with different superscript letters are significantly different ($P < 0.05$).
LB = Lohmann Brown; AS= Atak-S

CONCLUSIONS

In conclusion, our results showed that strain selection is important for productivity of laying hens rearing in free-range system. Furthermore it can be concluded that performance of LB laying hens was better than AS hens in free-range system.

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IMPLEMENTATION OF THE GENETIC POTENTIAL OF PRODUCTIVITY OF COWS OF HOLSTEIN BREED IN THE SOUTH OF R. MOLDOVA

Aleksandra KONSTANDOGLO[1], V. FOKSHA[1], A. KENDIGELYAN[2], I. AKBASH[2]

[1]Scientific and Practical Institute of Biotechnologies in Zootechny and Veterinary Medicine, Republic of Moldova
[2]Society of limited liability „DokSanCom" v. Tomay, Administrative and Territorial Unit Gagauzia, Republic of Moldova
Corresponding author email: aconstandoglo@yahoo.com

Abstract

In the article are shown the materials of estimation of cows of Holstein breed on milk productivity for I-III finished lactations. It was found that the cows for the first lactation had a milk productivity at the level of 85% from the full-aged cows (at a rate of 70-75%), which amounted to an average of 8980.2 kg of milk with a fat content of 3.83%. Milk productivity of cows of III lactation averaged 10530.1 kg of milk.
The parental index was at the level of 9720 kg by milk yield and 4.27% by fat content. Realization of genetic potential for milking of first-calves was 8980.2 kg, and cows for II-III lactation - 10081.8 and 10530 kg of milk, respectively. It was marked the cow nr. 5736, from which for 7 completed lactations (2135 days) was received 65274 kg of milk, the average daily milk yield for the whole period was 30.5 kg of milk.

Key words*: Holstein breed, lactation, lactation curve, realization of the genetic potential of productivity.*

INTRODUCTION

In the XX century the Holstein breed became the dominant one in the world dairy cattle breeding. The world population of Holstein cows is 25 million heads, or 72% of the eight world's most common dairy breeds (Jansen, 2009). Canadian cattle breeders believe that for the Holstein cows, a yield of 45 kg per day is good, but not sufficient. In the future, they plan to bring a yield for lactation to 12,000 kg, and daily - to increase to 80 kg and more (Wilson, 1985).

Using the high genetic potential of Holstein in purebred breeding and crossing with other breeds allowed in many countries to create highly productive herds and increase milk production while reducing the number of dairy cows. Over the past 40 years, milk yields in many European countries increased more than twice as a result of achievements in breeding, feeding and management of the herd. Currently, the average growth in milk productivity is 1.5% per year, the main role is given to the effective use of artificial insemination, which predetermines the high genetic potential of the herd (Sartori, 2006).

A dairy cattle breeding is the leading branch of livestock production in the Republic of Moldova and many countries of the world. The main task of dairy cattle breeding is to increase the productivity of animals and improve the quality of products. In increasing the production of milk, an important role is assigned to selection and breeding work, as it determines the genetic progress of breeds and herds. All other factors, including elements of technology, provide only the implementation of this process.

Currently, in the Republic of Moldova, as in other Eastern European countries, new herds of Holstein breeds with high productivity are created by importing livestock. In the Republic of Moldova, dairy cattle are imported from different countries, including Holland and Germany. These animals combine high milk production, are able to give high milk yields (Foksha et al., 2017). It should be noted that at the beginning of 2016 there were 1,421 heads of Holstein cattle in the republic, including 932 cows, whose milk productivity averaged 7612 kg of milk with a fat content of 3.81% (Foksha et al., 2016).

One of the most important factors of determining the value of imported livestock is the genetic potential of acquired animals, which is determined by the productivity of the mother's ancestors. Each regular import of

animals needs to study the realization of their genetic potential for economic-useful signs under new conditions. Therefore, the purpose of the research was to assess the dairy productivity of Holstein cows in the south of the Republic of Moldova on an example of a herd of Society of limited liability "Doksancom".

MATERIALS AND METHODS

Research on the study of the productivity of Holstein cows of Dutch breeding were conducted in the herd of Society of limited liability (SLL) „Doksancom" v. Tomay, Administrative and Territorial Unit Gagauzia, Republic of Moldova.

For studying the productivity of cows, the data of primary zootechnical and pedigree records were analyzed: journals on the accounting of dairy productivity, pedigree cards of cows, pedigree certificates on animals and other documents of primary zootechnical accounting. The yield for lactation, for 305 days, was calculated on the basis of control milking, which was performed 3 times a month, taking into account the fat content in milk. During the research, lactation curves of some cows were studied. The conditions for feeding and keeping the imported animals from Holland were the same. Feeding rations, depending on the level of milk productivity, the physiological state of the animals and the quality of the feed were periodically adjusted.

The coefficient of milk yield of cows was determined by the formula: MR = M/LW, where: MR – milk ratio, kg; M – milk for 305 days or a shortened lactation, kg; LM – live weight, kg.

To study the influence of the genotype on the part of the father, the parent bull index (PBI) was calculated. The parent bull index (PBI) was calculated according to the method proposed by Kravchenko: PBI= 2M + MM + FM/4, where: M – the highest productivity of the mother; MM – the highest productivity of mother's mother; FM – the highest productivity of the father's mother.

The calculation of selection and genetic parameters of productivity and static processing of research materials were carried out according to the methods of Plohinsky (1970), Merkuryev (1983). The digital data obtained during the studies were processed biometrically on a personal computer using Microsoft Excel programs.

RESULTS AND DISCUSSIONS

Indicators of milk production, fat content in milk of breeding cows of farm SLL "Docsancom" in three lactations are presented in Table 1.

Table 1. Dairy productivity of cows of SLL "Docsancom" for three complete lactations

Lactation by account	The number of cows, heads	Live weight, kg	Milk yield, kg	Fat content		Coefficient of milk, kg
				%	kg	
the first	168	648.9±6.7	8980.2±122.8	3.83±0.01	343.2±4.3	1383.7
the second	108	658.2±5.1	10081.8±159.3	3.77±0.01	379.7±5.7	1520.6
the third	36	702.9±8.2	10530.1±351.8***	3.74±0.02	390.9±11.7***	1497.9

Note: *** - P<0,001

Analysis of the milk yield of cows of different ages showed that less milk was obtained from the cows of the first lactation.

It was established that the cows for the first lactation had milk production at the level of 85% of the full-aged cows (at a rate of 70-75%), which averaged 8980.2 kg of milk with a fat content of 3.83%.

It should be noted that with an increase in the number of lactations, there was an increase in milk yield for lactation. Thus, the increase in milk yield for the second lactation was 1101.6 kg of milk and amounted to an average of 10081.8 kg of milk per lactation, to the third – to 1549.9 kg of milk, the difference is highly reliable, at P<0.001.

The amount of milk fat also increased with the increase of the number of lactations. The amount of milk fat at cows in the second lactation was by 36.5 kg, and at cows of third lactation was by 47.7 kg more than at cows of

the first lactation, the difference is highly reliable at P<0.001.

For a more complete assessment of cows for milk production, we calculated the output of dairy products per 100 kg of live weight. The ratio of milk yield for lactation to the live weight of a cow, or the milk ratio, characterizes the economics of milk production. The coefficient of milk of Holstein cows, both for the first and second lactation, was at the level of 1383 - 1497 kg. When the analysis was performed, depending on the age in lactations, was observed an increase in the live weight of cows of the second lactation by 9.3, on the third, 54 kg or 1.4-8.3%.

For a more complete assessment of cows for milk production, we calculated the output of dairy products per 100 kg of live weight. The ratio of milk yield for lactation to the live weight of a cow, or the milk ratio, characterizes the economics of milk production. Normal value is considered to be close to 1000. From the data of Table 1 it is seen that for the whole population of the analyzed cows this coefficient exceeds the norm, the cows of the second lactation have the highest coefficient of milk – 1520.6 kg. As it is known, high-yielding cows within each breed are characterized by a large increase in productivity during the II-III month of lactation and a slow decrease in subsequent. Studies by American scientists and All-Russian institute of livestock production (Aldrich, 1987; Nekrasov et al., 2011) in experiments on highly productive Holstein cows with milk yield of 8500 to 10,000 kg and more confirmed the opinion of most scientists and practitioners about the typical lactation curve.

In figures 1 and 2 are shown lactation curves of cows' at numbers 1016 and 0685.

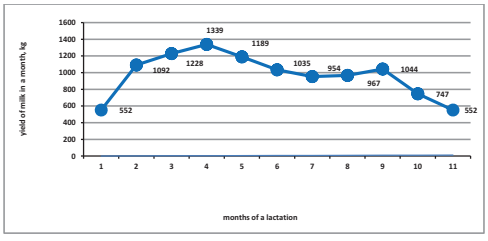

Figure 1. Lactation curve of a cow 1016, first lactation

The analysis of the lactation curve of the cow's number 1016 clearly showed that with a monthly milk yield at the peak of the first lactation, 1339 kg of milk it can be attributed to highly productive. At the same time, the productivity of the heifer by the end of lactation was almost equal to the full-aged cows of similar lactation terms. In the future, its lactation on the second lactation was 12054 kg of milk.

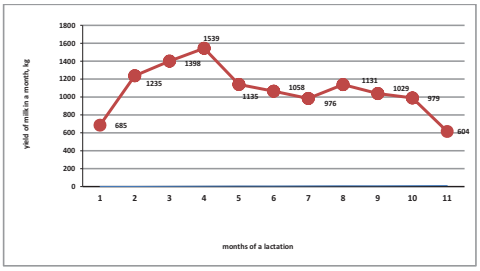

Figure 2. Lactation curve of a cow 0685, sixth lactation

Main peak of cow milk yield nr. 0685 for 4 months VI lactation was 1539 kg of milk, and then it is observed a gradual decrease until the end of lactation. In general, the yield for VI lactation amounted to 11769 kg of milk.

In the herd, were detected 16 cows with four and more lactations, the milk productivity of which remained at a high level in the dynamics of lactations. So, the most quantity of lactations was at the cow 5736. For seven finished lactations (2135 days), 65274 kg of milk was received from it, on average; the daily milk yield for the entire period was 30.5 kg of milk. For six lactations from the cow under the number 2918, was milked 63361 kg of milk. The highest milk yield for lactation from this cow was obtained by the last lactation, that is, for the sixth – 14,396 kg of milk. By 7514 kg of milk, less than six lactation was obtained from the cow 8545 - 55847 kg of milk.

It should be noted that both heifers and fully-aged cows in terms of milk productivity for 305 days of lactation exceeded the minimum standard requirements for Holstein breed.

For a more complete assessment of the potential capabilities of animals for all indicators of female ancestors, we calculated the parent index of cows (PIC) (Table 2).

Table 2. Realization of the genetic potential of cows 1-3 lactations

Account lactation	Indicators					
	Parent index of cows (PIC)		Own productivity		Realization of genetic potential (RGP),%	
	milk yield, kgM±m	fat, % M±m	milk yield, kg M±m	fat, % M±m	milk yield	fat
the first	9720±189.7	4.27±0.05	8980.2±122.8	3.83±0.01	92.4	89.7
the second			10081.8±159.3	3.77±0.01	103.7	88.3
the third			10530.1±3515.8	3.74±0.02	108.3	87.6

As a result of the analysis of the obtained data, it was established that the indicator of the parental index (PIC) was at the level of 9720 kg by milk yield and 4.27% by fat content.

The implementation of genetic potential (IGP) by milk yield was higher at cows of the third lactation and amounted to 108.3%, an average of 7.1% more than that of the cows of the first two lactations. On the content of fat in milk (%), the realization of genetic potential was higher at cows of the first lactation (89.7%). With an increase in the number of lactations, this indicator tends to decrease (87.6%), which is probably due to the existing direct correlation between the level of milk production and fat content in milk – with an increase in the milk production of cows, the fat content in milk decreases and vice versa.

Consequently, in the new conditions of keeping Holstein cows of Dutch breeding realize their genetic potential at a high level, which is facilitated by the appropriate conditions for keeping and feeding animals. The animals acquired in Holland combine high milk production, are able to give high milk yields, have good health, and have also successfully acclimatized and adapted to the conditions of the south of the Republic of Moldova, in particular in the SLL "Docsancom" herd.

CONCLUSIONS

A high level of milk productivity of cows was revealed for the first complete lactation, which amounted to 85% of full-aged cows. On average from cows by first lactation was milked 8980.2 kg of milk with a fat content of 3.83%.

The coefficient of milk yield of cows for the first two lactations was at the level of 1383-1497 kg, the cows of the second lactation had the highest coefficient of milking – 1520.6 kg.

The implementation of genetic potential by milk yield was higher at cows of the third lactation and amounted to 108.3%, an average of 7.1% more than at the cows of the first two lactations. On the content of fat in milk (%), the realization of genetic potential was higher at cows of the first lactation (89.7%).

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EXTENSION AND CURLING MODELLING AT THE MOLDAVIAN KARAKUL LAMBS

Ion BUZU

Institute of Zoology of Academy of Sciences of Moldova (ASM)
1, Academiei Street, MD 2028, Chișinău, Republic of Moldova

Corresponding author email: ionbuzua@mail.md

Abstract

The purpose of the research was to highlight the peculiarities of the extension and modelling of the curling at the Moldavian Karakul lambs and to reveal the relations of these properties with other qualities and characteristics of the skin for the improvement of the lamb selection procedures of the requested type. The researches were carried out on batch of Moldavian Karakul lambs from sheep flock of the Experimental Household of the National Institute of Animal Husbandry and Veterinary Medicine, Maximovca village, Anenii Noi district. From the nonparametric properties of the curling, have been investigated the extension, modelling and types of curling modelling, as well as their relations with other properties and characters of the skin, which ultimately determines the commercial value of the skin. The researches were carried out by the methods elaborated or perfected by us, according to the Sheep Karakul evaluation Instructions with principles of improvement in the Republic of Moldova. As a result of the research it was concluded that the curling extension, the modelling and the type of curling modelling at the lambs and skins Moldavian Karakul are very important properties, which directly or indirectly determine the commercial qualities and the market value of the skin as a whole. The excellent and appropriate extension of the curling is characteristic for the lambs and skins Karakul with the jacket, Kaukasian and coastal curling type. For lambs and skins Karakul with the flat curling type is characteristic of the weak extension of the curling. The highest excellent modelling rate has lambs with curling type flat (58.5%), coastal (50.4%) and jacket (26.4%). The most suitable modelling rate is lambs with the type of curling jacket (73.4%), costal (46.1%) and flat (35.0%). Curling modelling is in positive relationship with the length of the curls, the silk and the luster of the fibbers, and in the negative relation with the length of the fibbers. The lambs with jacket curling type, usually, have the parallel-concentric type curling modelling (48.9%) and mixed (49.9%). The lambs with the type of coastal and flat have predominantly parallel-scale modelling (81.4%) and (62.4%), respectively. The type of curling modelling is in a positive relation to the silk and luster of fibbers, and in a negative relationship with leather thickness and fibber length. Knowing the particularities of extension and modelling of the curling at the Moldavian Karakul lambs and the relations of these properties with other qualities and characters of the skin allow for the efficiency of lamb selection of the requested type.

Key words*: extension, modelling, type of modelling, curling, lambs, Moldavian Karakul.*

INTRODUCTION

The Karakul lamb curling consists of an integral complex of curls of different types (wave, bob, horns, peas, rings) and shapes (tubular, flattened, coastal, moire), which have a string of metric properties (characters) - the size (width), the height and length of the curls, as well as nonparametric properties - the curls extension, the modelling and the type of curling modelling. All these characters and properties of the loyalty directly or indirectly determine the commercial qualities and market value of the skin as a whole.

Extension of the curls represents the degree of their spreading on the body extremities of the lamb (head, tail, limbs, abdomen) or skins. At the Karakul lambs is characteristic existence of the ruffled curling or of moire drawing not only on the main body parts of the lamb, but also on the extremities.

This character serves as the basis for differentiate the Karakul-pur from the Karakul-metis.

The expanded curling on the extremities may consist of curls of different types (tubular, coastal or flat, bobsleigh, or blooming, rings, semi-timbers, peas, corkscrew) or die-drawn drawing. Typically, waves, grains, or coats are spread along the tail.

On the head can be waves, bobs, horns or moon drawing. On the abdomen can be seen waves, bobs, horns, rings, peas, corkscrew, smooth. On the forelegs, to the knees, and to the hindquarters, up to the glow, there can be waves, bobs, horns, rings, peas, smooths.

Below knee, as a rule, are smooths or moarat drawing. At the some lambs, the moire drawing can also be seen on the cheeks (Figure 1).

Figure 1. Moldavian Karakul lamb with excellent extension of curling

Modelling curling is the models of curls placement to each other and their configuration on the body surface of the lamb or skin.

At different lambs, shaping occurs in varying degrees of clarity. Well-shaped modelling forms so-called "drowing" - models. The value of modelling is conditioned by the qualities of the curls, such as the type and shape of the curls, their size and length, their strength and elasticity, the width of the seam between the curls. The value of the model depends on the degree of grading and pronouncing the drawing, which is closely related to the width of the seam. The wide seam with the large space between the adjacent curls usually forms a well-defined and pronounced modelling. The narrow seem does not show clearly modelling and is not visually observed.

The type of curling modelling is the orderly placement of curls to each other, on the skin surface, which form different modelling configurations (patterns) or types of curling drawing. The aesthetic aspect of the type of loop modelling is primarily due to the parallelism of the fibres and their linear arrangement, which form the curls and the curling as a whole.

In the special profile literature, most scientific papers are devoted to the research of the parametric properties of the curls and the curling as a whole. Particularly these were investigated by academician Иванов М.Ф. and his collaborators at the Moscova Institute of Oviculture (Иванов, 1964a, 1964c, 1964d, 1964e) and the Askania Nova Step-by-Step Animal Breeding Institute, Herson (Перегон, 1972), by academician Vasin - at the Moscow Fursking Institute, by professor Гигинейшвили, 1975, 1976 at the Union Institute of Animal Husbandry in Dubroviţi (Moscow suburby) by the researchers of the Union Institute for Scientific Research on Karakulture in Samarkand, Uzbekistan: Дъячков and Письменная, 1951, 1952, 1980; Закиров, 1987; Кошевой, 1975; Ролдугина, 2003), as well as researchers at the Neidam Research Station in Namibia, South Africa (Маттер, 1975; Нел Дж., 1975a, 1975b; Филлингер, 1975; Шеффер, 1975). The nonparametric properties of the Karakul lambs laughter were less analysed in our research (Buzu, 2017a, 2017b, 2015, 2012, 2001, 1999b, 1998), and in the work of other researchers (Pascal, 2015; Прманшаев et al., 2016a, 2016b; Туекбасов, 2011).

In the territory of the Republic of Moldova, the most profound research of the curling of Karakul lamb was carried out by Nicov (1936), who for the first time described the parametric and non-parametric properties of the curls. Subsequently, Ильев (1957) and zootechnist engineer Богданович (1957) described the variability of the curling properties at the metises lambs (Ţuşca x Karakul), resulting from the crossing of the local Tuşca ewes with the Karakul rams Asiatic type up to the third generation. Therefore, we can see that the level of knowledge about the properties curling of lamb loaf for the skin is a valuable database in the field and refers predominantly to the Asian Karakul race and less to the metis lambs of local races.

Appreciated positively the existing researches value in the field, we can also mention that the variability of the nonparametric properties of the curling (extension, curls modelling and the type of curling modelling) at the Karakul lamb, especially to the Moldavian types, is not sufficiently elucidated.

Correlative relationships and factors that influence the manifestation of non-parametric properties of the curling are not fully revealed. Based on these, the knowledge of the degree of

manifestation of the non-parametric properties of the curls and the relationships of these attributes with other skin features is a current problem for the efficiency of the selection process.

In this context, the purpose of this work was to highlight the peculiarities of resistance and modelling of the curling at the Moldavian Karakul lambs and to reveal the relations of these properties with other qualities and characteristics of the skin for the improvement of the lamb selection procedures of the requested type.

MATERIALS AND METHODS

The researches were carried out on batch of Moldavian Karakul lambs from sheep flock of the Experimental Household of the National Institute of Animal Husbandry and Veterinary Medicine, Maximovca village, Anenii Noi district. The nonparametric properties of the curling (extension, modelling and tipe of modelling) were investigated by the methods elaborated or perfected to us (Buzu, 2012), in accordance with the Sheep Karakul evaluation Instructions with principles of improvement in the Republic of Moldova (Buzu et al., 1996).

The extension of the curls has been visually appreciated. Depending on the degree of curls spreading on the corporal extreme of the lamb (head, tail, limbs, abdomen) or skins, the following degrees of curls extension have been differentiated: *excellent, appropriate, weak and insufficient*.

Excellent *extension* - it was considered when on the extremities of the lamb (head, forehead, cheeks, tail, tops, abdomen and limbs to hooves) there is a clear presence of valuable, less valuable types of curls or the moon drawing. This degree of curl extension was the most requested. This type of extension met with valuable lambs with the typical expression of Karakul-pure race. Lambs with excellent curls extension were required for selection and breeding. The *appropriate* extension - was found when the laces had a moderate degree of spreading on the ends of the lamb or on the extremities of the skin. The curling was present on the tail, head, abdomen and limbs, slightly below the knee and hocks. Lambs with the suitable curls extension were also required for selection and breeding. The *weak* expansion - was considered when the extremities of lamb/ skins, carling, or moire drawing, had a low spread. At the lambs with weak extension, the loops could be seen only half the tail surface. The head was usually „licked" to the ears. The abdomen had few unrolled curls. On his feet, the curling did not extend even to the hocks. On the rest of the surface extremities, there were unrolled fibbers or glow, and without moire drawing. The weak extension of the curls was not desirable to the lambs Karakul, although in some sorts of skins with coastal and flat type curling was not considered a big drawback. *Insufficient* extension - was found when there was a total lack of curls or a moire drawing on the extremities of the lamb and skins. The hair cover on the extremities was made up of straight and swollen fibres. This unwanted type of curls extension was characteristic of the metis lambs (T x K) and their skins.

Curling modelling represent the mode of curls placement to each other, their configuration on the lamb or skin surface, and the drawing clarity of the curling modelling as a whole. The value of modelling depends on the degree of grading and pronouncing the drawing. The following degrees of loyalty modelling have been differentiated: *excellent, appropriate, weak* and *insufficient*. *Excellent* modelling was considered when the model of the curls was highlighted and very clearly. The types, shapes and dimensions of the curls were well visible on the skin surface. Relief of the curls was well expressed not only on the crotch and back, but also on the sides, abdomen and extremities. This degree of curling modelling was associated with the superior qualities of the skin. The excellent modelling reflects a well-clear drawing, accompanied by an excellent luster and silk. The lambs with excellent loyalty modelling were the most demanded for selection and breeding.

The *appropriate* modelling - was found when the model of drawing curling was highlighted and clearly. On the surface of the skin were valuable and less valuable curls of different types and shapes. Relief of the curls was well expressed on the bodily regions of the lamb, such as the croup, the back and, less, the lateral sides. The right modelling was generated by

hollow, flat or flat waves, bobs, furrows. Lambs with this loyalty modelling were also requested for selection and breeding.

Weak modelling - was considered when the curling relief and the drawing were superficial or poorly understood, barely observed. The seam between the curls was thinly contoured; the curls were of lesser quality, bob type, the ridge blossoms. Relief of the curs could be poorly observed only on the crotch and the back. Lambs with poor loyalty modelling were not required for breeding. *Insufficient* modelling was found when the curling and drawing of curls were totally absent. The seam between the curls was not observable or contoured. Lambs with insufficient loyalty modelling were not allowed to breed.

Types of modelling curling. The orderly placement of the loops on the body surface of the lamb or skin forms different modelling configurations, called loop modelling models (model, drawing). Four main types of curls modelling have been differentiated: *parallel-concentric (of lira), parallel-scale, mixed (miscellaneous) and indeterminable* (Figure 2).

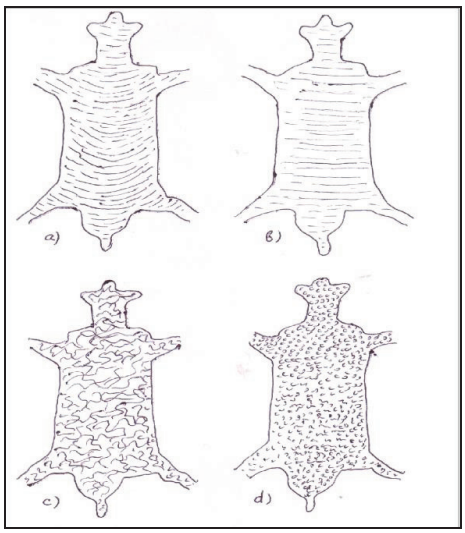

Figure 2. Types of curling modelling on skins Karakul. a) parallel-concentric; b) parallel-scale; c) mixed (miscellaneous); d) indeterminable.

The parallel-concentric modelling pattern was considered when the curls on the back of the skin, from the crotch to the thighs, the backs and the back were parallel to each other, bent in the bow shape with the convex side to the tail, forming parallel-concentric rows. On the front of the back, on the back and the withers, the parallel-concentric model could run in parallel-scale or mixed. Lambs with parallel-concentric shaping of the curls were most required for selection and breeding.

The type of *parallel-scale* modelling was found when the curls on the main parts of the skin were placed parallel to one another in a straight, like-scale form. As a rule, these curls, called by practicing karakulists "whip" (Buzu, 1999a), were very long and expanded continuously from one side to the other side of the lambs body. Relief of the curls was clearly manifested. Lambs with such curling modelling were retained for selection and breeding.

The type of *mixed* modelling was considered when the curls on most of the skin surface were not located parallel to each other but were mixed and had a mixed sinusoidal arrangement. At the same time, curl shaping was well understood, forming an Asian ornament drawing. Lambs with this type of loyalty modelling are admitted for breeding. The type of *indeterminate* modelling was found when the curls on the entire lambs surface, including the main parts of the skin, were disordered and undefined, and the model as a whole was absent. This type of loyalty modelling was not wanted. Lambs with this type of loyalty modelling were not allowed to breed.

The data obtained as a result of the research were statistically processed using the computerized software "STATISTICA - 12" and their certainty was assessed, according to variational biometric statistics, by to Плохинский (1989).

REZULTS AND DISCUSSIONS

The extension of the curls, like other skin types, is hereditary determined and conditioned to a large extent by the genotype of the lamb's parents, their genetic compatibility.

This properties to lambs Karakul is in correlation with the type of curling, the shape of the curls, the purity of the breed (the metiers generation), the quality of the fibres (silk, luster), etc (Иванов, 1964b; Васин, 1971; Гигинейшвили , 1976; Дъячков, 1980; Buzu, 2012). It was established that the extension of the curls on the lamb's extremities is most dependent on the type of curl (Table 1).

Table 1. Extension of curls by type of curling,
the silk and the luster of fibres at the Moldavian Karakul lambs

Specification	Effective of lambs, head	Extension of curls							
		Excellent		Appropriate		Weak		Insufficient	
		head	%	head	%	head	%	head	%
Depending of the type of curling									
Jacket (Tubular)	429	105	24.5	255	59.4	57	13.3	12	2.8
Coastal	231	36	15.6	121	52.4	69	29.9	5	2.1
Flat	123	6	4.9	36	29.3	63	51.2	18	14.6
Kaukasian	195	59	30.3	97	49.7	24	12.3	15	7.7
Brac	5	-	-	-	-	1	20.0	4	80.0
Depending of silk fibbers									
Excellent	432	80	18.5	207	47.9	124	28.7	21	4.9
Appropriate	416	81	19.5	242	58.2	70	16.8	23	5.5
Reduced	128	44	34.4	64	50.0	13	10.1	7	5.5
Insufficient	6	2	33.3	2	33.3	1	16.7	1	16.7
Depending on luster fibbers									
Intense	342	61	17.8	171	50.0	89	26.0	21	6.2
Appropriate	486	95	19.5	266	54.7	103	21.2	22	4.5
Reduced	134	47	35.1	63	47.0	14	10.4	10	7.5
Insufficient	19	3	15.8	10	52.6	5	26.3	1	5.3

The excellent extension of the curls is more characteristic for lambs with jacket and Kaukasian type of curling, which have tubular curls, and is rarely found at the lambs with the coastal, flat or Karakulcea curling type. We found that at the lambs with jacket curling type the majority of individuals had the right extension (59.4%) and excellent (24.5%) of the curls on the extremities of the body. Share of lambs with weak extension and insufficient curls in this batch is unimportant and represents 13.3% and 2.8%, respectively. Characteristic for lambs with jacket curling is the extension of tubular curls (short wave, bean, peas) and small blossom ridge on the extremities. In the batch of lambs with the Kaukasian type of curl, the individuals with the right extension (49.7%) and excellent (30.3%) of the curls also predominate. Lambs with weak and insufficient curls in this batch are in the minority. Characteristic of the lambs in this batch is the extension of the small curls on the extremities, worthless and defective, such as rings, peas, corkscrew.

In lambs with the type of coastal curling, the appropriate extension (52.4%) of the loops also prevails, but an essential share of individuals with the weak extension (29.9%) of the loops is also found. The slight extension of the loops to the lambs with the coastal type curling is characterized by the appearance on the extremities of some of the linings made of undrained fibres and placed on the skin, especially on the head, legs (above the knees and hocks), partly on abdomen and tail.

The worst extension of the curls was found in lambs with flat type curling. In this group predominates the individuals with weak extension (51.2%) and insufficient (14.6%) of the loops. Lambs with the appropriate loops extension in this group occupy a minority share (29.3%) and are the smallest compared to the other batch. The weak extension of lambs with a flat type curling is characterized by the presence on the skin of the edges of the mirror-linen, consisting of short, un-ruled short fibres leather, with intense luster and silk. For lambs of these types, weat extension is not considered a major drawback and therefore does not diminish their class.

For brac lambs, 80% of cases are characterized by the insufficient extension of the curls to the body extremities and are characterized by the presence of un-ruled fibbers (straight) on the abdomen, legs (above knees and hocks), tail. Un-ruled fibres at these lambs are not skinned, they are long and high, like moustaches. Such fibres are characteristic of the lambs of Metis origin and the skins of the specification "Karakul-metis" and "Smuşka".

At the type Karakulcea lambs, it is more often the proper extension of the curls with the moire drawing. In some geographic regions and countries of the world (South Africa), the Karakulists have created new type sheep of Karakul with a hair cover, so-called "smooth",

with the moire drawing and weak extension of the (Филлингер, 1975; Шеффер, 1975). This kind of skins has an increased demand on the world markets. From their extremities (especially from the legs) with a smooth and glossy glow are made quite elegant and original coats, with the type "fir" arrangement in combination with "muton".

Research has shown that the extension of the curls is in negative correlation with the silk and luster of the fibres of the hair cover. It has been found that the higher the silk and the luster of the fibres, the more extension the curls on the extremities of lambs Karakul is weaker. Thus, lambs with better fibre silk possess a lesser extension of the curls. The largest share of individuals with excellent curl extension was found in the batch of lambs with low silk (34.4%) and insufficient (33.3%) of fibres, and the lowest share - lambs with excellent fibre silk (18.5%). Lambs with excellent fibre silk yield, with the percentage of individuals with excellent curling extension, with low silk, with 15.9% ($P < 0.001$). The largest proportion of individuals with the weak extension (28.7%) of the curls was registered at the lamb batch with excellent fibre silk, and the smallest share (10.1%) - in the lamb batch with low silk. The first batch of lambs, exceeded after this index the second by 18.6% ($P < 0.001$).

Extension of the curls on the extremities is also indirectly related to the fibre luster. Research has shown that the lambs with the intense and proper luster of the fibres have an extension of the curling on the weaker ends compared to congeners with reduced fibre luster. Thus, in the lamb batch with excellent fibre luster, the share of individuals with excellent curling extension was 17.3% lower compared to the lamb batch with reduced fibre gloss ($P < 0.001$). At the same time, the weight of the individuals with the reduced extension of the curling in the lamb group with the intense luster of the fibres was 15.6% higher compared to the reduced luster fibre lamb batch ($P<0.001$). In the batch of lambs with intense luster of fibres there was the lowest registered (17.8%) of individuals with excellent extension and the highest percentage (26.0%) of individuals with the weak extension of the curls on the extremities. Inversely, in the group of lambs with reduced luster fibbers was recorded the highest share (35.1%) of individuals with excellent extension and the lowest percentage (10.4%) of individuals with low extension curls extremities.

In practice, when lamb Karakul evaluation, curls extension is appreciated as an important feature in high quality skins and, as an ancillary character, in lower quality skins. Knowing the reciprocal correlation of this character with other skin properties allows the selector to direct the selection process in the desired direction.

The modelling curling, as well as other skin characters, is hereditary and is correlated with many other features, such as the type of curling, type and shape of curl, size and length, fibre length, luster and silk, body regions lambs or leather quality areas, the width of the stitch, the linear arrangement of the fibbers, etc.

An evident link of curling modelling is observed with the type of curling (Table 2).

We have found that the best modelling of the curling possesses the lambs with type flat, coastal and jacket. Lambs with the Kaukasian curling type possess the worst model of curling. The largest and predominant proportion of individuals with the excellent modelling of curling (58.5%) was recorded in the lame batch with the flat type curling.

At the lambs with the costal type curling, also, predominate the individuals with excellent modelling (50.4%) and less than half of this group (46.1%) constituted lambs with the appropriate modelling curling. Lambs with jacket type curling have, for their part, the appropriate modelling (73.4%) and excellent (26.4%) of the curling. After to the weight of the individuals with excellent modelling, the lambs with flat type curling overcome the conjuganers with the jacket type curling by 32.1% ($P<0.001$) and the ones with the and those with coastal type of curling - by 8.1% ($P<0.1$).

The excellent curling modelling is present only at the lambs, with the type jacket, coastal and flat curling.

The lambs with Kaukasian type of curling, for the most part, the curling modelling is weak (66.0%) and insufficient (13.7%).

The modelling of the curling depends largely on the length of the curls.

Table 2. Curling modelling according to the type of curling, qualities of curls and hair fibre at the Moldavian Karakul lamb

Specification	Effective of lambs, head	Modelling of curling							
		Excellent		Appropriate		Weak		Insufficient	
		head	%	head	%	head	%	head	%
Depending of curling type									
Jacket	429	113	26.4	315	73.4	1	0.2	-	-
Coastal	232	117	50.4	107	46.1	8	3.5	-	-
Flat	123	72	58.5	43	35.0	8	6.5	-	-
Kaukazian	197	-	-	40	20.3	130	66.0	27	13.7
Depending on length curls									
Very log	35	31	88.6	4	11.4	-	-	-	-
Long	251	204	81.3	47	18.7	-	-	-	-
Medium	495	70	14.2	411	83.0	13	2.6	1	0.2
Shot	190	-	-	38	20.0	138	72.6	14	7.4
Very shot	10	-	-	-	-	2	20.0	8	80.0
Depending on length fibbers									
16-17 mm	19	-	-	7	36.9	10	52.6	2	10.5
14-15 mm	60	9	15.0	38	63.3	10	16.7	3	5.0
12-13 mm	153	40	26.1	94	61.4	18	11.8	1	0.7
10-11 mm	195	62	31.8	108	55.4	25	12.8	-	-
8-9 mm	144	60	41.7	69	47.9	14	9.7	1	0.7
6-7 mm	59	21	35.6	31	52.5	5	8.5	2	3.4
4-5 mm	3	3	100	-	-	-	-	-	-
Depending on silky fibbers									
Excellent	427	221	51.8	186	43.6	19	4.4	1	0.2
Appropriate	424	77	18.2	276	65.1	61	14.4	10	2.3
Reduced	120	3	2.5	38	31.6	68	56.7	11	9.2
Insufficient	6	-	-	-	-	3	50.0	3	50.0
Depending on luster fibbers									
Intense	337	189	56.1	138	40.9	10	3.0	-	-
Appropriate	489	108	22.1	310	63.4	59	12.1	12	2.4
Reduced	145	6	4.1	52	35.9	76	52.4	11	7.6
Insufficient	7	-	-	1	14.2	3	42.9	3	42.9

The highest proportion of individuals with excellent curling modelling was recorded at the lamb batch with very long curls.

The highest proportion of individuals with weak and insufficient curling modelling was recorded at lambs with short and very short curls. It was found that in the batch of lambs with very long curls, the overwhelming majority of individuals have an excellent modelling (88.6%) and only 11.4% of the lambs have an appropriate modelling of the curling. Lambs with long curls are predominantly an excellent modelling (81.3%) and in the minority (18.7%) - a proper modelling of the curling. In the batch of lambs with medium long curls, the share of lambs with excellent curls modelling decreases to 14.2%, and the overwhelming majority (83.0%) possesses appropriate modelling of the curling, and some individuals with a weak modelling. In the batch of lambs with short curls, there are no individuals with excellent curling modelling, and the proportion of individuals with the proper loyalty modelling decrease to 20.0%. The majority of the individuals in this batch have poor modelling weak (72.6%) and insufficient (7.4%) modelling. Among lambs with very short curls predominate individuals with insufficient (80.0%) and poor (20.0%) modelling of curling. Therefore, the longer the curls are, the better the modelling. The long and very long curls form, as a rule, excellent curling modelling. Medium and long curls form the appropriate modelling, and short and very short curls form the weak and insufficient curling modelling. The curling modelling also depends on the length of the fibres.

Research has shown that the best modelling of the curling is formed at lambs with a moderately short length of fibre - 8-9 mm (not considering the batch of lambs with very short

fibre lengths of 4-5mm, because they were very small number - 3 heads).

In this batch was recorded of individuals with the excellent curling modelling (41.7%) and the lowest share of individuals with a weak and insufficient curling modelling (10.4%) were registered. The Lambs with short lengths of fibres, of 6-7mm, also possess a particularly good modelling of the curling. Among these are 35.6% of the lambs with excellent modelling and 52.5%, with the appropiate curling modelling. The lambs with medium length (10-11 and 12-13mm) also have a good curling modelling. The majority lambs of these batch have an appropriate and excellent curling modelling. In the batch of lambs with the length fibres of 10-11 mm predominates individuals with the appropriate (55.4%) and excellent (31.8%) modelling curling. Among the lambs with the length of the fibres of 12-13mm, the share of the lambs with the appropriate shape of the appropriate curling constituted 61.4% and the one with the excellent modelling - 26.1%. The share of individuals with the weak curling modelling among the lambs of these lots is unimportant. With the increase in fibre length in lambs from 6-7mm to 14-15mm, the share of individuals with excellent modelling decreases from 35.6% to 15.0%, and at the same time increases the share of individuals with weak modelling to 8.5% to 16.7%. At the lambs with the length of the fibres 16-17mm, the excellent curling modelling is no longer found, and the weight of the individuals with the appropriate modelling decreases to 36.9% and at the same time considerably increases the weight of the individuals with the weak and insufficient curling modelling until 52.6% and 10.5%, respectively.

The modelling of the curling is also indirectly related to the qualities of the hair cover, in particular the silk of the fibres.

It was found that the best curling modelling was recorded at lambs with excellent fibre silk. Thus, in this batch of lambs predominates individuals with excellent (51.8%) and then appropriate (43.6%) of the curling modelling. Individuals with weak and insufficient curling modelling in this batch of lambs rarely meet. In the batch of lambs with the appropriate silk fibres, their overwhelming majority have an appropriate (65.1%) and excellent (18.2%) modelling, and a minority share of individuals with weak (14.4%) and insufficient (2.3%) of the curling.

The lambs with low silk fibre have, for the most part, a weak modelling (56.7%) and less, appropriate (31.6%). With increasing the degree of fibre silk, from reduced to appropriate and excellent, the share of individuals with excellent curling modelling increases from 2.5% to 18.2% and 51.8%, respectively ($P<0.001$), and vice versa, with the decrease of the degree of silk fibre from excellent to the appropriate and reduced, respectively the share of individuals with a low and appropriate low curling modelling increases from 4.6% to 16.7 and 65.9%. Hence the conclusion that the higher the fibre silk, the better is the curling modelling.

Such correlation of the curling modelling at the lambs Karakul also occurs according to the luster of the fibres. We have found that lambs with intense luster of fibres have predominantly excellent (56.1%) and appropriate (40.9%) modelling of the curling. Individuals with a weak curling modelling in this batch of lambs occupy an insignificant weight (3.0%). In the batch of lambs with the best appropriate of fibres predominates, in their overwhelming majority, individuals with an appropriate (63.4%) and excellent (22.1%) modelling of the curling. In this batch, in minority, individuals with a weak (12.1%) and insufficient (2.4%) modelling already appear in the minority. The lambs with reduced fibre lambs have, in most cases, a weak modelling (52.4%), appropriate (35.9%) and insufficient (7.6%) of the curling, and those with insufficient luster fibres, and a weak (42.9%) and insufficient (42.9%) modelling of the curling. With the increase of the degree of fibbers luster from insufficient to excellent, the lambs summary weight with excellent and appropriate curling modelling increases from 14.2% to 97%, or 82.8% ($P<0.001$) and, vice versa, with the decrease of the degree of fibbers luster from intense to insufficient, increases the share of lambs with weak and insufficient modelling curling from 3.0% to 85.8%, or by 82.8% ($P<0.001$). Therefore, the harder the fibber luster to lambs is better, the more qualitative is the modelling of the curling.

Superior modelling (excellent) is always determined by curls valuable (waves), long and very long, medium and high size, with excellent resistance and elasticity, short and medium, silky and luster fibres, broad curls seam. Good modelling is associated with jacket, coastal and flat curling types. The most expressive modelling is usually seen on the crotch, tailgate, back, then on the sides, descending to the extremities. The higher is the quality of modelling, the higher is the lamb's ranking and the range of skins. So, the more valuable the lamb's skin qualities, the better the modelling degree. And vice versa, the better the skin qualities, the lower the degree of curling modelling. Therefore, the selection in the direction of improving the modelling outlook has an additive effect on all the other skin qualities, thereby, increasing the efficiency of the selection.

The type of modelling, as well as other attributes of the loop, is determined hereditary and is in several correlative relationships with other kelly characters (Table 3).

Table 3. Type of curling modelling at the Moldavian Karakul lambs depending on the thickness of the skin and the qualities of the hair

Specification	Effective of lambs, head	The type of curling modelling							
		Parallel-concentric		Parallel-scale		Mixed (sinusoid)		Indeterminable	
		head	%	head	%	head	%	head	%
Depending on the curling type									
Jacket	425	208	48.9	5	1.2	212	49.9	-	-
Coastal	226	33	14.6	184	81.4	7	3.1	2	0.9
Flat	117	29	24.8	73	62.4	14	12.0	1	0.8
Kaukazian	192	-	-	-	-	59	30.7	133	69.3
Depending on thickness skin									
Subtle	301	102	33.9	76	25.2	111	36.9	12	4.0
Medium	419	110	26.3	123	29.3	127	30.3	59	14.1
Thickened	245	56	22.9	66	26.9	64	26.1	59	24.1
Thick	12	-	-	1	8.3	3	25.0	8	66.7
Depending on length fibbers									
16-17 mm	20	2	10.0	1	5.0	6	30.0	11	55.0
14-15 mm	57	12	21.0	9	15.8	25	43.9	11	19.3
12-13 mm	152	41	27.0	30	19.7	69	45.4	12	7.9
10-11 mm	193	59	30.6	50	25.9	59	30.6	25	12.9
8-9 mm	145	50	34.5	60	41.4	25	17.2	10	6.9
6-7 mm	60	12	20.0	29	48.4	14	23.3	5	8.3
4-5 mm	3	1	33.3	2	66.7	-	-	-	-
Depending on silky fibbers									
Excellent	415	148	35.7	149	35.9	104	25.1	14	3.3
Appropriate	415	113	27.2	93	22.4	156	37.6	53	12.8
Reduced	121	2	1.7	19	15.7	34	28.1	66	54.5
Insufficient	6	-	-	-	-	1	16.7	5	83.3
Depending on luster fibber									
Intense	347	134	38.6	131	37.8	76	21.9	6	1.7
Appropriate	504	141	28.0	112	22.2	193	38.3	58	11.5
Reduced	135	4	3.0	23	17.0	34	25.2	74	54.8
Insufficient	14	-	-	4	28.6	4	28.6	6	42.8

The closest connection of the modelling type is with the type of curling of the skin. Typically, parallel-mixed and mixed modelling patterns are characteristic for lambs with jacket curling. The type of parallel-scale modelling, usually, encountered at the lambs with coastal, flat or Karakulcea type curling. The type of indeterminate modelling is typical for lambs with the Kaukasian type of curling.

Research has shown that almost half of the lambs with the jacket curling type have a parallel-concentric type modelling (48.9%) and the other half - a mixed type modelling (49.9%). In this batch of lambs, practically rare individuals are encountered with parallel-scale modelling (1.2%) and do not encounter individuals with indeterminate type modelling. In the batch of lambs with the coastal type

curling, overwhelming majority, predominates individuals with parallel-scale type modelling (81.4%), followed by individuals with parallel-concentric curling modelling (14.6%). Mixed and indeterminate type modelling lambs are found in this batch in rare (3.1%) and very rare (1.9%) cases. At the lambs with flat type curls, there was, also, the predominance of individuals with parallel-scale type curling modelling (62.4%), followed by individuals with parallel-concentric type curling modelling (24.8%). A mixed-type modelling of the curling is found, in this batch, at the some individuals with a minority share (12.0%), and the indeterminable one is found in very rare cases (0.8%).

Lambs with Kaukasian curling have a curling modelling of only two types. In the vast majority (69.3%), their curling modelling is indeterminate and in the minority (30.7%) the modelling is of mixed type. Therefore, the lambs with the valuable types of curling (jacket, coastal, flat), usually, have the curling modelling of valuable types (parallel-concentric, parallel-scale and mixed).

Since the type of curling is determined by the type and shape of the curls, it results that the tubular curls (tubular wave, bob) usually form parallel-concentric and sinusoidal (mixed) type modelling. The waves by coastal and flat forms, furrows generate parallel-scale and boulder shaping. Lower quality curls (rings, peas, corkscrew) usually form a non-durable modelling of indeterminate type.

The type of curling modelling also depends on the quality of the derm, especially its thickness. It was found that lambs with the thin derm have the largest share of individuals with valuable types of curling modelling and make up 96.0%, including parallel-concentric modelling - 33.9% with parallel-scale modelling - 25.2% and with mixed type modelling - 36.9%. Lambs with medium thickness derma have a high summary weight of individuals with valuable types of curling modelling and make up 85.9%, including the parallel-concentric curling modelling - 26.3%, with parallel-scale modelling – 29.3% and with mixed type modelling - 30.3%. Lambs skin has thickened a summary weight of individuals with valuable lower curling modelling models compared to the first two batches and make up 75.9%, which is 20.1% less compared to the thin lamb batch ($P<0.001$) and 10% less than the medium thickness lamb batch ($P<0.001$). The lambs with thick leather have, predominantly, a model of non-value, indeterminable (66.7%) and less valuable of mixed type (25.0%). Therefore, thin and dense leather generates a loop with the value modelling model (parallel-concentric, parallel-scale, mixed), and the thick and loose leads to the formation of a non-value (indeterminable) type of curling modelling.

The type of curling modelling also depends on the quality of the fibres, especially their length. It was found that the highest proportion of individuals with a parallel-concentric curling modelling was recorded in the group of lambs with a moderately short length of fibre - 8-9mm (34.5%), of the individuals with curling modelling parallel-scale type was recorded at the lamb batch with the short fibre length, 6-7mm (48.4%) (not considering the batch of lambs with a very short length of 4-5mm fibres, because they were very small - 3 heads), of the individuals with curling modelling of the mixed type was recorded in the batch of lambs with the average length fibres of 12-13mm (45.4%) and individuals with indeterminate curling modelling were recorded at the lamb batch with the overlapping length of the fibres, 16-17mm (55.0%).

It has been observed, that with the reduction of the length of the fibres from 16 - 17 mm to 8-9 mm, the share of lambs with parallel-concentric modelling increases from 10.0% to 34.5%, or 3.4 times ($P<0.001$). With decreasing fibre lengths up to 6-7mm, lambs with parallel-scale modelling increase from 5.0% to 48.4%, or 9.7 times ($P<0.001$). The weight of the lambs with the modelling of the mixed type curls is optimal if the fibre length is medium (12-13 mm) and decreases, both if the length of the fibres increases from this optimum, and if the length of the fibres decreases. With the increase in fibre length at the lambs from 6-7 mm to 16-17 mm, the proportion of individuals with indeterminable curling-type modelling increases from 8.3% to 55.0%, or 6.6 times ($P<0.001$). Therefore, the shorter is the hair fibres at the Karakul lambs, at birth, is shorter, the proportion of the individuals with the shaping of the valuable types (parallel-concentric, parallel-scale,

mixed) of the curling, and vice versa, as the fibres the lambs pilots are longer, the lower the weight of individuals with the shaping of valuable types of curling modelling, and the proportion of individuals with the indeterminate (invalid) modelling increases. So, short fibres form a better quality of curling and modelling than long and over-drawn fibres. The curling with the valuable modelling pattern is usually formed from the hair cover with good fibre silk qualities. We have found that lambs with excellent fibre silks have the largest proportion of individuals with the most valuable parallel-concentric and parallel-scale curling modelling, representing 35.7% and 35.9%, respectively. In the lamb batch with the appropriate silk fibre, the highest proportion of individuals with the mixed type modelling was registered - 37.6%.

Among the lambs with reduced and insufficient silk of the curling there was the largest proportion of individuals with the model of the non-value curling modelling type, indeterminable, representing 54.5% and 83.3%, respectively. It has been noticed that with the increase of the silk of the hair fibres at the lambs from the low degree to the excellent grade, the proportion of the individuals with the parallel-concentric type curling modelling increases, from 1.7% to 35.7%, or 21 times ($P<0.001$) and those with parallel-scale curling modelling, from 15.7% to 35.9%, or 2.3 times ($P<0.001$). At the same time, with the diminishing of the silveryness of the hair fibres at the lambs from the excellent degree to the insufficient degree, the share of the individuals with the non-worthless modelling of the indeterminate type curling considerably increases from 3.3% to 83.3%, or 25.2 times ($P < 0.001$).

The type of curling modelling is closely related to the luster of the hairpiece. I have found that lambs with intense luster of fibres possess the most valuable types of curling modelling. The total share of individuals with the modelling of the curling of valuable types is 98.3%, including the most valuable type of parallel-concentric curling modelling is 38.6%, with the parallel-scale modelling type - 37.8% and the type mixed curling modelling - 21.9%. The lambs with invalid modelling, indeterminate, in this batch are very rare (1.7%).

In the batch of lambs with the appropriate luster predominates the individuals with modelling of mixed type curling (38.3%), parallel-concentric (28.0%) and parallel-scale (22.2%). Individuals with indeterminable curling modelling, in this batch are in the minority - 11.5%.

The lower is the lambs fibre luster, the lower is the proportion of individuals with the modelling of valuable types, and the proportion of individuals with the non-indeterminable type curling modelling increases. Thus, in the batch of low luster fibres lambs, the share of individuals with parallel-concentric modelling decreased to 3.0% and in the batch of lambs with insufficient luster, they practically no longer meet. At the same time, the share of individuals with invalid, undeterminable modelling increased in these batch until 54.8% and 42.8%, respectively. Therefore, the most valuable types of curling modelling are formed at the lambs with the type curling jacket, coastal and flat, with thick, thin and medium gross leather, with short and medium long hair fibres, with excellent hair or appropriate silky, with intense or appropriate luster.

Knowing the particularities of extension and curling modelling of at the Moldavian Karakul lambs and the relations of these properties with other qualities and characters of the skin allow for the efficiency of lamb selection of the requested type.

CONCLUSIONS

The extension of the curls, the modelling and the model of curling modelling at the lambs and skins Moldavian Karakul are very important characters (attribute) that directly or indirectly determine the commercial qualities and the market value of the skin as a whole.

The excellent and appropriate extension of the curling is characteristic for the lambs and skins Karakul with the type curling jachet, Kaukasian and coastal curling type. For lambs and skins Karakul with the flat curling type is characteristic of the weak extension of the curling.

The highest excellent modelling rate has lambs with flat curling type (58.5%), coastal (50.4%) and jachet (26.4%). The most appropriate modelling rate is lambs with the type of curling

jachet (73.4%), costal (46.1%) and flat (35.0%).

Curling modelling is in positive relationship with the length of the curls, the silk and the luster of the fibres, and in the negative relation (but beneficial for selection) with the length of the fibres.

The lambs with jachet curling type, usually, have the parallel-concentric type curling modelling (48.9%) and mixed (49.9%). The lambs with the type curling of coastal and flat have predominantly parallel-scale type modelling (81.4%) and (62.4%), respectively.

The type of curling modelling is in a positive relation to the silk and luster of fibbers, and in a negative (but beneficial to selection) relationship with leather thickness and fibber length.

Knowing the particularities of extension and modelling of the curling at the Moldavian Karakul lambs and the relations of these properties with other qualities and characters of the skin allow for the efficiency of lamb selection of the requested type.

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THE EVALUATION OF THE PRESENT STAGE OF FORMATION OF THE GRIZZLED COLOURED VARIETY FROM THE KARAKUL DE BOTOŞANI BREED

Ionică NECHIFOR[1], MARIAN FLOREA[1,2], Constantin PASCAL[2]
[1]Agricultural Station of Research and Development for Sheep and Goat Growing Popauti-Botosani, Romania
[2]University of Agricultural Sciences and Veterinary Medicine Iași,
M. Sadoveanu nr. 5-8
Corresponding author email: pascalc61@yahoo.com

Abstract

The aim of the research was to evaluate the current stage of the formation of a new variety of colour within the Karakul de Botoşani breed. In this respect, the research objectives were represented by the assessment of the effective size of the active population, the inbreeding rate and the subdivision of the analysed population, as well as the determinations regarding the degree of reproductive isolation and the intergenerational interval. The biological material was represented by males and females of known origin, which form the active population of the Karakul de Botoşani breed and as working methods were used mathematical techniques as they provide solutions to predict the frequencies of genetic constituents specific to animal populations. The conducted research shows that there is a difference in the inbreeding rate from the active population. This aspect is due to the fact that the population has a live stock of a small number of reproductive individuals, fact confirmed by the ratio of sexes that had very high values, from 1 to 12.62 in the population from the forming area, to a ratio of 24.46 females at one nursery ram in the other areas. The existence and keeping of this report at a rather tight level is a consequence of the fact that the reproduction is based on the directed mount, but also of the high degree of subdivision of the breed in relation to the colour shades which appear in this variety. The present inbreeding degree specific to the active population is of 7.41 with variations from 8.48 at the grizzled Karakul population from coterminous areas and of 7.31at the population within the forming area. The analysis of the genealogical files and of the records of the mounts and of the bringing forth showed that the intergenerational interval has an average duration close to 4 years, arguing that the population has a rather high degree of precocity in comparison with other sheep breeds.

Key words*: Karakul de Botoşani, pelts, genetic parameters.*

INTRODUCTION

Karakul de Botoşani represents an animal population characterized through distinct phenodeme and genome and has as a basic objective in formation, improvement and breeding especially pelt production.

The first grizzled colour individuals from the Karakul de Botoşani breed appeared after the crossings practiced between brown rams with females of black colour and grey heterozygous (Ursu, 1998). This colour is better known in the speciality literature and under the name of agouti or lupe colour. To obtain the present type, from the early stages of the formation of this new variety a rigorous selection has been applied and has intervened in conducting mattings to enhance the colour shades and the desired type of loop. Presently, the population represented by individuals who have a composed colour of dark heterochromatic type evolves reproductively, the purpose being of to increase the degree of resemblance and fixation of the main characters on which the pelt quality depends.

MATERIALS AND METHODS

The biological material under investigation belongs to the Karakul de Botoşani breed of known origin, included in one of the forms specific to the control of the production performances with proven belonging to the grizzle colour variety. In order to assess the actual population size, inbreeding rate and the degree of subdivision, were subjected to research the sheep of grizzle colour from the forming county of the Karakul de Botoşani as well as other populations from holdings situated in adjacent areas.

The working methods used were specific to the field of pelt production and statistical data

processing. To accomplish the research objectives, a complex task was initiated to identify the data related to the estimation of the degree of reproductive isolation.

For this purpose, analysis of the zoo technical records have been carried out from several active populations from the Karakul de Botoşani breed of grizzle variety, located in the breeding area of the respective breed. The population surveyed was of 747 adult females and daughter of the 47 rams, and for calculating the effective population size, the following calculation relation was used:

$$Ne = \frac{4NmxNf}{Nm+Nf};$$

where: Nm = number of males

Nf = number of females

Based on the value determined for the actual size of the population, it was possible to highlight the homozygous growth rate for each new generation of animals (ΔF) and the genetic effect due to inbreeding, knowing that ΔF = 1/2 Ne.

In order to determine the present status of reproductive isolation of the effective belonging to the grizzle colour variety, from the Karakul de Botoşani breed, based on the data from husbandry records, the value of the reproductive isolation coefficient was calculated, the formula used being the one described by Wright, 1921 , cited by Drăgănescu in 1972 and 1979, namely:

$$CIR = \frac{AA-(AI+II)}{AA+AI+II};$$

where: CIR = reproductive isolation coefficient;

AA = the number of individuals admitted to the range, studied from the core nucleus and having both native parents;

AI = the number of individuals admitted to the range, studied from the core nucleus and having one native parent and another immigrant;

II = the number of individuals admitted to rang, studied from the core nucleus and having both immigrant parents.

All the data utilized to establish the effective size of active population, and also the ones based on which was determined the reproductive isolation degree and kinship degree with other varieties of the breed were taken from Register of mating and calving which is in the archive of SCDCOC Popăuți-Botoşani, as well as from Genealogical Register of Karakul de Botoşani breed.

In determination of genetic distance between actual population represented by individuals which form the gray variety, in according with evolutionary theory, we suppose that genetic similarity is close not only to founding breeds represented by Asian Karakul and German Karakul but also with other Karakul populations located in different rearing units from forming area. To estimate this genetic parameter was utilized the standard method described by Nei (1972) based on construction of dendrograms in which the data were organized in subcategories till reaching of the desired detail level.

Dendogram was constructed using the neighbour-joining (NJ) method (Saitou and Nei 1987). Nei's standard distances (Ds; Nei 1972), observed heterozygosity (Ho) and expected heterozygosity (He), neighbour-joining trees and bootstrap values were computed using the computer package DISPAN (Ota 1993).

RESULTS AND DISCUSSIONS

The grizzle variety belonging to the Karakul de Botoşani breed is at this moment in the stage of increasing the degree of similarity between individuals of this population and the fixing of the valuable characters specific to pelts, which is why the population evolves reproductively closed. In order to assess the current stage of formation of a new variety of colour inside the Karakul de Botoşani breed, a detailed analysis of some important aspects was carried out, such as: evaluating the effective size of the population, determining the degree of reproductive isolation, intergenerational interval assessment.

Actual size, inbreeding rate, and subdivision of analysed population. The size and rhythm of inbreeding in different populations is extremely important because it influences not only the evolution but also the changes occurring in its genetic structure and has direct consequences on the effects due to inbredness, genetic derivation and costs necessary to implement improvement programs and indirectly on the effect of selection (Mochnacs et al., 1978; Vicovan, 2008; Popa et al., 2012).

If the size of the effective (Ne) is high, it also attracts a more intense effect due to the selection, and the chances that there may be effects in such populations due to genetic derivation are extremely limited.
In diminished populations, the evolutionary effects will be affected by effects due primarily to genetic derivation and to a lesser extent than those due to strict selection. In this last case, the effect of selection tends to zero, because the depressive effects lead to an increase in homozygosity and a strong restriction of genetic variation (Pipernea, 1979).
In the case of the researches carried out, the actual size of the active population belonging to the grizzle variety from the Karakul de Botoşani breed was determined. It also sought to determine the structure of the active population by gender and to determining the actual rhythm of inbreeding.

Table 1. Actual size, sex ratio and inbred rate in the population represented by Karakul de Botoşani – grizzle variety

Specifi-cation	Rams (Nm)	Females (Nf)	Sex ratio Nm/Nf	Actual size (Ne)	Inbred rate (ΔF)	Repro-ductive isolation index
In the forming area	34	429	12.62	126.01	7.31	-
In adjacent areas	13	318	24.46	49.96	8.48	-
Total population	47	747	15.89	176.87	7.41	0.68

The analysis of the data obtained shows that the values of the actual size of the active populations varies, depending on the holding and the area, from the minimum of 49.46 to the ones located near the forming area to 126.1 in the holdings within the forming area of the Karakul de Botoşani breed, which corresponds to a growth rate of inbreeding ranging from 0.48 to respectively 0.73. For the entire population, the actual size was of 176.87 and the inbreeding rate was 7.41.
The existence of a difference in inbreeding rate is due to the fact that the gender ratio is 15.89 females to a male with variations from a ratio of 1 to 12.62 and mother sheep in the forming area, to a ratio of 24.46 females to a nursery ram in other areas. The existence and keeping of this report at a rather tight level is a consequence of the fact that the reproduction is based on the directed mount, but also of the high degree of subdivision of the breed in relation to the shades of colour appearing in this variety.
Knowing the degree of inbreeding in animal populations where the effective size is small is an extremely important activity as this .indicator is due to mating to more closely related animals than the average population. Any "in-endogamy reproduction of a population leads to inbreeding, the extent of which depends on the size of the population, selection and management of mating.
All individuals in "closed populations" are, after a certain number of generations, the more closely related to each other as the effective size is reduced (Popescu Vifor, 1979). The degree of kinship is determined with the coefficient of inbred (F) that can be estimated on the pedigree, as it represents 1/2 of the degree of kinship.
In the case of the present researches it was found that at this moment, the inbreeding degree of the population is of 7.41 and shows variations from 8.48 at the population of grizzle Karakul from the breeding areas placed near Botoşani County and of 7.31 at the population within the forming area.
On the basis of all these values, it is concluded that at this point the population is at a moderate to close inbred level. The existence of this indicator at values higher than 6.0 is a consequence of the fact that in the activity of forming, improvement and fixation of the desired characters is made easier when there is a moderate inbreeding within the population. Subsequently, after reaching the desired type, the reduction of this indicator can be achieved by bringing or exchanging breeders from different ecological areas and the mating control is done after careful analysis of the individual pedigree.

Analysing the degree of kinship of individuals or reproductive isolation. The problem of determining the degree of reproductive isolation is complex, but it has the role of highlighting the proportion of blood characteristic to the analysed population (Drăgănescu, 1979; Popescu Vifor, 1979; Pipernea, 1979). Reproductive isolation depends on several aspects, being influenced by the age structure, average duration of exploitation and

reproduction applied in the analysed population (Popa, 2009).

The analysis of the evolutionary dynamics of the degree of kinship or genetic similarity with other founding breeds shows that as the generation passes between the current grizzle breeds and other breeds that participated in the formation of the Karakul de Botoşani breed, there is an increase in the genetic distance. The assessment of the degree of kinship shows that if in 2007 the proportion of blood belonging to the Ţurcana breed was 11, in the genetic structure of the current type of grizzle Karakul de Botoşani its gene pool is reduced enough, which makes the genetic distance to register a tendency from 0.036 in 2007 to 0.024 in 2016 (Table 2).

The results also highlight a genetic distance from the Asian Karakul and the Karakul brought from other units from the country (Dulbanu, Ruşeţu), because in 2016 the genetic similarity R_{xy} was of 0.002 with the Asian type and R_{xy} = 0.0081 with the one from other units.

The analysis of the degree of kinship or genetic similarity of the current grizzle type, formed within the Karakul de Botoşani breed, with the German Karakul is not recorded because the breeders have activated in more distant periods and were black and grey.

Table 2. The degree of kinship of the individuals Karakul de Botoşani – grizzle variety with the founding breeds

Breeds	2007	2016	Difference	R_{xy} with breed (%)	
				2007	2016
Ţurcana	11	3	- 8	0.036	0.024
Asian Karakul	14	19	+ 5	0.012	0.002
Dutch Karakul	3	1	- 2	-	-
Karakul from other units	2	-	- 2	0.0110	0.0081

Analysis of the evolution of the intergenerational interval. This indicator is extremely important because it is also an essential factor of improvement, influencing directly the effect of selection on each new generation (Pascal, 2015). The analysis of genealogical records and evidence records of the mounts and bring forth highlights that the active population of the grizzle Karakul de Botoşani breed the intergenerational interval has an average duration close to 4 years, showing a certain degree of high precocity compared to other native breeds.

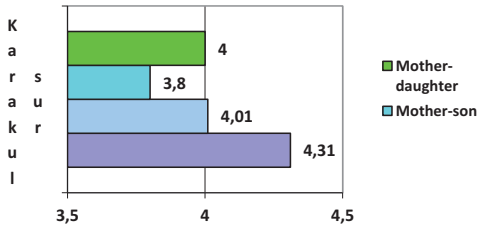

Figure 1. The intergenerational interval in some varieties of Karakul de Botoşani breed

By comparing these values to those determined by other authors, but for other sheep breeds, this intergenerational interval is longer with 6-12 months as a direct effect due to the prolongation of the duration of exploitation of females and rams in the living stock.

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CONCLUSIONS

1. The calculation of the actual size of the active population indicates a variation from a minimum of 49.46 determined for the active population in exploitations near the forming area to a maximum of 126.1 determined by the total flock from the Karakul de Botoşani breeding area.
2. For the entire active population while the degree of reproductive isolation was 0.68, the actual size was 176.87, and the rate of inbreeding was of 7.41.
3. In the active population the sex ratio was 1 to 12.62 in the forming area and 24.46 females to a nursery ram in the other areas.
4. The existence and keeping of a fairly tight relationship is a consequence of the fact that reproduction is based on the directed mount, but also on the high degree of subdivision of the breed in relation to the nuances of colour which appear in this variety.
5. The actual inbreeding degree determined on the total active population for this variety is 7.41 with variations from 8.48 at the grizzle Karakul population in coterminous growth areas and 7.31 at the population within the forming area.

6. The research results show that if in 2007 the proportion of blood belonging to the Ţurcana breed was 11, the genetic structure of the current type of grizzle Karakul de Botoşani its gene pool is reduced quite a lot, which makes the genetic distance to show a distance tendency from 0.036 in 2007 to 0.024 in 2016.
7. In the active population of the grizzle Karakul de Botoşani breed, the intergenerational interval has an average duration close to 4 years, highlighting a high degree of precocity compared to other indigenous breeds.

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THE IMPLICATIONS OF GENETIC MATERIAL ON THE PARAMETERS OF RAW MILK OBTAINED UNDER IDENTICAL GROWTH CONDITIONS FROM ROMANIAN BLACK SPOTTED AND HOLSTEIN-FRIESIAN COWS

Lucian-Ionel ILIE

University of Agronomic Sciences and Veterinary Medicine Bucharest, No. 59,
Blvd. Mărăşti, 011464, Bucharest 1, Romania, Phone: +4021.318.22.66, Fax: +4021.318.28.88,
email: www.usamv.ro

Corresponding author email: drlucianilie@yahoo.com

Abstract

Romanian Black Spotted and Holstein-Friesian cows are breeds that are frequently found on the dairy farms in Romania. The aim of the study was to evaluate the main sensory, physical-chemical and microbiological parameters of the milk coming from these cows grown under identical conditions of housing, microclimate and nutrition. In terms of most sensorial parameters (appearance, colour, smell, density, taste) the recorded values did not differ significantly from one breed to the other. For the total number of germs and the number of somatic cells the values varied quite a lot, higher values being recorded by Romanian Black Spotted cows' milk, but without exceeding the legal limits. Significant differences were recorded for some physical-chemical parameters for which the milk samples were analyzed. Thus, the amount of milk harvested from Holstein-Friesian cows was up to 35% higher than from Romanian Black Spotted, milk to which the dry substance was in larger quantity (up to 14%) due to higher protein content (4%) and fat (2-5%). At the same time, values for acidity, density and freezing point were close for the two types of milk analyzed.

Key words: *Holstein-Friesian cows, Romanian Black Spotted cows, milk composition, milk quality.*

INTRODUCTION

Cow milk represents a system with a yield and chemical composition that may be affected by various factors, including here the genetic and the environmental ones.

Some authors claim that the mammary diseases (clinical mastitis) represents the main cause of losses on milk yield production, losses that are mainly due to the growth of the total number of germs and the number of somatic cells in milk, accompanied by a decrease of chemical components values in the milk yield. Other authors believe that low milk production is due to unfavorable environmental conditions, which do not allow full expression of the genetic potential of the cows (Neijenhuis, 2008; Timms, 2004).

From the nutritional point of view, milk is a valuable food, because it contains most of the nutrients needed by the body, in a balanced proportion and which are well assimilated by the organism (Arghiriade et al., 2013). From a chemical point of view, milk is mostly formed from a dispersed medium – water (89.7 – 86%), in which the other components are dispersed which forms the dry substance (10.3 – 14%; the average dry substance is 12.5%) (Tăpăloagă, 2014).

The dry substance consists of main components: proteins, fats, carbohydrates and small components: mineral salts, vitamins, enzymes, gases and pigments. Fat varies between very wide limits, between 2.5 and 7.5% and is represented by triglycerides, complex lipids and free fatty acids. Proteins are in proportion 3.4%, contain about 22 essential amino acids, giving to the proteins a high biological value. Lactose varies widely, between 2.84 – 7.46%, with an average of 4.55%, is and its the main milk sugar (Tudor, 2009).

In addition to the important source of calcium, phosphorus and B complex vitamins, milk contains large quantities of potassium but low sodium, which is manifested by increased diuresis. Also in milk there are low amounts of essential fatty acids, iron and vitamins A and D. Milk pigments are mainly carotene, lactochrome and lipocrom.

The diversity of microorganisms present in milk can be explained by multiple contamination sources: mammary contamination,

unhygienic milking or external factors that act after milk (contact with the outside or cooling and storage temperature) (Kalac, 2011; Lembeye, 2016). So, somatic cells count can be viewed as an indicator of udder health status and milking hygiene (Ilie, 2011). Human nutrition is the basis of its construction. Wellbeing, balance and health of each human being are in close touch with food. Developing appropriate feeding brings together with other correct behaviour items a healthier life for everyone (Tăpăloagă, 2017).

MATERIALS AND METHODS

The study was conducted on two groups of bovine breeds, Romanian Black Spotted and Holstein-Friesian (20 cows of each breed), grown under identical conditions of housing, microclimate and nutrition. The milk collected from them has been evaluated in terms of sensorial parameters (appearance, colour, smell, consistency, taste), of some physical-chemical parameters (fat, protein, dry matter, acidity, density, freezing point) and hygienic parameters (the total number of germs and the number of somatic cells). The samples were collected and analyzed during 2017, a number of 724 samples, making statistical comparative evaluations between the average values obtained by the two batches of cows. Concerning the data collected for this survey, there were used the results of the control of cow milking, determined during test milking. The methods of analysis used were those mentioned and described in the specific normative acts (Reg. (EC) No. 853/2004; Reg. (EC) No. 854/2004; Reg. (EC) No. 882/2004; AOAC, 2012), the samples being harvested twice a day in the morning and evening meals.

RESULTS AND DISCUSSIONS

From the point of view of sensorial parameters, the recorded values did not differ significantly from one race to the other, the samples being of colour white-yellow or even yellowish fat for fatty milk; the smell was normal with a weak flavour of fresh protein; normal taste is sweet due to lactose; the opacity being higher as the milk is fatter.

It is well known that the smell of raw milk is typical for each ruminant species, which is considered dependent on some quantitative differences in the volatile profile and on the presence of specific compounds associated with each type of milk (Toso, 2002).

Table 1. Milk yield and composition of milk values

Cow's groups	Milk yield (kg x 100 l)	Fat (%)	Protein (%)
Holstein-Friesian	5.77	5.16	4.0
Romanian Black Spotted	4.28	4.02	3.4

For parameters that characterize the hygienic quality of milk, the total number of germs and the number of somatic cells, the values varied quite a lot, higher values being recorded by cow's milk Romanian Black Spotted (NTG = 83,000 cell/ml and respectively SCC = 365,000 cell/ml), but without exceeding the legal limits (NTG = 100,000 cell/ml and CCS = 400,000 cell/ml).

Table 2. NTG and CSS values of milk samples

Cow's groups	NTG (cell x 10.000ml)	CSS (cell x 100.000ml)
Holstein-Friesian	6.7	3.12
Romanian Black Spotted	8.3	3.65

The significant differences were recorded for some physical-chemical parameters for which the milk samples were analyzed. Thus, the amount of milk harvested daily from the Holstein-Friesian cow's (average value 577 kg of milk/day/20 cows) has been with up to 35% was greater than to Romanian Black Spotted cow's (average value 428 kg of milk/day/20 cows).

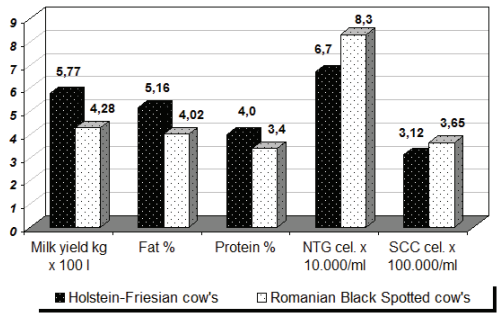

Figure 1. Values of milk yield, composition of milk, NTG and SCC

For milk samples from the Holstein-Friesian cows the dry substance was in greater quantity (up to 14%) due to higher protein content (4%) and fat (2-5%).

The researches concerning this thematic (cow productivity, content of somatic cells) performed by other scientific teams, demonstrated a highly significant effect of the herd on the count of somatic cells, while others presented no significant effect of the first factor on the other parameter.

At the same time, values for acidity, density and freezing point were close for the two types of milk analyzed and did not exceed the maximum admissible limits provided by the legislation.

The stage of lactation and animal health play an important role in the balance which must exist between milk components (Tăpăloagă, 2014).

Among sensory characteristics of milk, flavor is one of the most important attributes for acceptability and preference by consumers (Kim, 1996).

CONCLUSIONS

The values of the chemical compounds present in milk depend primarily on the genetic potential of the cows. Secondly, expressing the maximum productive potential of cows is dependent on microclimate conditions. Without a superior genetic potential, excellent microclimate conditions will generate good parameters, but not extraordinary for milk. The genetic potential also influences the hygienic quality of milk, an increased immunity will make the rate of mammary affection low, so rare cases with increasing number of somatic cells.

For establishing the productive performance is mandatory, additionally to the conditions of housing, microclimate and nutrition, the quality of the genetic material, which will increase the feed conversion rate in higher quantities of animal food, compared to similar costs. In addition, although the amount of milk is higher, its quality is clearly superior, giving a higher value to the use of this food of a population category of various ages, without having associated food problems.

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ANTIOXIDANT ROLE OF THE GRAPE SEEDS MEAL IN PREVENTING THE DEGRADATION OF FATTY ACIDS-HIGH DIET FORMULATIONS FOR BROILERS

Margareta OLTEANU[1], Rodica Diana CRISTE[1], Dumitra Tatiana PANAITE[1], Raluca Paula TURCU[1,2], Mariana ROPOTA[1], Petru Alexandru VLAICU[1,2], Cristina SOICA[1], Monica MITOI[3]

[1]National Research-Development Institute for Animal Biology and Nutrition-IBNA-Balotesti, Calea Bucuresti nr. 1, Tel: (+4021)3512082; Fax: (+4021)3512080, Ilfov, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Marasti, 59, Romania
[3]Institute of Biology Bucharest, Splaiul Independentei, no 296, 060031, Romania.

Corresponding author email: margaretaolteanu@yahoo.com

Abstract

The production of foods rich in polyunsaturated fatty acids is influenced by the structure and nature of the feeds given to animals. The use of feed ingredients rich in polyunsaturated fatty acids causes the feeds to get rancid due to fat oxidation. Hence, the need to use antioxidants blocks oxidation by their reaction with the free radicals. The aim of the work was to investigate ways to prevent the degradation of high-polyunsaturated fatty acids diets used for Hubbard broilers feeding. Two compound feeds formulations, grower and finisher, were used. The control compound feed used corn, wheat, soybean meal and gluten as basic ingredients, plus 2% flax meal which is rich in polyunsaturated fatty acids. The experimental compound feeds differed from the control diet by the use 2% (E1) and 3% (E2) grape seeds meal, which has antioxidant properties. The flax meal had 30.01% protein, 15.59% fat, 75.76 g PUFA/100 g total fatty acids, of which 57.87 g omega -3 fatty acids, and 17.89 g omega-6 fatty acids/100 g total fatty acids, and omega-6/omega-3 ratio of 0.31; the energy level was 20.94 Mj/kg. The grape seeds meal had 12.76% protein, 7.13% fat, 3.68 mg EAG/g polyphenols and 35.30 mM Trolox equivalents/g sample, as antioxidant capacity. The compound feeds for the grower stage had between 20.19% (C) and 20.44% (E2) protein, between 5.44% (C) and 6.06% (E2) fat, between 65.33 g% (C) and 70.10 g% polyunsaturated fatty acids, of which omega-3 polyunsaturated fatty acids between 4.29 g% (E1) and 5.01 g% (E2). The compound feeds for the finisher stage had between 19.84% (C) and 20.09% (E2) protein, between 8.13% (E1) and 8.74% (E2) fat, an average value of 60.80% of polyunsaturated fatty acids, of which omega-3 polyunsaturated fatty acids between 7.31 g% (C) and 7.92 g% (E2). We analysed the evolution of the compound feeds fat degradation indices at 0 and 14 days after manufacture and noticed that the peroxide index at 14 days, compared to 0 days, had the lowest increase in group E2, 4.00%, for the grower compound feeds, compared to 14.63% for group C, and 10.00% for the finisher compound feeds, compared to 26.82% in group C. fat acidity displayed the same evolution, with the lowest increase in group E2: 13.86%, compared to 20.10% in group C for the grower stage, and 1.83% (E2), compared to 4.27% in group C for the finisher stage. The Kreiss reaction was negative for all compound feeds. The use 3% of grape seeds meal as natural antioxidant in the compound feed for group E2, inhibited the reactions of lipid degradation.

Key words*: antioxidants, winery by-products, fatty acids, lipid degradation.*

INTRODUCTION

The consumers are increasingly interested to use in their daily diet foods (vegetal or animal) rich in polyunsaturated fatty acids, omega 3 particularly, because of their beneficial effects on human health (Huang, 2010; Pilkington et al., 2011; Flachs et al., 2014). Animal products can be enriched in polyunsaturated fatty acids through animal nutrition, using diets which include feed ingredients high in polyunsaturated fatty acids. However, the higher proportion of these fatty acids in animal diets also enhances the effect of their lipid oxidation (Ren et al., 2013), which makes it necessary to use feed additives with antioxidant properties. Hence, the increasing interest of the nutritionists and animal producers towards the use of agro-industrial by-products rich in bioactive compounds, as natural antioxidants in animal feeding.

The winery by-products, grape marc, grape seeds and peels, as well as the grape seeds cakes and meal, are a natural source high in

polyphenols, which bestows them with antioxidant properties. The abundance of bioactive polyphenols in these by-products (Radovanovic et al., 2009; Granato et al., 2010), is of real interest for many researchers and companies from the food industry and from the feed additives industry.

MATERIALS AND METHODS

Our feeding trial studied ways to prevent the degradation of the dietary polyunsaturated fatty acids added to the compound feeds for slow-growing Hubbard broilers. We used grape seeds meal as natural antioxidant and manufactured two types of compound feeds, grower and finisher. The diets were formulated with corn, wheat, soybean meal and gluten as basic ingredients, plus 2% flax meal, which is high in polyunsaturated fatty acids. The experimental diet formulations differed from the control compound feed by the addition of 2% (E1) and 3% (E2) grape seeds meal. Compound feeds samples were collected and assayed for the main nutrients and for the evolution of the fat degradation indices at 0 and 14 days from manufacture. We also performed mycological, bacteriological and mycotoxicological analyses to check compound feeds salubriousness.

Standardized methods were used to determine the concentration of the basic nutrients, as follows:

- the dry matter (DM) was determined using the gravimetric method, whose working principle involves the determination of sample mass by drying at 103^{o}C, according to Regulation (CE) 152/2009 and standard SR ISO 6496:2001. We used a Sartorius (Gottingen, Germany) scale and BMT drying closet, ECOCELL Blueline Comfort (Neuremberg, Germany);
- the crude protein (CP) was determined using the Kjeldahl method, whose working principle involves sample digestion by heating with sulphuric acid in the presence of a catalyst, for the conversion of the protein nitrogen into ammonium sulphate. The reaction products are alkalinized with sodium hydroxide, for the release of the trapped ammonia, by distillation in a solution of boric acid in excess, followed by titration in a solution of hydrochloric acid. The method complies with Regulation (CE) 152/2009 and standard SR ISO 5983-2:2009. We used a semiautomatic KJELTEC auto 2300 system – Tecator (Sweden);
- the ether extractives (EE) were determined by extraction is organic solvents, which involves fat extraction by petrol ether, removal of the solvent by distillation, drying and weighing the residue. The method complies with Regulation (CE) 152/2009 and standard SR ISO 6492:2001. We used a SOXTEC-2055 FOSS system – Tecator (Sweden);
- the fatty acids were determined using the chromatographic method, which involved the transformation of the fatty acids from the sample in methyl esters, followed by the separation of the compounds in a chromatographic column and their identification by comparison with standard chromatograms. The method complies with standard SR CEN ISO/TS 17764 -2: 2008. We used a Perkin Elmer-Clarus 500 chromatograph, fitted with a system for injection into the capillary column, with high polarity stationary phase (BPX70: 60m x 0.25mm inner diameters and 0.25µm thick film); or high polarity cyanopril phases, which have similar resolution for different geometric isomers (THERMO TR-Fame: 120m x 0.25mm ID x 0.25 µm film);
- the crude fibre (CF) was determined with the method with intermediary filtration, whose working principle involves weighing the sample boiled successively with solutions of sulphuric acid and sodium hydroxide. The resulting residue was filtered, dried, burnt and weighed. The method complies with Regulation (CE) 152/2009 and standard SR EN ISO 6865:2002. We used a FIBERTEC 2010 system – Tecator (Sweden);
- the ash (Ash) was determined by the gravimetric method, which involves sample decomposition by burning and weighing of the resulting ash. The method complies with Regulation (CE) 152/2009 and standard SR EN ISO 2171:2010. We used a Caloris CL 1206 furnace.
- the gross energy was determined by calculation using the gross chemical analysis (dry matter, protein, fibre, fat, nitrogen-free extractives and ash), using the equations of Burlacu et al. (2002).

The fat degradation indices, peroxide value, fat acidity and Kreiss reaction were determined by the volumetric method, according to STAS 12266-84. The principle of the peroxide value determination presumes the treatment of the fat solution in acid medium with potassium iodide, where the peroxides release the iodine, which is titrated with sodium thiosulphate. The principle of fat acidity determination presumes the neutralization of the free fatty acids from the fat by titration with potassium hydroxide solution. The Kreiss reaction is a qualitative method whose principle involves the condensation reaction between the epihydrinic aldehyde and fluoroglucine, with the formation of a coloured compound whose intensity varies from pink to violet-red, depending on the concentration of epihydrinic aldehyde.

To determine the concentration of polyphenols and the antioxidant capacity of the samples, we first extracted the phenol compounds in acidified methanol (methanol:HCl = 80:20).

The polyphenol content of the methanol extracts has been determined according to the method described by Mihailovic et al. (2013), modified. The reaction mixture consisted of: the methanol extract in proper dilution according to the analysed sample, Folin-Ciocâlteu reagent and a solution of 7.5% de Na_2CO_3. The reaction mixture was maintained for 30 min. at room temperature, thereafter absorbance was read at 765 nm. The total concentration of phenols, was expressed in equivalents gallic acid/g fresh matter (mg EAG/g sample). We used a UV-VIS Thermo Scientific spectrophotometer.

The determination of the antioxidant capacity of the methanol extracts has been done using the DPPH method proposed by Marxen et al. (2007). The antioxidant capacity has been estimated by calculating the difference between the control and the sample, compared to a standard curve which used Trolox (synthetic antioxidant analogue to α-tocopherol), as standard antioxidant. The antioxidant capacity has been expressed in Trolox equivalents/g fresh matter (mMTrolox/g sample). We used a UV-VIS Analytik Jena Specord 250 Plus spectrophotometer with thermostatic carousel.

RESULTS AND DISCUSSIONS

The addition of raw materials rich in polyunsaturated fatty acids to the compound feeds for animals, with the purpose of producing functional foods, favours the lipid oxidation phenomenon, which can be slowed down using antioxidants, whose high content of polyphenols react with the free radicals.

Flax meal, feed ingredient high in polyunsaturated fatty acids, is used the compound feeds for slow-growing Hubbard broilers. The flax meal we used had 30.01% protein, 15.59% ether extractives and 20.94 MJ/kg gross energy, similar to the results reported by Mironeasa et al. (2010) and by Elagamey et al. (2013), i.e. 86.74 – 89.17% dry matter, 6.26 - 9.01% protein and 2.14 – 8.28% ash, similar to the data obtained by researchers Criste et al. (2009) and Panaite et al. (2016).

The grape seeds meal had 12.76% protein, 7.13% fat (Table 1), similar to those reported by Elagamey et al. (2013), Panaite et al. (2016) or by Vlaicu et al. (2017). The polyphenols content of the grape seeds meal was 26.65 mg EAG/g sample and the antioxidant capacity was 148.35 mM Trolox equivalents/g sample. These results are close to those reported by Poudel et al. (2008), cited by En-Qin Xia et al. (2010) or by Ky et al. (2014), who reported polyphenols concentrations in the range of 23.8 – 44.5 mg EAG/g sample in the grape pulp, to 31.6 - 374.9 mg EAG/g in the grape peel samples; they also reported values of the antioxidant capacity in the range of 16.8 - 92 mM Trolox equivalents/g sample in the grape seeds, to 15.7 - 113.3 mM Trolox equivalents/g sample, in the grape peel samples.

Table 1. Feed ingredients content of main nutrients

Sample	DMr (%)	OM (%)	CP (%)	EE (%)	CF (%)	Ash (%)	GE (Mj/kg)
Flax meal	90.75	88.28	30.01	15.59	11.47	4.96	20.94
Grape seeds meal	90.76	88.35	12.76	7.13	35.30	2.92	18.49

Chemical composition expressed on dry matter (DM); DMr-real dry matter; OM-organic matter; CP-crude protein; EE-ether extractives; CF-cellulose; Ash-ash; Ca-calcium; P-phosphorus; GE-gross energy

The values of the lipid degradation indices for the flax meal and for the grape seeds meal (Table 2), which are below the admitted level, show that that could be added to the compound feeds for broilers.

Table 2. Evolution of the fat degradation indices in the feed ingredients

Items	Flax meal	Grape seeds meal	Admitted limits
Peroxide value (ml thiosulfate 0.1 N/g fat);	0.350	0.445	1.2
Fat acidity (mg KOH/g fat);	9.10	11.24	50
The Kreiss reaction	negative	negative	negative

We also determined the fatty acids content in the fat of the feed ingredients in terms of the level of fatty acids saturation (Table 3).

Table 3. Fatty acids content in the fat of the feed ingredients in terms of the level of fatty acids saturation (g fatty acid/100 g total fatty acids)

Items	Flax meal	Grape seeds meal
SFA, %	7.43	14.82
MUFA, %	16.81	17.91
UFA, %	92.57	84.93
PUFA, % of which:	75.76	67.02
Ω3, %	57.87	0.78
Ω6, %	17.89	66.24
Ω6/Ω3	0.31	85.28
SFA/UFA	0.080	0.174
PUFA/MUFA	4.506	3.742

SFA= saturated fatty acids; MUFA= mono-unsaturated fatty acids; PUFA= poly-unsaturated fatty acids; UFA= total unsaturated fatty acids; Ω3-omega 3 poly-unsaturated fatty acids; Ω6- omega 6 poly-unsaturated fatty acids

There was obtained a value of 0.31 for the omega-6/omega-3 (Ω6/Ω3) ratio in the flax meal, which is below 1, value considered to be ideal. These results are close to those reported by Panaite et al. (2016) and by Vlaicu et al. (2017).The evaluation of the basic chemical composition of the compound feeds for broilers, tested for the growing stage, showed a protein content ranging between 20.19% (C) and 20.44% (E2) and a fat content ranging between 5.44% (C) and 6.06% (E2). The compound feeds for the finishing stage had protein content ranging between 19.84% (C) and 20.09% (E2), and a fat content ranging between 8.13% (E1) and 8.74% (E2).

The total content of polyunsaturated fatty acids, PUFA (Table 4) of the studied compound feeds ranged between 60.75 g/100 g fat, in group E1 –finishing stage, and 70.10 g/100 g fat, in group E2 –growth stage. The content of omega-3 fatty acids ranged between 4.29 g/100 g fat, in group E1 –growth stage, and 7.92 g/100 g fat, in group E2 –finishing stage. The omega-6/omega-3 ratio was between 6.68 in group E2 –finishing stage, and 14.59 in group E1 – growth stage. These results are higher than those reported by Olteanu et al. (2017) and Turcu et al. (2017).

As it is known, peroxide concentration is a major indicator of fat degradation by autooxidation, so that the peroxide value must be determined in the samples with more than 4% fat.

Table 4. Fatty acids content of the compound feeds fat, depending on their level of saturation (g acid/100 g total fatty acids)

Items	Growth stage			Finishing stage		
	C	E1	E2	C	E1	E2
SFA, %	11.04	11.14	9.72	14.74	14.75	14.87
MUFA, %	23.62	21.94	20.18	24.11	24.23	24.14
UFA, %	88.96	88.86	90.28	84.96	84.98	84.95
PUFA, % of which:	65.33	66.92	70.10	60.86	60.75	60.82
Ω3, %	4.46	4.29	5.01	7.31	7.36	7.92
Ω6, %	60.87	62.62	65.09	53.55	53.39	52.90
Ω6/Ω3	13.65	14.59	13.00	7.33	7.25	6.68
SFA/UFA	0.124	0.125	0.108	0.17	0.17	0.18
PUFA/MUFA	2.765	3.050	3.474	2.52	2.51	2.52

SFA= saturated fatty acids; MUFA= mono-unsaturated fatty acids; PUFA= poly-unsaturated fatty acids; UFA= total unsaturated fatty acids; Ω3-omega 3 poly-unsaturated fatty acids; Ω6- omega 6 poly-unsaturated fatty acids

The acidity index shows the amount of free fatty acids in the fat.

The Kreiss reaction too is an indicator of the fat getting rancid, as it shows the presence of epihydrinic aldehyde and of other carbonyl compounds resulting from fat degradation. Thus, we evaluated the effect of the grape seeds meal, as antioxidant, added to the compound feeds for the two experimental groups (E1 and E2), compared to the control group (Table 5), by determining the indices of fat degradation in the compound feeds samples collected at 0 and 14 days from manufacture.

Table 5. Evolution of fat degradation indices in the compound feeds

Items		Growth stage			Finishing stage			Admitted limits
		C	E1	E2	C	E1	E2	
Peroxide value (ml thiosulfate 0.1 N/g fat);	0 days	0.41	0.54	0.50	0.41	0.40	0.50	1.2
	14 days	0.47	0.59	0.52	0.52	0.50	0.55	
	Difference (%)	14.63	9.26	4.00	26.82	25.00	10.00	
Fat acidity (mg KOH/g fat);	0 days	15.71	14.42	14.35	17.34	17.42	18.60	50
	14 days	20.10	17.27	16.34	18.08	18.00	18.94	
	Difference (%)	27.94	19.76	13.86	4.27	3.33	1.83	
Kreiss reaction	0 days	negative	negative	negative	negative	negative	negative	negative
	14 days	negative	negative	negative	negative	negative	negative	

After 14 days of storage at room temperature, the lowest difference in the peroxide values, 4.00%, was noticed in the compound feed for group E2, followed by 9.26%, in the compound feed for group E1, compared to 14.63% for group C, for the growth stage. In the compound feed for the finishing stage, the lowest difference in the peroxide values, 10.00%, was noticed also in the compound feed for group E2, followed by 25.00% in the compound feed for group E1, compared to 26.82% for group C. The lowest difference in fat acidity, 13.86%, was noticed in the compound feed for group E2, followed by 19.76% in the compound feed for group E1, compared to 27.94% for group C, for the growth stage. In the compound feed for the finishing stage, the lowest difference in fat acidity, 1.83%, was noticed also in the compound feed for group E2, followed by s 3.33%, in the compound feed for group E1, compared to la 4.27% for group C. As Table 5 shows, the absolute values for the fat degradation indices are below the admitted limit in all compound feeds, for both stages.

Table 6. Mycological, bacteriological and mycotoxicological evaluation of the compound feeds

Items	Growth stage			Finishing stage			Admitted limits *(MO 362bis/2003; 32 EC/2002)*
	C	E1	E2	C	E1	E2	
Mycological assessment							
TFC (col/g);	7000	2500	4000	7000	10500	5250	max. $5x10^4$ col/g;
Bacteriological assessment							
TGC (col/g);	$35x10^3$	$35.5x10^3$	$37x10^3$	$40x10^3$	$38.5x10^3$	$37.5x10^3$	max. $15x10^6$ col/g;
Total coliform(col/g)	0.9	9.5	9.5	0.7	150	110	max. $3x10^3$ col/g
E. coli (col/g)	0.4	4.5	0.9	0.7	0.0	3	max.100 col/g;
Salmonella (col/g);	Absent	Absent	Absent	Absent	Absent	Absent	0 col/g;
Mycotoxicological assessment							
AFLA-total (ppm)	$0.5x10^{-3}$	$0.7x10^{-3}$	$0.8x10^{-3}$	0	$0.1x10^{-3}$	$0.1x10^{-3}$	0.02 ppm

TFC–total fungal count; TGC –total germs count; *E.coli – Escherichia coli*

We also evaluated the sanitary quality of the compound feeds (Table 6), as they may bring risk for the animals, therefore for the foods (Frazzoli and Mantovani, 2010). Hence, the permanent feed safety concern of the food industry and of the compound feeds producers.

The results of the mycological, bacteriological and mycotoxicological determinations on the compound feeds show that they were all below the admitted levels. ANSVSA norm regarding the quality and salubriousness parameters for the production, import, quality inspection, selling and using simple concentrate feeds, compound feeds, feed additives, premixes, energy substances, minerals and special feeds, published in MO 362 bis/2003 and in EC Regulation 32 bis/2002.

CONCLUSIONS

The 2% grape seeds meal added to the compound feeds enriched them in omega-3 polyunsaturated fatty acids, in both stages, with higher values in the finishing stage.

The use of the natural antioxidant, 2% grape seeds meal in the compound feeds for group E1, and 3% grape seeds meal in the compound feeds for group E2, improved the feeding qualities of the compound feeds in both stages, by inhibiting the reactions of lipid degradation. Thus, the highest values of total polyunsaturated fatty acids, of omega-3 fatty acids, were determined in the compound feeds for group E2, higher than in the compound feeds for group E1, and for the control group (C).

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THE USE OF SOME SUPPLEMENTARY FEEDS AND THEIR INFLUENCE ON THE LONGEVITY AND WAX PRODUCTION OF CAGED HONEYBEES

Dan-Iulian EŞANU, Cristina Gabriela RADU-RUSU, Ioan Mircea POP

University of Agricultural Sciences and Veterinary Medicine Iaşi, 8, Mihail Sadoveanu Alley, Iaşi, Romania

Corresponding author email: popmirceais@yahoo.com

Abstract

Replacing natural honeybee nutrients sources (pollen, nectar) with different types of alternative feed (sugar syrup, corn syrup, protein flour) has different consequences on bee health and their production, either maintained in colonies or isolated from them. In this context, this study analyzed the influence of using of some energy and energy-protein resources on longevity and wax production of caged honeybees, isolated from the colony. Thus, the introducing of protein substances into alternative feed, especially pollen substitutes (beer yeast, powdered milk), has led to a decrease in the life of bees. The increase in the amount of bee wax was favoured by the introduction of protein sources into the bee`s diets, with the best results generated by pollen; instead, the pollen substitutes determined the lowest values of this parameter. The bee`s energy-protein mixtures consumption also affected the overall health of those.

Key words*: caged honeybees, longevity, pollen, wax.*

INTRODUCTION

Natural bee nourishment contains energy components (mana, nectar, honey) and protein components (pollen, bee bread), all of which are quantitatively determined by the climatic conditions of the apicultural season (Moraru, 2006). As with other animals, bee nutrition is also studying how energy and nutrients are used in the body or entire colony, as well as the yield of their use in different functions or their productions (Pop, 2006).

When there are not enough natural food sources for bees, beekeepers administer them different food recipes to provide the nutrients needed for bee colonies based on energy syrups or pollen substitutes(beer yeast, milk powder) and whose consequences on bee development are still study subjects of research papers.

The bee family is an autonomous biological unit, the development of which is based on the continuous interaction between individuals (Rinderer, 2012). The isolation of bees from colony also involves the aggravation of nutritional relationships between individuals, which negatively influences some morpho-productive characteristics of the solitary bee, particularly its longevity.

The isolated bee has a shorter lifespan than the one inside of a colony, where division of labor implies, among other activities, food exchange (trofalaxia) from one individual to another (Sammataro, 2013); the lifespan is also determined by presence of brood and adult bee workers (Eyer, 2016). Some studies are demonstrating that even among bees introduced into cages creates a type of nutritional relationship dependent on the number of individuals, which is reflected in their consumption of food (Brodschneider, 2017).

This method of bee nourishment research which involves introducing them into cages of different dimensions was also necessary for elucidating problems related to bee psychology, toxicology or parasitology (Glavinic, 2017), as well as problems related to the activity of colony castles and the analysis of some productive parameters (Williams et al., 2013).

The method of feeding bees isolated from colonies allows several experiments to be carried out over a short period of time compared to feeding bees in the field, and this is particularly relevant in studying the impact on bee lifespan of chemicals substances used in agriculture, like pesticides, fungicides, neonicotinoids (Fries, 2013).

Concerning of some parameters of appreciation of their quality, the bees within a colony react differently in so-called food diets compared to isolated bees (Altaye, 2010); the presence of

pollen in nature increases the vitality of bees from field, while the introduction of pollen into diets of caged honeybees decrease their longevity (Pirk, 2010).
Differences between field results and those obtained from caged honeybees have also occurred in the case of feeding with energy diets. For example, the use of honey in diets has determined bigger longevity of field bees than the values obtained from caged bees, while the use of sugar syrup in diets determined smaller longevity of field bees than the one of caged honeybees (Abou-Shaara, 2017).
Another problem related to the research of additional feeding of caged honeybees and the consistency of the obtained results is represented by the cage constructive model and the feeding regimen adopted. Some studies show significant differences between morphological parameters obtained on bees fed with same recipes but kept on different cages or fed with different regimens; even the feeders dimensions used for syrup can influence the results of an experiment (Huang, 2014).
The determination of the characteristics of supplementary feed recipes still remains the subject of more researchers. In the context of attempting to determine the impact of the various ingredients used on morpho-productive characteristics, this study aims to establish the influence of 8 types of feed recipe on longevity and wax production of caged honeybees.

MATERIALS AND METHODS

This study used as biological material bees of *Apis mellifera* (Carpathian ecotype), maintained in 3 experimental series, June 2017-September 2017, each series containing 8 experimental groups of 3 cages/each group (100 bees /cage). All groups were provided with specific food recipes and *ad libitum* water, so a total of 8 food recipes.
Determination of the target indicators (longevity and wax production) involved daily count of dead bees and weighing the wax combs from cages at the beginning and end of the experiment; we had also counted the quantities of each food recipe.
The experiment implied running 3 experimental series, with a 51 days period/series. Each series was formed from 8 experimental groups, consisting of 3 cages/group, 100 bees/each; we ensured one feed recipe for each group of research (total of 8 recipes).
The scheme of experimental research is presented on Table 1.

Table 1 Experimental research scheme

Specification (bees and conditions)	*Apis mellifera carpatica* - caged honeybee(0-24 h age)- 3 experimental series x 8 groups/series x 3 cages/group(100 bees/cage) -30 days in the dark, 34ºC, 55% U	
Feed recipes for each group (B1...B8)	B1	Honey
	B2	Sugar syrup 2:1
	B3	Corn hydrolyzate
	B4	Enzymatic invert sugar syrup
	B5	Sugar syrup 2:1 + pollen
	B6	Sugar syrup 2:1 + beer yeast
	B7	Sugar syrup 2:1 + milk powder
	B8	Energy-protein cake
Measured parameters	- bee longevity	
	- the amount of wax deposited	

The newly emerged adult worker bees (0-24 h age) were obtained from 3 different bee colonies in Deleni (Vaslui, Romania) using a specific method for obtaining them:
- Day 1: the queens of those3 bee families were isolated on 2 frames with the Haneman wall boxes and placed back in hives for 24 h;
- Day 2: the queens were removed from the Haneman boxes and placed back to hive;
- Day 18: the brood frames were collected from the hives and placed in the incubator (34°C);
- Day 21: the newly emerged bees were collected from the frames and placed in boxes. Each box was populated with individuals from a single bee family (each group was formed with bees from those 3 different colonies).
The boxes (210x60x120 mm) were made of wood, with two transparent plastic moveable walls (for better observation) and at the bottom had holes covered with wire mesh to allow ventilation. Inside each box was placed a wax frame foundation (about 3 g) on which the bees later deposited the wax. On bottom we placed 2 water feeders (10 ml each), one for water and one for feed recipe; these were plastic
After introducing of bees inside, all cages of a series (24 units) were kept for 30 days in an

incubator (dark, 34°C, 55% U), for reproducing the physical conditions from anormal beehive.
Specifications of feed recipes:
B1-honey (collected from our apiary);
B2- 2:1 sugar syrup (2 parts of sugar mixed with 40°C water);
B3-corn hydrolyzate (from the market);
B4-enzyme invert sugar syrup (from the market);
B5-sugar syrup 2:1 + bee-collected pollen (the pollen was grinded and then mixed with sugar syrup 2:1, pollen: syrup = 1:3 based on weight)
B6 - sugar syrup 2: 1 + beer yeast (inactivated beer yeast, yeast: syrup = 1:3)
B7-sugar syrup 2:1 + milk powder (skimmed milk powder, milk powder: syrup = 1:3)
B8- energy-protein cake (from the market for bees, mixed with water, 6 g cake/3 ml water).
After introducing bees into the cages, each bee feeder was filled with the specific feed recipe, and the water feeders were also filled.
Every day the feed recipes and water were refreshed, the quantities were determined and recorded and the dead bees were removed and counted as well.
In the end of last day of incubation we released the bees who had been still living, we counted them and also we weighed the wax frames from each cage of experiment.
In the same way we went through hall 3 experimental series of this research study.
The data was recorded and then statistically analyzed for discussions and conclusions.

RESULTS AND DISCUSSIONS

The results of this study relate to the bee longevity and the amount of beeswax deposited by bees in each cage.
Knowing the number of bees at the beginning of the experiment and the number of dead bees collected daily from cages, we have established the evolution of the number of individuals in each group during of the 30 days (incubating period) of each experimental series.
The cumulated data of those 3 series are presented in figure 1, where we tried comparing groups fed with energy syrups (Figure 1, a) as well as those fed with energy-protein mixtures (Figure 1, b); we also tried to establish the influence of introducing of some protein sources in bees feed recipes (Figure 1, c).

Analyzing Figure 1, we observe that bee fed with energy recipes had a longer lifetime than those who received energy-protein feed. This can be seen by analyzing the results obtained from group B2 (sugar syrup) and those obtained from lots that were fed with a mixture of sugar syrup and different protein ingredients (B5, B6, B7).

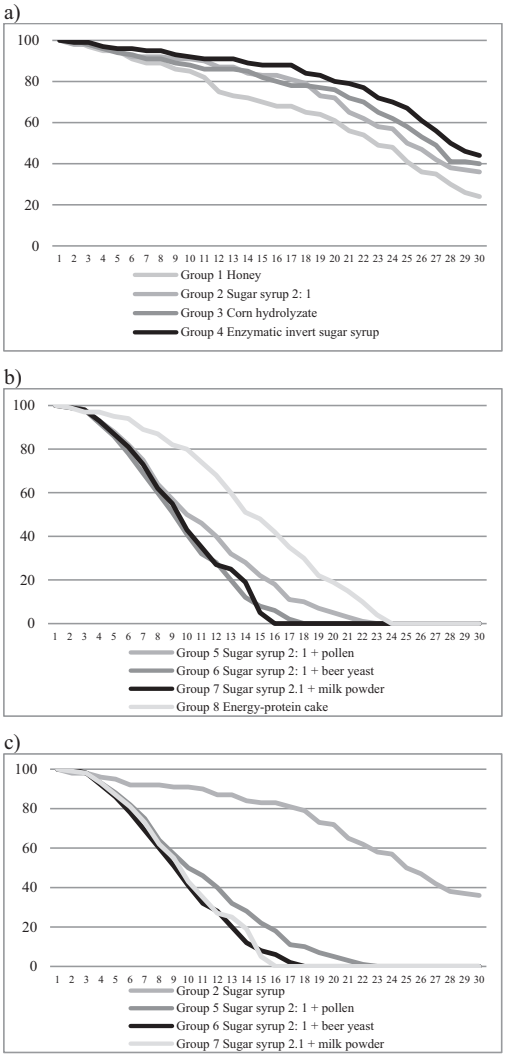

Figure 1. Evolution of bees numbers

Analyzing the result of groups fed with energy recipes (B1, B2, B3, B4) we can see that the least influence on the bee longevity produced the enzyme invert sugar, while honey determined the highest losses bees on experiment days. The low bee longevity caused

by the introduction of proteins into food can be seen in the B5, B6, B7 and B8 values that are inferior to those obtained from the first four groups. Taking into account the results obtained in the last four lots we notice that the group fed with sugar syrup and milk powder lost the earliest all bees in cages (day 16). A similar involution of the number of bees was registered also in the group fed with sugar syrup and beer yeast (lost all bees on the day 18). Among the fed energy-protein groups, the one who received the energy-protein cake (B8) showed the longest life of the bees in the cages. The wax frame foundations from cages were weighed before introducing and also when we finished the experimental series. By the difference of those two values we determined the amount of wax (mg) deposited by bees of each group; the average values of this parameter are presented in figure 2.

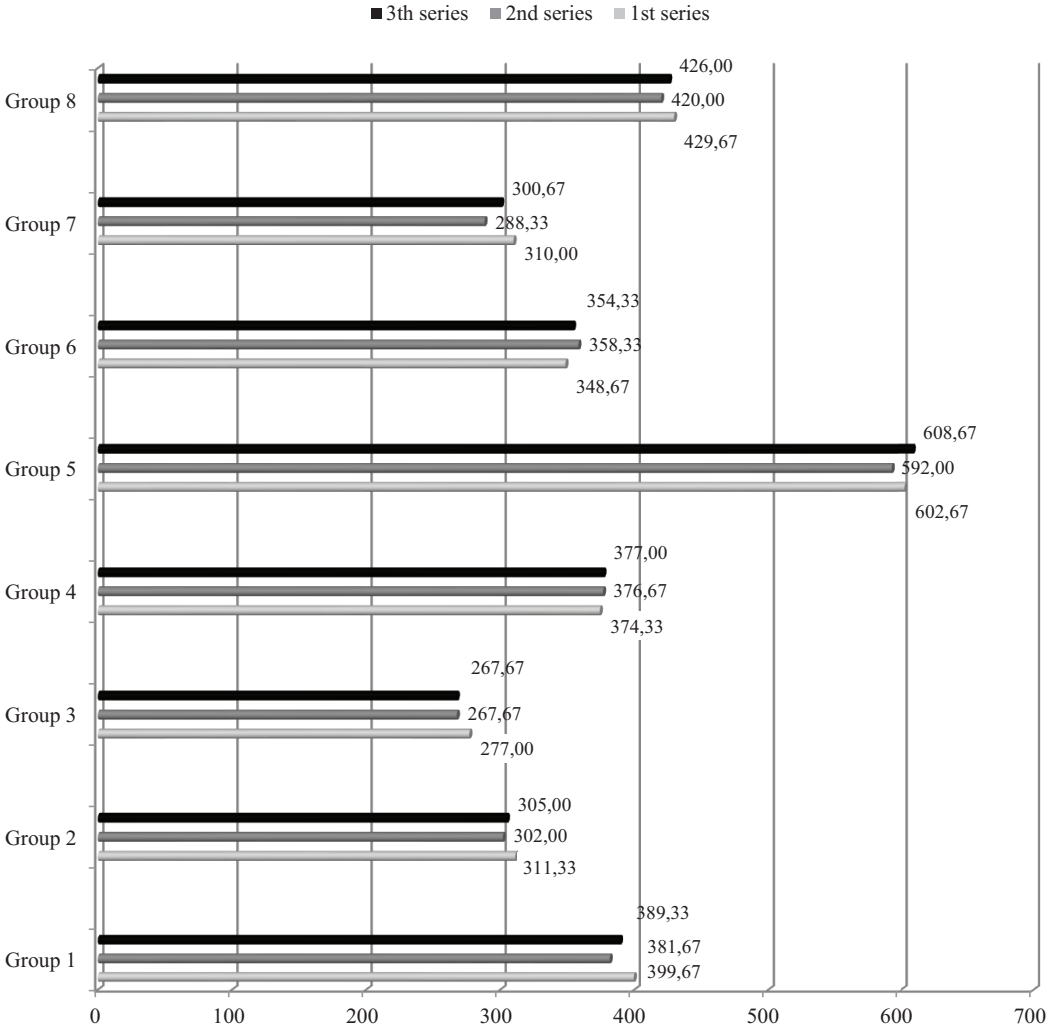

Figure 2. Amount of beeswax (mg)
Group 1- honey; Group2: sugar syrup; Group 3 - corn syrup; Group 4 - enzymatic invert sugar; Group 5 - sugar syrup 2:1 + pollen; Group 6 - sugar syrup 2:1 + beer yeast; Group 7 - sugar syrup 2:1 + milk powder; Group 8 - Energo-Protein Cake

In the Figure 2 it can see that this indicator had the highest values at the group fed with sugar syrup and pollen (608.67 mg), and the lowest values in the corn syrup group (267.67 mg). Analyzing this indicator we can state that pollen was much more valuable than the pollen substitutes used.

The importance of pollen in increasing the amount of wax can be seen by comparing the results of the group fed with sugar syrup (302 mg) and that fed with mixture of pollen and sugar syrup (608.67 mg).

Although proteins are important in the amount of beeswax, we can see that the lots fed with sugar syrup mixed with yeast (B6) or powdered milk (B7) recorded low values (348.67 mg and 288.33 mg); the main cause was the negative influence of recipes used on bee longevity and general health status (all the cages of these groups presented at the end of the control period traces of diarrhea).

A ranking, shown in descending order, of the values of this indicator obtained in groups fed with energy recipes would reveal the B1 group (399.67 mg), then B4 (377 mg), B2 (311.33 mg) and finally B3 (267.67 mg). Honey is therefore the best source of energy for bees kept in cages (isolated from the colony) to deposit a higher amount of wax.

CONCLUSIONS

The results obtained from this study show that caged honeybees bees react differently at some feed recipes to bees maintained in the field regarding the life span and the amount of wax deposited on frames.

After analyzing the results of the energy-fed groups, we noticed that honey (the natural source of bee's energy) determined the values of bee longevity lower than the values obtained from feeding with enzymatic inverted sugar syrup, which proved to be the best source of energy for caged bees. The importance of inverting of sugar in the bees nourishment quoted in many studies was also highlighted by this research.

Analyzing the results obtained from groups that received energy-protein recipes, we noticed that processing of proteins by bees has the consequence of decreasing their longevity. There are also another experiments related to feeding caged honeybees claim that protein processing by bees has a negative impact on their longevity.

At the same time, we should also note the lifetime of bees that received mixtures of sugar syrup with pollen substitutes (beer yeast, milk powder), which was lower than that of bees that received recipes with pollen; we can state that pollen was the best source of protein from this point of view.

The highest amount of wax was recorded in the group fed with sugar syrup and pollen (608.67 mg), and the smallest in the group fed with corn-hydrolysed syrup (267.67 mg).

Comparing the results obtained in the group fed with sugar syrup (311.33 mg) and the one fed with a mixture of sugar syrup and pollen, we noticed the influence of proteins on this production indicator.

Groups fed with mixture of sugar syrup and pollen substitutes recorded low values of wax amount (348.67 mg at mixture with beer yeast and 288.33 mg at mixture with milk powder), due to the general health status of bees that have consumed those recipes.

The results of this study contribute to the assessment of the impact of different feed recipes on some quality parameters of caged honeybees, like wax production and longevity. The characterization of the ingredients used in bees nourishment continues to be an interesting subject for researchers around the world.

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SOURCES OF CAROTENOIDS AND THEIR USES AS ANIMAL FEED ADDITIVES – A REVIEW

Diana PASARIN, Camelia ROVINARU

National Institute for Research and Development in Chemistry and Petrochemistry
202 Spl. Independentei, Bucharest, Romania

Corresponding author email: rovinarucamelia@yahoo.com

Abstract

Carotenoids are natural pigments, widely distributed in nature, synthesized by plants, algae, fungi, and phototrophic bacteria. Carotenoids have coloring power and antioxidant properties, being used as colorants for foods, cosmetics and feeds. Nowadays, a small part of industrial carotenoids is obtained from algae and plants, and the most part is chemically synthesized, but with high production cost. Taking into account consumer's demand for natural compounds, there is an increased trend for products containing natural ingredients including those used in animal feed. Products quality and animal health are influenced by carotenoids added in feed as important component in daily diet. This paper reviews aspects regarding the main sources of carotenoids used as feed additives for nutritional and coloring properties and their impact on animal and human health.

Key words*: carotenoids, feed additives, sources of carotenoids, pigmentation.*

INTRODUCTION

Carotenoids gained great interest lately for food and feeding, as their functions related to nutrition, health and wellbeing are well appreciated. As consumers become more and more aware of the importance of natural instead of synthetic and artificial ingredients, they tend to change preferences and replace products in their diets according to norms which no longer respond to good, tasty and affordable consumptions, yet to higher, long-term perspective standards (García-Chavarría and Lara-Flores, 2013; Carotenoids Market by Type, 2016; Mohiseni., 2017).

In this line, arguments related to the replacement of synthetic carotenoids with natural ones in case of animal feed additives lay in multiple health impact through environment and animal to human health (Kaur and Shah, 2017).

As such, many researches have been directed to in-depth knowledge on carotenoid sources, especially natural ones, for their use as animal feed additives, so as to benefit from their functions including skin and tissues pigmentation (especially when it comes to animals/birds grown in captivity), as growth promoters (in terms of mass production), for reproduction and survival goals, sensory properties and deposition of carotenoids crucial for consumers (egg yolks and other products). In fact, as studies show, there is a strong relationship between diets/foods rich in carotenoids and the risk reduction of various diseases. Carotenoids increase the immune response, are excellent antioxidants, scavenging and inactivating free radicals. This is why both vitamin A precursor carotenoids and the non-precursors ones play an important role in the protection and inhibition of serious diseases as cancer, atherosclerosis, cataracts, macular degeneration, multiple sclerosis, degenerative diseases, and cardiovascular diseases (Mezzomo et al., 2016).

On the other hand, there is no novelty feeding animals with carotenoids, these have always been part of their daily diet. Astaxanthin is the major carotenoid used for pigmentation of fish muscle, mainly salmons (Bhosale, 2004). Beta-carotene, lycopene, and lutein increase growth performance of animals and quality of pigmented products (Nelis and De Leenheer, 1991). Animals and humans are unable to synthesize carotenoids, they have to meet their daily nutritional needs from the products ingested (Priyadarshani and Rath, 2012). Carotenoids, as part of the nutrients in the feed, support animal health and animal products quality (Amaya et al., 2014). They have an

important role in molecular processes of cell membranes whose structure, properties and stability can be modified, leading to possible beneficial effects on human health (Zaheer, 2017).

Out of high production and marketability reasons, carotenoids are present in the animal kingdom, playing functions such as coloring (pets/ornamental birds and fish, mimicking), flavoring (scents and pollination in nature), reproduction (bird feathers and finding mates; development of embryos), resistance to bacterial and fungal diseases, immune responses (lutein connected to anti-inflammatory natural substance in poultry), as well as normal development of skin and mucosa.

The aim of this review is to highlight the various sources of carotenoids and their application in animal feed additives. This topic has rarely been described in a general matter, and as such we endeavor to provide an overview of published works on the subject.

DEFINITION, CHEMICAL STRUCTURE AND CLASSIFICATION OF CAROTENOIDS

Carotenoids are natural pigments, widely distributed in nature, responsible for the coloring of fruits, vegetables and flowers. Carotenoids take their name from carrot (*Daucus carota*), which accumulates high levels of these pigments in the root, being an exception among plants (Stange, 2015). They are used as food colorants, feed supplements, for cosmetic and pharmaceutical purposes.

Most carotenoids are made up of eight condensed 5-carbon-containing isoprene molecules, joined in a head to tail pattern (Mattea, 2009; Domonkos, 2013). Structurally, carotenoids take the form of a polyene chain that functions as a chromophore, due to 9-11 conjugated double bonds and possibly terminating in rings, what determines their characteristic color in the yellow to red range (Vershinin, 1999). The presence of different number of conjugated double bounds leades to various stereoisomers abbreviated as E- and Z-isomers, depending on whether the double bonds are in the trans (E) or cis (Z) configuration (Vincente et al., 2017). Carotenoids are synthesized by all photosynthetic organisms and some non-photosynthetic bacteria and fungi **(**Ruiz-Sola, 2012). Due to length of the polyene tail, carotenoid compounds are involved in capturing energy from light, absorbing wavelengths, ranging from 400-550 nanometers (violet to green light), thus assisting in photosynthesis and causes the compounds to be deeply colored yellow, orange, or red (Vershinin, 1999; Zakynthinos, 2016).

There have been identified over 750 naturally occurring carotenoids, biosynthesized *de novo* by plants, algae, fungi and bacteria (Okada et al., 2008), among which only 50 have significant biological activity for animals and humans (Mezzomo et al., 2016).

Animals cannot synthesize carotenoids (Rock, 2009), obtaining them through dietary intake, with a few exceptions, represented by aphids and spiders which acquired the ability and genes from fungi (Moran, 2010; Nováková, 2012) or are produced by endosymbiotic bacteria in whiteflies (Sloan, 2012). Carotenoids are classified in Table 1.

Table 1. Classification of carotenoids

Classification criteria	Major classes	Structure	Examples	Attribute	References
Chemical structure	Carotenes	Hydrocarbons (constituted by carbon and hydrogen) that can be cyclized at one or both ends of the molecule	α-carotene β- carotene γ-carotene lycopene torulene	-color range from pale yellow, bright orange to deep red -maintains eye health -does not color the fish	Delgado-Vargas, 2000 Tinoi, 2005 Aizawa, 2007 Kaur and Shah, 2017
	Oxycarotenoids (xanthophylls)	Oxygenated hydrocarbon derivatives that contain at least one oxygen atom as hydroxyl groups, keto, epoxy, methoxy, or carboxylic acid	lutein, zeaxanthin, β-cryptoxanthin, astaxanthin, canthaxanthin, violaxanthin,neoxanthin, antheraxanthin	-generally yellow in color -antiallergic and anti-cancerous actions -can color the fish -colors egg yolks	Delgado-Vargas, 2000; Bhosale and Berstein, 2005; Kushwaha et al, 2014.

Classification criteria	Major classes	Structure	Examples	Attribute	References
Functionality	Primary	Xanthophylls	β-carotene violaxanthin neoxanthin	-structural and functional components of the cellular photosynthetic apparatus	Delgado-Vargas, 2000
	Secondary		α-carotene β-cryptoxanthin zeaxanthin antheraxanthin capsanthin capsorubin	-localized in fruits and flowers -encompasses carotenoids produced at large levels	Delgado-Vargas, 2000
Nutritionally	Precursors of vitamin A	Have at least one ring of β-ionone not replaced and side polyenic chain with at least 11 carbons	α-carotene β-carotene β-cryptoxanthin	-helps maintain eye health, healthy mucus membranes and immunity	Ambrosio et al., 2006; Premkumar, 2014
	Non provitamin A		lutein zeaxanthin lycopene		Premkumar, 2014

β-carotene is used as food colorant and food supplement having antioxidant capacity and provitamin A activity, in a concentration ranges from 2 to 50 ppm. Once converted to vitamin A, health benefits include maintenance of normal eye health, epithelial function, embryonic development, and immune system function. Currently, the application of carotenoids in food formulations is limited because of it poor water solubility, high melting point and chemical instability (Qian, 2012a; Piorkowski, 2014), promoted by heat, light, singlet oxygen, acid, iron and iodine, and free radical, because of conjugated polyene chain which is characteristic of carotenoids (Dutta, 2005; Boon, 2010). Good sources of beta-carotene are cantaloupe, mangoes, papaya, carrots, sweet potatoes, spinach, kale and pumpkin (Premkumar, 2014).

Lycopene is an aliphatic carotenoid which dissolves in the oil, and that is why the presence of oils greatly increases its absorption by the digestive system (Story, 2010). Lycopene is abundant in tomatoes, watermelons, pink grapefruit, papaya and are the most effective singlet oxygen scavenger in vitro (Sies and Sthal, 1995). Epidemiological studies concluded that diets rich in high lycopene foods may reduce risk of cardiovascular diseases and different cancer types (Boon C., 2010).

Torulene has one β-ionone ring and a longer polyene chain than that of β-carotene; it has 13 double bonds and red color, in contrast to other carotenoids produced by the microorganisms. Torulene is found in red yeasts such as *Rhodotorula glutinis, Sporobolomyces ruberrimus.* Its structural characteristics make possible antitumoral and immune enhancing activities and could be used as food, feed and cosmetic additives (Zoz et al, 2015).

Lutein is a dihydroxy-carotene with an alcohol group containing hydroaromatic α structure (Kim, 2006) being in covalent interactions within fatty acids (Mezzomo, 2016). Lutein is found in leaves of green vegetables (spinach, kale, pumpkin, turnip greens) and is yellow-colored. Lutein gives color to chicken fat, egg yolk, and chicken feathers and also increase the efficacy of vaccination against infectious bronchitis virus in laying hens (McGraw, 2003).

Lutein and zeaxanthin are associated with eye health, because they are the only carotenoids found in the retina in macula lutea, being likely effective in age-related macular degeneration (Premkumar, 2014).

Astaxanthin is a dark-red organic pigment being the most valuable microalgal compounds. *Haematococcus pluvialis* strain is the richest source of astaxanthin (Mezzomo and Ferreira, 2016; Zaheer, 2017). This substance gives the pinkish-red hue to the salmonids (salmons and trout), shrimps, lobsters and crabs and has an important role in their immune-system and fertility. Nutritionally, astaxanthin is the most powerful antioxidant in the nature, with role to scavenge efficiently free radicals from the human body (Koller et al., 2014).

Similarly to lutein, it was proved to have a positive role in many human health problems, such as: UV-light protection, anti-inflammatory properties, support for eye health (helps diabetic retinopathy, macular degeneration,

eye strain and fatigue, and seeing in fine detail), immune-boosting effects (improves the ability of protective white blood cells to surround and destroy infecting organisms, especially fungi), prevention of different types of cancer (colon and breast cancer) (Amaya et al., 2014).

Canthaxanthin is an orange-red oxycarotenoid found in crustaceans, fungi and algae, particularly used in color feeding. Canthaxanthin has an antioxidant activity and enhances junctional communication between cells directly or through the formation of 4-oxo-retinoic acid, which is also able to stimulate the retinoic acid receptor (Amaya et al., 2014).

SOURCES OF CAROTENOIDS FOR FEED ADDITIVES

As have been pointed out above, there has been a change lately in the perspective towards the ingredients of foods, what is considered healthy for humans and for the environment and what should constitute the food of animals and birds. Yet, researches are still conducted to efficiently obtain natural carotenoids and optimal feed formulas for animals/birds. (Kaur and Shah, 2017).

According to the new study by *reportsnreports.com* (2016), the carotenoid market is led by the feed segment. The demand for incorporation of carotenoids in animal feed is growing due to consumers preference for good quality and meat, meat products, and fish aspect. Carotenoids also increase the palatability of feed, aquaculture being currently the fastest growing sector. It is known that the farming of salmon and shrimp grew exponentially in the last years and the trend is ascendant, so the market of feed carotenoids, especially of astaxanthin, expands rapidly (Anonimous, 2010).

With the growth of world population and increased awareness and demand for better animal nutrition (in terms of quality and quantity), carotenoid additives seem to be the solution (García-Chavarría and Lara-Flores, 2013; Mohiseni, 2017).

According to global trends and forecasts (https://www.marketsandmarkets.com/Market-Reports/carotenoid-market-158421566.html), global carotenoids market is estimated to be valued at USD 1.53 Billion by 2021 and this is mainly due to the increasing number of health-conscious consumers and the rising demand for natural coloration. The quality of food is associated with color, flavor, texture, and odor, humans considering color as the most relevant aspect due to its appealing nature (Amaya et al., 2014).

If, today, carotenoids produced commercially are mostly by total synthesis to meet the demands, intention is to growingly substitute these with natural ones, if possible obtained by as mild as possible methods and processes (Mezzomo and Ferreira, 2016).

Synthetic carotenoids

For carotenoid synthesis, one need to construct the polyene chain, meaning the hydrocarbon skeleton conjugated by alternating double and single bonds. For the specific location of the double and single bonds, different reactions have been developed along time, the first to be obtained being the β-carotene (Amaya et al., 2014).

Out of economic and technological reasons, although various carotenoids have been synthesized at laboratory level, few of them went to commercial scale, among which are the following: astaxanthin, astaxanthin-dimethyl-di-succinate, as well as β-carotene, β-apo-8'-carotenal, also canthaxanthin, citranaxanthin, and the ethyl ester of β-apo-8'-carotenoic acid. For commercial use in animal nutrition, they are usually formulated as 10% concentrated products as a powder or beadlet (Amaya et al., 2014).

Currently, carotenoids synthetic production is the most common manufacturing method. Over 95% of the feed market uses synthetic astaxanthin, mainly from major producers BASF (Germany) (Lucantin pink) and Hoffman-La Roche (Switzerland) (Carrophyll pink), which contains only 25% of the biologically active stereoisomer found naturally. For replacing, only in aquaculture feed additive, the synthetic pigment with natural astaxanthin produced by *Haematococcus*, the biomass of this microalga should be increased to 10 000 ton per year, the price of astaxanthin being too high (Lorenz, 2000; Minyuk, 2008).

The addition of synthetic carotenoids to the diet of laying hens and broilers is a common

practice, for improving yolk color (Santos-Bocanegra, 2004). Canthaxanthin is the preferred synthetic red xanthophyll in poultry farming available as Carophyll® Red (DSM Nutritional Products, Switzerland) or Lucantin® Red (BASF, Germany). The preferred yellow xanthophyll is β-apo-8´-carotenoic acid ethyl ester available as Carophyll® Yellow and Lucantin® Yellow (Marounek, 2015).

Synthetic carotenoids are technologically feasible and cost-effective. These are standardized and more stable, i.e. apo-carotene-ester concentration in feed samples showed very little variation from expected values, whereas it was 30% below expectations for marigold extracts.

It has been determined that 1 ppm of apo-carotene-ester is equivalent to about 2 ppm of lutein-zeaxanthin from marigold. Canthaxanthin is the red carotenoid which dominates pigmentation of broilers, whereas paprika xanthophylls which require two to three times the amount compared to canthaxanthin, receives little attention.

The stability of various red carotenoids products in premixes was checked after three months of storage and recovery varied considerably: 66% to 92% for canthaxanthin, 76% for citranaxanthin, and 39% for capsanthin from paprika, which shows that carotenoids are very unstable on their isomeric forms. Another property is the transfer efficiency from feed to egg, which is better for the synthesized ones. Apo-carotene-ester has a 55% transfer efficiency to egg yolk, as compared to only 17% for marigold carotenoids and canthaxanthin an average deposition rate of about 40% (www.poultryworld.net/Broilers/Nutrition/2012/8/The-effect-of-carotenoids-on-yolk-and-skin-pigmentation-WP010752W/).

The consumer's demand for "non-synthetic" colorants has driven the industry to identify and develop natural sources of carotenoids to replace chemical synthesis (Olesen, 2010).

Natural sources of carotenoids

Plants

The majority of naturally occurring carotenoids are found in vegetables and fruits, dark green leaves of plants and seeds, flowers and roots (Amaya et al., 2014). Carotenoids found in plants differ in concentration depending on plant varieties, degree of ripeness, time of harvest, growing and storage conditions, etc. (Zakynthinos et al., 2016).

Feed ingredients such as corn, maize, tomato, green beans and cabbage, pumpkins, prunes or red pepper all are sources of carotenoids with benefits for animals (Amaya et al., 2014; Zakynthinos et al., 2016; Mezzomo and Ferreira, 2016,).

Red pepper or paprika (*Capsicum annuum*) is reported to be a rich, abundant and inexpensive source of keto-carotenoids (capsanthin). It has been studied as a fish feed additive but with a lower efficacy in comparison to commercially available astaxanthin and a less desirable coloration in comparison to canthaxanthin in rainbow trout (Akhtar et al., 1999). In addition to pigmentation, paprika increased growth rate and improved the reproductive performance in yellow tail. Good results have also been reported for laying hens' yolk pigmentation (Yanar et al., 2016).

Marigold flower (*Tagetes erecta*) is rich in lutein and zeaxanthin and it is efficient for poultry skin and egg coloration. Lutein from marigold is also used as a yellow coloration for cultured marine fish yellow tail and red sea bream (Maoka T, 2011). As consumers associate color to nutritive value, healthiness, freshness and taste, this may count as an interesting dietary alternative (García-Chavarría and Lara-Flores, 2013).

Medicinal plants are known to be sources of natural antioxidants used in treatments for human and animal diseases. Because of their fair price and strong effects, medicinal plants are rapidly becoming a safe alternative for antibiotics and chemical drugs in aquaculture. Furthermore, these can help increase growth as they maintain healthy intestinal microflora (Mohiseni, 2017).

Some valuable natural supplements for pigmentation of chicken eggs, broilers and fish are presented in the following Table 2.

Table 2. Natural animal feed supplements

Natural sources	Main carotenoids	Role as feed additive	References
Carrots	β-Carotene α-Carotene	Increases yolk color parameters and carotenoid contents Coloration of skin and flesh of food fish	Isler, 1967 Hammershoj et al, 2010 Weerakkody et al., 2016
Alfalfa (*Medicago sativa*)	Lutein Zeaxanthin β-apo-8'-carotenal β-apo-8'-carotenoic acid Lycopene	Pigmentation of broilers Improve egg quality	Amaya et al., 2014 Varzaru et al., 2015
Marigold flower (*Tagetes erecta*)	Lutein Zeaxanthin	Poultry skin and egg coloration	Rajput et al, 2012 García-Chavarría and Lara-Flores, 2013
Yellow maize	Lutein Cryptoxanthin, Zeaxanthin	Boosts carotenoids in eggs and improve egg yolk color	Breithaupt, 2007
Natural sources	Main carotenoids	Role as feed additive	References
Saffron (*Crocus sativus)*	Crocetin β-Carotene Zeaxanthin	Oxidative stability of shell eggs and liquid yolks	Botsoglou et al, 2005
Seeds of *Bixa orellana*	Bixin and decomposition products	Animal nutrition	Amaya et al., 2014
Paprika (red pepper) (*Capsicum annuum*)	Capsanthin Capsorubin	Pigmentation and weight gain of rainbow trout Pigmentation of salmonids Pigmentation of Pacific white shrimps Yolk pigmentation in laying hens Weight gain in broilers	Yanar, 2016 Talebi et al., 2013 Arredondo-Figueroa et al., 2003 Vicente et al., 2007 Galib et al., 2011

There are some problems regarding carotenoids production from plant origin, due to seasonal and geographic variability that cannot be controlled. A better option, regarding yields and costs, is the microbial production of carotenoids on agro-industrial wastes which are cheap substrates (Mata-Gómez, 2014).

Crustacea and marine animals

Marine animals accumulate various carotenoids from foods (bacteria, algae, other animals) and modify them through metabolic reactions (oxidation, reduction, translation of double bonds, oxidative cleavage of double bonds, cleavage of epoxy bonds). Many of these carotenoids are metabolites of β-carotene, fucoxanthin, peridinin, diatoxanthin, alloxanthin, and astaxanthin (Maoka, 2011).

Astaxanthin is distributed in both marine and fresh water fish. *Cyprinidae* fishes synthesize (3S,3′S)-astaxanthin from zeaxanthin by oxidative metabolic conversion. *Perciformes* and *Salmonidae* fish cannot synthesize astaxanthin from other carotenoids (Matsuno, 2001). Astaxanthin present in these fishes originates from crustacean sources (Atlantic krill, crayfish meal, crab meal) used in aquaculture feed formulation as additive. Crustacean by-products represent an "attractive ingredient for industrialization, since around 70% of the raw weights of the catch are processing discards" reducing at the same time an environmental problem (Shahidi et al., 1998). Lobsters, crabs and shrimps, together with their processing waste are sources of astaxanthin (Mezzomo et al., 2016).

Supply of marine animal based natural carotenoids are limited because of declining trend in catches of crustaceans from marine resources. These sources of carotenoids are very expensive and thus aquaculture feed production involves high production costs (García-Chavarría and Lara-Flores, 2013).

Microbial sources of carotenoids

Interest in microbial sources of carotenoids was renewed due to consumers opinions against synthetic additives. Types of carotenogenic microorganisms, like bacteria and fungi, can be stimulated to produce cost-effective carotenoids in response to environmental stress (light, temperature), chemical compounds or modification of metabolic pathway using recombinant DNA technology (Bhosale, 2004). Advances have been made through genetic manipulation of some non-carotenogenic microbes such as *Escherichia coli*, *Saccharomyces cerevisiae*, *Candida utilis* in terms of carotenoids production (Das et al., 2007).

Several bacteria have biotechnological potential for the production of carotenoids. Some thermophilic halophilic bacteria such as *Halococcus morrhuae* and *Halobacterium salinarum* develop red and orange colonies, bacterioruberin synthetized by *H. salinarum* being the most found carotenoid (Asker and Ohta, 1999). *Haloferax alexandrines* has good industrial perspective for the production of canthaxanthin (Asker and Ohta, 2002) and the marine bacterium *Flavobacterium* sp. produces zeaxanthin, being considered one of the best microbial sources of zeaxanthin (Masetto et al., 2001). Other bacteria with ability to synthesize carotenoids are *Agrobacterium aurantiacum, Mycobacterium brevicaie*, *Mycobacterium lacticola*, *Rhodobacter sphaeroides*, *Rhodococcus maris*, *Streptomyces chrestomyceticus* (Dannert, 2000).

Some examples of carotenoid-producing microorganisms are presented in Table 3. Practical obstacles still need to be overcome for their commercial exploitation, though developments in recent years have proven that, in both farmed fish and poultry, carotenoids like astaxanthin, canthaxanthin, β-carotene, zeaxanthin and lycopene from bacterial sources impart color to skin, flesh and eggs (Bhosale, 2004).

Table 3. Microorganisms sources of carotenoids and their application as feed additives

Bacteria and Yeasts	Carotenoids/Activity	Application as animal feed additives	References
Mycobacterium lacticola	Astaxanthin/ antioxidant, photo- protectant	Fish feeds	Kushwaha et al., 2014
Paracoccus carotinifaciens (commercially sold as Panaferd)	Astaxanthin/ pigmentation	Coloration of farm raised salmon and trout	Bories, 2007
Spongiococcum excentricum	Lutein/antioxidant	Poultry feeds	Kushwaha et al., 2014
Flavobacterium sp.	Zeaxanthin/ pigmentation	Additive in poultry feed to increase yellow color of animal's skin and egg yolk Fish feed	Alcantara and Sanchez, 1999 Masetto et al., 2001 Bhosale, 2004
Haloferax alexandrines	Canthaxanthin/ pigmentation	Feed in salmon farming to guarantee the flesh color of fish	Asker and Ohta, 2002
Dietzia natronolimnaea HS-1	Canthaxanthin/ pigmentation, antioxidant	Egg yolk pigmentation	Esfahani-Mashhour et al., 2009 Gharibzahedi et al., 2012
Phaffia rhodozyma	Astaxanthin/ pigmentation	Pigmentation of salmon, trout, and red sea bream (*Pagrus* sp.) Pigment source for egg yolk of laying hens	Maoka, 2011 Kushwaha et al., 2014
Blakeslea trispora	β-carotene/growth performance	Chickens for fattening Shrimp feed Feed for dairy cows	Sales et al., 2008
Rhodotorula spp.	β-carotene Torulene Torularhodin/ antioxidant, precursor of vitamin A	Nutrition of laying hens	Bhosale P., 2004 Kushwaha et al., 2014
Xanthophyllomyces dendrorhous	Astaxanthin	Feed supplement for salmons, crabs, shrimps, chickens and egg production	Bhosale P, 2004 Kushwaha et al., 2014
Mushrooms			
Cantharellus cinnabarinus	Canthaxanthin	Poultry feeds and fish feeds	Kushwaha et al., 2014

Microbial sources are an environmental-friendly method for the production of carotenoids, which meet the increasing demand of these natural products (Das, 2007).

Microalgae, rich source of carbohydrates, protein, enzymes and fiber, have been a major source of food for humans in Asian countries. They have been naturally taken over, researched and commercialized both for nutritional supplements for humans and as animal feed additives to replace synthetic components (Priyadarshani and Rath, 2012). Microalgae play an important role in the high-level nutrition for both aquaculture and farm animals. The most famous sources of microalgae, which accumulate carotenoids in

their biomass, are *Chlorella, Chlamydomonas, Dunaliella, Muriellopsis* and *Haematococcus* spp, offering economical alternatives to chemical synthesis (Bhosale and Berstein, 2005).

Carotenoids, even at levels of parts per million, are strong dyes, especially canthaxanthin, astaxanthin and lutein from *Chlorella,* which have been used as pigments and included as ingredients in feed for salmonid fish, trout, and poultry to enhance the reddish color of fish and egg yolk yellowish color (Plaza et al., 2009). Broiler chickens fed with algae have yellow skin and shanks, and the egg yolk is darker indicating higher carotenoids content (Becker, 2004). Microalgae like *Chlorella vulgaris* and *Haematococcus pluvialis* synthetize large amount of astaxanthin (Yin, 2013; Kim, 2016) and *Dunaliella salina*, *Spirulina maxima and S. platensis* are biotechnologically relevant, as sources of natural pigments (β-carotene, α-carotene, β-cryptoxanthin and zeaxanthin, respectively) for the culture of black tiger prawns (*Penaeus monodon*), salmonid fish and ornamental fish (Priyadarshani and Rath, 2012). Zeaxanthin from *Spirulina* is used as a red coloration for goldfish and ornamental carp. Astaxanthin became the most important carotenoid in salmons and rainbow trout with the development of salmonid farming. Astaxanthin can be incorporated in feeds up to 100 mg/ kg and canthaxanthin up to 25 mg/kg (high accumulation in humans can be toxic). Animal feed is the largest field of commercial application of microalgae carotenoids due to the importance of astaxanthin and canthaxanthin in aquaculture (Britton, 2004).

Bellow given is Table 4, including the sources of carotenoids from microalgae with application as feed additives.

Table 4. Microalgae sources of carotenoids

Algae and cyanobacteria	Carotenoid	Application as feed additives	References
Haematococcus pluvialis (commercially sold as NaturRose)	Astaxanthin	Animal feed additives Aquaculture Used for culture of prawns, salmonid fish and for ornamental and tropical fish	Priyadarshani and Rath, 2012 Mezzomo and Ferreira, 2016 García-Chavarría, Lara-Flores, 2013 Bhosale, 2004 Amaya et al., 2014
Dunaliella sp.	β-carotene	Animal feed additives Used as natural food coloring agent in aquaculture feed industry	Priyadarshani and Rath, 2012 Mezzomo and Ferreira, 2016 García-Chavarría, Lara-Flores, 2013 Bhosale, 2004 Amaya et al., 2014
Chlorella vulgaris	Lutein Astaxanthin Fucoxanthin	Used in diets of rainbow trout yielding both muscle and skin pigmentation effects Animal feed additives	Priyadarshani and Rath, 2012 Mezzomo and Ferreira, 2016 García-Chavarría M., Lara-Flores M, 2013
Chlorella protothecoides	Lutein	The reddish color of salmonid fish and yellowish color of egg yolk	Guedes et al., 2011 Mezzomo and Ferreira, 2016
Algae and cyanobacteria	Carotenoid	Application as feed additives	References
Chlorella zofingiensis	Lutein	Enhances the reddish color of salmonid	Guedes et al., 2011
Muriellopsis sp.	Lutein	Poultry farming and/or aquaculture	Blanco et al., 2006
Scenedesmus obliquus	Lutein	Fish feed	Mezzomo and Ferreira, 2016
Tetraselmis sp	Lutein	Aquaculture and larval feeds	Priyadarshani and Rath, 2012
Nannochloropsis oculata	Astaxanthin	Aquaculture & larval feeds Hen supplements - increases egg nutritional value	Priyadarshani and Rath, 2012 Zaheer, 2017 Gładkowski et al, 2011
Spirulina sp	*β*-carotene	Animal feed additives	Indira Priyadarshani and Biswajit Rath, 2012

The market demand for pigments natural sources has promoted large-scale cultivation of microalgae for biosynthesis of such compounds (well-established are β-carotene and astaxanthin), so significant decreases in production costs are expected in coming years.

The European legislation specifies that before their use in EU, carotenoids have to be subjected to an authorization process.

A general overview of the current legal situation of the different carotenoids used as feed additives in compound feed in European

Union is shown in Table 5 (Vincent et al., 2017), with added data regarding what carotenoids are totally synthesized and which are extracted or biosynthesized come from (Pérez-Gálvez, 2003; Baldo, 2011; Amaya et al., 2014; Rodriguez-Amaya, 2015; Mezzomo and Ferreira, 2016).

Table 5. Carotenoids used in the feed industry as feed additives in the European Union

Carotenoids feed additive	Target animal	Total synthesized	Extracted	Biosynthesized	EURL-FA evaluation report
Astaxanthin	Fish (salmon, trout)		Crustaceans Pink shrimp (*Penaeus paulensis*) processing waste	*Phaffia rhodozyma* *Haematococcus pluvialis* *Chlorella vulgaris*	EURL-FA Feed Additives (2014)
Astaxanthin	Fish Ornamental fish Crustaceans	X			EURL-FA Feed Additives (2010a)
Astaxanthin dimethyldisuccinate[1]	Salmon and trout	X			EURL-FA Feed Additives (2007a)
β-apo-8'-carotenoic acid ethyl ester (apoester)[2]	Poultry	X			EURL-FA Feed Additives (2011c)
Astaxanthin, adonirubin, canthaxanthin	Salmon and trout			Red carotenoid-rich *Paracoccus carotinifaciens*	EURL-FA Feed Additives (2007b)
Canthaxanthin[2]	Chickens for fattening and minor poultry species for fattening; Laying poultry and poultry reared for laying; Ornamental fish and ornamental birds; Ornamental breeder hens	X		*Chlorella vulgaris*	EURL-FA Feed Additives (2011a)
Capsanthin[3]	Poultry		Red peppers		EURL-FA Feed Additives (2012b)
Citranaxanthin[2]	Poultry	X			EURL-FA Feed Additives (2016)
Lutein[3]	Poultry		Marigold (*Tagetes erecta and Tagetes patula*) Rose fruit (*Rosa canina*) Carrot		EURL-FA Feed Additives (2012a)
β-carotene (solid form)	All animal species	X		*Chlorella vulgaris* *Dunaliella salina* *Arthrospira maxima*	EURL-FA Feed Additives (2010b)
Zeaxanthin[3]	Poultry		Marigold (*Tagetes erecta* and *Tagetes patula*) Paprika (*Capsicum annuum*)		EURL-FA Feed Additives (2012a)

[1]formulated in an organic matrix
[2]formulation containing the active substance and other ingredients
[3]formulation containing a natural source of the active substance and other ingredients
EURL-FA - EU Reference Laboratory for Feed Additives

In the EU, carotenoids used as feed additives are mainly authorised under the category „sensory additives", as colorants (Vincente et al., 2017). The addition of natural and synthetic carotenoids in layer feeds in the European Union is currently limited to 80 ppm (mg/kg), excepted canthaxanthin, whose limit is 8 ppm, because excessive exposure in humans can lead to the development of precipitated crystals in the retina (Baker, 2001).

CONCLUSIONS

Beyond the properties related to health, various natural sources of carotenoids have been and still are investigated as they represent renewable raw materials, are profitable in terms of culture/cultivation costs, as well as for their replicability in terms of processes, and they act synergistically – complementing one-another in diets.

Even though the high demand of carotenoids has long been met by synthetic technology, because of by-products with undesirable effects on consumption, production of carotenoids from natural sources, improvement of bioproduction techniques and development of extraction methods, became the focus of extensive research.

In order to reach industrial production, more optimization studies and also validation methods are required to scale up the processes.

A most important part in animal nutrition is represented by feed additives, including especially carotenoids, which are mainly sensory additives used in the coloring of fish, birds and food of animal origin.

Many natural sources with high potential for animal feed additives have already been identified and studied, some of these being highlighted in this article.

According to market reports, demand for carotenoids in animal feed is increasing due to the increasing preference for good quality and appearance of meat, meat products, and fish. Global carotenoids market is expected to reach USD 1.53 Billion by 2021 and, due to the increasing number of health-conscious consumers and the rising demand for natural coloration, the use of natural carotenoids will contribute to the enhancement of the market growth.

The potential health benefits of microalgae represents the main drive behind the new developments in their usage in feed and foods. Even so, carotenoid production from algae is not as cost effective as the synthetic counterpart.

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SEASONAL CHANGES IN THE MORPHOLOGICAL TRAITMENTS AND FORAGE QUALITY OF KERMES OAK (*Quercus coccifera* L.)

Mevlüt TÜRK[1], Mehmet ALAGÖZ[1], Emre BIÇAKÇI[1]

[1]Suleyman Demirel University, Faculty of Agriculture, Department of Field Crops, Isparta, Turkey

Corresponding author email: mevlutturk@sdu.edu.tr

Abstract

This study was conducted in order to determine the seasonal changes in the forage quality of Kermes Oak under Mediterranean region of Turkey in 2013-2014 years. Leaf samples were taken as 150 number in the middle of each month from the same geographical sides and altitude. The leaf width, leaf length, crude protein (CP) ratio, neutral detergent fiber (NDF), acid detergent fiber (ADF), total digestible nutrient (TDN) and relative feed value (RFV) were determined in this research. According to results, the highest leaf width and leaf length were determined on samples taken in July and August. The highest ADF and NDF values were obtained from samples taken in October and November. The samples taken in February, March, April and May had the highest TDN and RFV values.

Key words: crude protein, goat, kermes oak, relative feed value, shrubs.

INTRODUCTION

The areas where the Mediterranean climate type is seen in the world are around 100 million hectares. 32 million hectares of these areas are located in the Mediterranean coast. In Turkey has approximately 7 million hectares of vegetation formed under the influence of Mediterranean climate (Baytekin et al., 2005).

The Mediterranean climate is described as hot and dry in summers and moist and cool in winters. The hot and dry summer months severely limit the development of especially herbaceous species. However, shrubs with deep roots maintain the green appearance of the vegetation in this season. With the cooling of the air in the autumn and the start of the precipitation, especially the cool climate, the grassy species are developing again. In the winter months, the herbaceous species continue to grow as water and temperature become suitable for plant development. Depending on the climatic factors, this evolution of plants also affects the way animals benefit from these areas. According to these differences, animals prefer abundant or scarce, green or dry, grassy or woody species (Gökkuş et al., 2009).

Maquis shrublands are considered as natural feed areas especially for the goats at all times of the year. There is a close relationship between the maquis areas in the Mediterranean region and the goat flocks. As is the case in the world, goat breeding is most common on these areas also in our country and it is the main feed sources of the goats (Papachristou et al., 1999, 2003; Rogosic, 2000). It has been noted that over 60% of goats' feed were formed by shrubs in the Mediterranean maquis shrublands (Perevolotsky et al., 1998). The shrubs of the Kermes are seen as precious feed sources for domestic and wild animals, for the people in the area to burn and for preventing soil erosion (Vrahnakıs et al., 2005). Similarly, in Spain it is emphasized that the marble forest prevents erosion, contributes to the formation of the soil, recovers the area after the fires, and is important as a food and shelter for domestic and wild animals (Canellas and San Miguel, 2003).

Kermes oak thickets constitute more than 2.4 million hectares area in Turkey. This area constitutes 10% of the general forest area of the country. The kermes oak forms a grassland area for 9 million hair goats. (TÜİK, 2013). Kermes oak is the natural plant of the Mediterranean region. Where the Mediterranean climate is seen, the kermes oak is an important goat food. The Kermes oak provides the proportion of crude protein needed for goat feeding, except during the winter season (Alatürk et al. 2014).

This study was conducted in order to determine the seasonal changes in the forage quality of Kermes Oak under Mediterranean region of Turkey in 2013-2014 years.

MATERIALS AND METHODS

The research was conducted at Isparta (37°45′N, 30° 33′E, altitude 1035 m) located in the Mediterranean region of Turkey, between 2013 and 2014 years.

The climatic data are given in Table 1 for the experimental area.

Table 1. The climatic data

Month	Temprature (^{0}C)			Total rainfall (mm)			Average relative humidity (%)		
	LT*	2013	2014	LT	2013	2014	LT	2013	2014
January	1.9	2.8	3.7	72.4	58.6	61.3	73.1	72.5	76.7
February	2.8	4.9	5.2	65.5	101.9	23.4	70.4	70.6	60.8
March	6.1	7.3	7.3	53.8	25.1	78.6	65.4	59.7	63.3
April	10.7	12.1	11.7	56.2	59.9	44.8	61.3	56.5	59.5
May	15.6	18.0	15.1	50.4	66.5	107.0	57.4	50.4	60.3
June	20.2	21.0	20.0	29.6	34.4	42.8	51.2	47.4	49.8
July	23.6	23.5	24.5	14.9	88.2	0.8	45.4	41.5	43.5
August	23.2	24.1	24.9	10.5	15.4	10.2	46.4	39.1	44.2
September	18.6	18.9	18.4	15.4	3.0	99.2	51.9	43.3	58.6
October	12.9	10.7	12.9	37.6	104.0	57.1	62.0	54.0	64.7
November	7.4	8.7	6.8	46.5	67.6	37.0	68.5	65.5	69.2
December	3.4	1.0	6.0	84.5	29.4	108.6	74.7	64.2	75.2
Av./Tot.	**12.2**	**12.75**	**13.04**	**537.3**	**654**	**670.8**	**60.64**	**55.39**	**60.48**

(*) Long term average

The experiments were evaluated in a randomized complete block design with three replications. While sampling in the shrubs was done by imitating animal grazing habits from parts of animals that were accessible but not grazed by animals. For this purpose, 150 leaf samples were cut with a bond cutter from the same geographical side and the same height every month and put on the pouch paper.

Chemical analyzes were made in the laboratory of Field Crops Department at Süleyman Demirel University. At first morphological observations such as leaf width and length were measured with 0.01 mm sensitive caliper. The samples were dried at room temperature then dried in an oven at 65°C till they reached constant weight. After cooling and weighing, the samples were ground for crude protein, ADF and NDF content analyses. Nitrogen content was calculated by the Kjeldahl method; The ANKOM Fiber Analyzer was used for NDF and ADF analysis. ANKOM F57 filter bags were used for ADF and NDF analysis in this study. Total digestible nutrients (TDN) and relative feed value (RFV) were estimated according to the following equations adapted from Horrocks and Vallentine (1999):

TDN = (-1.291 x ADF) + 101.35,

RFV = %DDM x %DMI x 0.775,

The data were analyzed together using the Proc GLM (SAS 1998). Means were separated by LSD at the 5 % level of significance.

RESULTS AND DISCUSSIONS

The results of variance analysis are presented in Table 2. Changes in leaf width, leaf length, CP, NDF, ADF, TDN and RFV were found significant between the months. The means are indicated in the table 3 andresults of the Duncan test are indicated in Latin letters near the means.

Table 2. The results of variance analysis

	Df	LW	LL	CP	NDF	ADF	TDN	RFV
Block	2	0.083	0.0277	0.001	4.917	2.341	4.126*	41.600*
Vegetation Season	11	1.58**	3.444**	0.063**	86.743**	76.974**	128.106**	842.592**
Error	22	0.053	0.0277	0.002	1.045	0.534	0.869	10.518

df: degrees of freedom, LW: Leaf Width, LL: Leaf Length, *P<0.05 and **P<0.01.

Maximum leaf widthwas recorded (18.00 mm) in July and August while the minimum leaf widthwas recorded (16.00 mm) in November, December and January.leaf lengthwas at the maximum (27.00 mm) in July and August, at the lowest level (24.00 mm) in December, January and February.

Crude protein ratio of the plant showed a peak in April (11.63) and the crude protein content decreased as the growth progressed.As the development progresses, decreases in protein ratio and increases in cell wall materials are observed (Haddi et al., 2003).Our results are similar with the results of Parlak et al. (2011) and Gökkuş et al. (2009).

Neutral detergent fiber (NDF) and acid detergent fiber (ADF)exhibited a significant increase from July to November later started to decrease and kept its lowest level in February, March, April and May.Cell wall materials (NDF, ADF and ADL) varied according to plant species and growth periods. At the beginning of growth, it is known that the vast majority of cell protoplasm contents of plants are composed of water and cell wall materials are at the lowest level. Cell wall materials are more associated with the presence of mature cells than younger cells (Lyons et al., 1999).It is also known that there is an increase in cell wall materials and a decrease in leaf/stem ratio due to maturation in plants (Frost et al., 2008). These results are in agreement with those reported by Alatürk et al. (2014) and Kökten et al. (2012), Tolunay et al. (2009) and Bouazza et al. (2012).

The highest total digestible nutrient (TDN) and relative feed value (RFV) were obtained from samples taken in February, March, April and May. The TDN refers to the nutrient that are available for livestock and are related to the ADF concentration of forage. The RFV is an index that is used to predict the intake and energy value of the forages. Forages with an RFV value over 151, between 150-125, 124-103, 102-87, 86-75 and less than 75 are considered as prime, premium, good, fair, poor and reject, respectively (Lithourgidis et al., 2006).

Table 3. The LW, LL values and CP, NDF, ADF, TDN, RFVratios of Kermes oak at different moths

Month	LW	LL	CP	NDF	ADF	TDN	RFV
February	16.11 d	24.00 e	10.13 c	49.33 f	34.03 f	57.40 a	118.00 a
March	16.33 cd	25.00 d	10.87 b	48.13 f	33.20 f	58.47 a	121.83 a
April	17.00 b	26.00 b	11.63 a	48.20 f	33.26 f	58.41 a	121.55 a
May	17.00 b	26.00 b	11.89 ab	48.03 f	33.13 f	58.56 a	122.14 a
June	17.00 b	25.66 c	10.13 c	55.03 d	38.00 e	52.32 b	100.26 c
July	18.00 a	27.00 a	9.93 cd	57.63 c	42.66 c	46.29 d	89.85 d
August	18.00 a	27.00 a	9.44 de	58.03 c	42.93 c	45.91 d	88.86 d
September	17.00 b	26.00 b	9.06 e	60.06 b	44.46 b	43.96 e	84.81 de
October	16.66 bc	25.00 d	9.00 e	61.50 ab	45.50 ab	42.59 ef	80.82 e
November	16.00 d	25.00 d	8.88 e	62.16 a	46.00 a	41.96 f	79.44 e
December	16.00 d	24.00 e	9.19 e	55.13 d	40.83 d	48.68 c	96.35 c
January*	16.00 d	24.00 e	9.38 de	51.77 e	37.23 e	53.26 b	107.68 b

(*) 2014

CONCLUSIONS

The season is an important factor affecting the nutritional value of the kermes oak. The nutritive value of the leaves of the kermes oak changes as it matures.

Considering that the protein level of ruminants consumed by ruminants should be at least 10,60% (NRC, 2001), the Kermes oak produced sufficient crude protein feed for animals on February, March, April, May and June.The crude protein content of the kermes oak was reduced due to ripening, whereas the cell wall compounds (NDF, ADF) were increased.

It is not desirable for animals to consume more than 45.8% of NDF and 25% of ADF content per day (NRC, 2001). In general, the Kermes oak has included NDF and ADF over the border values throughout the year.

As a result, it is known that woody species are best appreciated by the goats, and when grazing is done with the goats, the feeding value of the kermes oak is generally sufficient, but supplementary feed should be given to meet the protein deficit in the winter. In addition,

Kermes is a green food source for grazing animals because of its evergreen characteristic. Therefore, if maquis, pastures in and over the forest, forest areas deteriorated in structure that covered with kermes oak are included in the grazing systems, livestock farming may be more profitable with the extension of the grazing period and the reduction of roughage costs.

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THE HAEMATOLOGICAL PROFILE OF NEWBORN CALVES OBTAINED BY CROSSBREEDING WITH MEAT SIRE BREEDS

Marinela ENCULESCU[1], Radu NEAMT[2], Daniela Maria VIDMICHI[1], Ioana NICOLAE[1]

[1]Research and Development Institute for Bovine, 21, Bucuresti-Ploiesti, 077015, Balotesti, Romania
[2]Research and Development Station for Bovine, 32, Bodrogului, 310059, Arad, Romania

Corresponding author email: marinelaenculescu2006@yahoo.com

Abstract

Hemogram is a basic test used to establish the haematological status and diagnosis of various haematological conditions in animals. The purpose of this study was to establish the physiological status of crossbred calves compared to Romanian Black Spotted calves at birth by determining their haematological profile. Our researches were carried out in the Dairy cows' Experimental Farm of I.C.D.C.B. Balotesti, on a number of 45 newborn calves. They have been distributed in four experimental groups, each one of 9 calves (E_1: F_1 Charolaise x Romanian Black Spotted, E_2: F_1 Blanc Blue Belgique x Romanian Black Spotted, E_3: F_1 Aberdeen Angus x Romanian Black Spotted, E_4: F_1 Limousine x Romanian Black Spotted) and the control group of 9 calves (M: Romanian Black Spotted). The haematological parameters (red blood cells, haemoglobin, hematocrit, red blood cells distribution width, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, mean platelets volume, platelets distribution width, total white blood cells, lymphocytes, monocytes, neutrophil) were determined using the automated hematology analyzer Abacus Junior Vet 5. The results were expressed as a mean (±standard error), standard deviation (sd) and coefficient of variation (V). The analysis of variance (ANOVA) single-factor was applied to test the significance of differences. The obtained results showed no statistical significant differences between experimental groups ($F_{critical}<F_{0.05}$) for all the haematological parameters studied. However, for MCHC (mean corpuscular hemoglobin concentration), significant differences ($p\leq0.05$) between the experimental groups was observed, as following: $F_{critical}=2.93$; $F_{0.05}$ (8; 44)=2.00; $F_{critical} > F_{0.05}$. The recorded values obtained in this work could help to a better interpretation of clinical pathology data and diagnosis of neonatal diseases in calves.

Key words*: calves, crossbreed, haematological profile, newborn.*

INTRODUCTION

Hematology has become an area of great interest, considering the increased incidence of haematological diseases in farm species. Haematological examinations data are analyzed corelatively, for the diagnosis diseases of the blood and hematopoietic organs or subclinical conditions. (Gherariu et al., 1985). The haematological profiles provide reliable information on the health and functional status of the organism (Kumar and Pauchaura, 2000). Erythrocytes/red blood cells are the main mass of the blood cells (Stancioiu, 1999). In cattle, erythrocytes have an average diameter of 5-6 µm (Roland et al., 2014) and a relatively long life span of 130-160 days (Brockus, 2011; John, 2010). The component by which erythrocytes exert their function as respiratory gas transporter is hemoglobin. In combination with hematocrit and hemoglobin concentration, the number of erythrocytes is useful in detecting and monitoring of anemia and erythrocytosis/polycythemia. For a better assessment, the erythrocyte mass is correlated with hematocrit. The red blood cells numbers is influenced by the plasmatic volume changes such as physiological status and hydro-electric balance (Means, 2004). The red blood cells distribution width (RDW) indicates whether all the red cells are about the same, width, size and shape (Terzano et al., 2005). Platelets are involved in haemostasis and of tissue repair processes (Russell, 2010). Leucocytes count/white blood cells, play an essential role in immune defense and include different subpopulations (lymphocytes, monocytes, neutrophil, eosinophil and basophil). They are the results of the dynamic production of bone marrow, the release of the cells to the peripheral circulation and the storage in different organs (Yaqub et al., 2013).

MATERIALS AND METHODS

The experimental procedures used in this study were in accordance with the Romanian Law no. 43/2014 and the Council Directive 2010/63/EU regarding handling and protection of animals used for scientific purposes. Forty-five calves, (crossbreed and Romanian Black Spotted, 9 calves/group), from the Research and Development Institute for Bovine Balotesti, were screened for haematological profile at birth. The analyses were carried out in the Animal Physiology and Biochemistry Laboratory of the institute. Blood samples were collected aseptically from the jugular vein (1-2 ml) of each animal, in vacutainer tubes with anticoagulant using potassium-ethylenediamine tetraacetic acid (EDTA/K3) with a concentration of 1.27 mg EDTA/K3 per ml of blood. Haematological parameters (red blood cells count, haemoglobin concentration, hematocrit percentage, red blood cells distribution width, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets count, mean platelets volume, platelets distribution width, total white blood cells count, lymphocytes percentage, monocytes percentage, neutrophil percentage) were determined using automated hematology analyzer Abacus Junior Vet 5. Results were expressed as a mean (±standard error), standard deviation (sd) and coefficient of variation (V). The analysis of variance (ANOVA) single-factor was applied to test the significance of differences.

RESULTS AND DISCUSSIONS

The recorded mean values for RBC and HGB (Table 1) were situated in the normal physiological limits, except whith groups E_2: F_1 BBB x BNR and M: BNR for red blood cell (the means values were above normal physiological limits), but without statistical significant differences ($F_{critical}<F_{0.05}$). The same mean values have been obtained by others authors in newborn calves (Botezatu et al., 2014; Anton et al., 2009).

Table 1. The result of RBC, HGB, HTC, RDW parameters in newborn calves

Groups/Breed[1]	Parameters[2]	$\ddot{X}\pm s_x$	sd	V%
E_1: F_1 *CH x BNR*	RBC, mil/mm^3	8.95±0.83	2.65	29.61
E_2: F_1 *BBB x BNR*		10.58±0.45	1.51	14.22
E_3: F_1 *AA x BNR*		8.99±0.34	1.09	12.12
E_4: F_1 *LI x BNR*		8.44±0.54	1.72	20.38
M: BNR		9.35±0.48	1.51	16.15
$F_{critical}=1.82$; $F_{0.05}(8; 44)=2.00$; $F_{critical} < F_{0.05}$; (p=0.144).				
E_1: F_1 *CH x BNR*	HGB, g/dl	10.34±0.34	1.11	10.74
E_2: F_1 *BBB x BNR*		11.54±0.55	1.56	13.52
E_3: F_1 *AA x BNR*		11.78±0.50	1.47	12.48
E_4: F_1 *LI x BNR*		10.08±0.58	1.86	18.45
M: BNR		10.61±0.59	1.89	17.81
$F_{critical}=1.97$; $F_{0.05}(8; 44)=2.00$; $F_{critical} < F_{0.05}$; (p=0.118).				
E_1: F_1 *CH x BNR*	HTC, %	29.15±1.30	4.81	16.50
E_2: F_1 *BBB x BNR*		33.85±1.52	4.15	12.26
E_3: F_1 *AA x BNR*		32.32±1.43	4.56	14.11
E_4: F_1 *LI x BNR*		29.48±1.25	3.98	13.50
M: BNR		30.82±1.58	5.02	16.29
$F_{critical}=1.71$; $F_{0.05}(8; 44)=2.00$; $F_{critical} < F_{0.05}$; (p=0.166).				
E_1: F_1 *CH x BNR*	RDW, %	23.36±0.50	1.58	6.76
E_2: F_1 *BBB x BNR*		25.60±0.23	1.08	4.22
E_3: F_1 *AA x BNR*		24.12±0.39	1.26	5.22
E_4: F_1 *LI x BNR*		24.48±0.94	3.01	12.30
M: BNR		24.45±0.22	0.71	2.90
$F_{critical}=1.96$; $F_{0.05}(8; 44)=2.00$; $F_{critical} < F_{0.05}$; (p=0.119).				

[1] CH=Charolaise, BBB=Blanc Blue Belgique, AA=Aberdeen Angus, LI=Limousine, BNR=Romanian Black Spotted.
[2]RBC= erythrocytes/red blood cells count, HGB=hemoglobin concentration, HCT= hematocrit percentage, RDW=red blood cells distribution width.

The average percentage for HTC was 33.85±1.52% for group E_2: F_1 BBB x BNR and 32.32±1.43% for group E_3: F_1 AA x BNR comparative with groups E_1: F_1 CH x BNR, E_4: F_1 LI x BNR and M: BNR where registered values below reference values indicated by the literature. Decrease of the HTC indicated anemia and impaired ability to carry oxygen from red blood cells. The RDW and MCV are useful for classifying the types of anemia. The increase of RDW is the first sign early in iron deficiency or other trace mineral deficiencies associated with macrocytic or microcytic anemia-the MCV is normal or lower and RDW is higher (Brockus, 2011; Brun Hansen, 2006; Glader, 2004; Kincaid, 1999). Together with MCHC and MCH (Table 2), MCV can permit the early detection of processes that will cause anemia. In newborn calves, the recorded means values for RDW were higher (from 23.36±0.50% to 25.60±0.23%) and obtained means values for MCV were below normal physiological limits (from 33.00±0.57 fl to 35.44±0.86 fl). The coefficient of variation calculated for RBC, HGB, HTC, MCH was lower than 20.38%, expressing a homogeneous population. However, for RBC (group E_1: F_1 CH x BNR) the coefficient of variation was 29.61%. For RDW, the values were below than critical threshold of 10% for groups E_1: F_1 CH x BNR, E_2: F_1 BBB x BNR, E_3: F_1 AA x BNR and M: BNR, indicated a very homogeneous population.

Table 2. The result of MCV, MCH, MCHC parameters in newborn calves

Groups/Breed[1]	Parameters[2]	$\ddot{X}\pm s_x$	sd	V%
E_1: F_1 *CH x BNR*	MCV, fl	34.33±1.06	3.39	9.87
E_2: F_1 *BBB x BNR*		32.33±1.01	3.43	10.61
E_3: F_1 *AA x BNR*		35.44±0.86	2.74	7.73
E_4: F_1 *LI x BNR*		34.66±0.89	2.29	6.61
M: BNR		33.00±0.57	1.65	5.00
$F_{critical}=1.86$; $F_{0.05}(8; 44)=2.00$; $F_{critical} < F_{0.05}$; $(p=0.137)$.				
E_1: F_1 *CH x BNR*	MCH, pg	12.57±0.46	1.54	11.25
E_2: F_1 *BBB x BNR*		11.41±0.43	1.39	12.18
E_3: F_1 *AA x BNR*		12.54±0.50	0.90	7.18
E_4: F_1 *LI x BNR*		11.98±0.44	1.41	11.77
M: BNR		11.56±0.22	0.59	5.10
$F_{critical}=1.78$; $F_{0.05}(8; 44)=2.00$; $F_{critical} < F_{0.05}$; $(p=0.160)$.				
E_1: F_1 *CH x BNR*	MCHC, g/dl	38.64±0.58	1.86	4.81
E_2: F_1 *BBB x BNR*		36.81±0.37	1.17	3.18
E_3: F_1 *AA x BNR*		37.12±1.08	3.44	9.27
E_4: F_1 *LI x BNR*		35.44±0.83	2.86	8.07
M: BNR		35.17±0.26	2.22	6.31
$F_{critical}=2.96$; $F_{0.05}(8; 44)=2.00$; $F_{critical} > F_{0.05}$; $(p=0.031)$.				

[1]CH=Charolaise, BBB=Blanc Blue Belgique, AA=Aberdeen Angus, LI=Limousine, BNR=Romanian Black Spotted.
[2]MCV=mean corpuscular volume, MCH=mean corpuscular hemoglobin, MCHC=mean corpuscular hemoglobin concentration.

In case of MCHC (Figure 1), significant differences ($p \leq 0.05$) between the experimental groups were observed, as follows:
$F_{critical}=2.93$; $F_{0.05}(8; 44)=2.00$; $F_{critical} > F_{0.05}$

Also, a very homogeneous population was obtained for MCHC (values between 3.18%-9.27%) for all experimental groups studied.

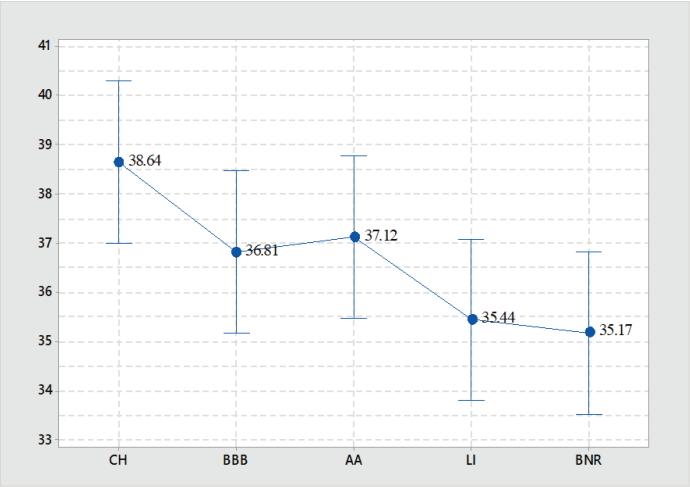

Figure 1. Average values of mean corpuscular hemoglobin concentration (MCHC) in newborn calves

In calves, platelets counts may be the same or above adult reference intervals (Brun Hansen, 2006). The obtained results for PLT, MPV and PDW were not statistically significant ($p>0.05$) between experimental groups (Table 3). An increased platelets count might be associated with an increased risk for thrombosis (Boudreaux, 2011), in our study the recorded means values for PLT were situated in normal physiological limits.

The obtained means values for MPV and PDW were below references values indicated by the literature. The MPV together with PDW can be used to differentiate conditions associated with lower platelets production and with increased platelets destruction.

The PLT and VTM are often low in conditions associated with alteration of platelets production (Perkins, 2004).

Table 3. The result of PCT, MPV, PDW parameters in newborn calves

Groups/Breed[1]	Parameters[2]	$\ddot{X}\pm s_x$	sd	V%
E_1: F_1 *CH x BNR*	PLT, thousands/mm^3	505.40±85.58	272.30	53.88
E_2: F_1 *BBB x BNR*		632.00±61.21	194.80	30.82
E_3: F_1 *AA x BNR*		542.80±53.30	169.60	31.25
E_4: F_1 *LI x BNR*		679.60±74.96	231.11	34.01
M: BNR		729.00±35.41	123.70	16.97
$F_{critical}$=1.86; $F_{0.05}$ (8; 44)=2.00; $F_{critical} < F_{0.05}$; (p=0.137).				
E_1: F_1 *CH x BNR*	MPV, fl	5.88±0.15	0.59	10.03
E_2: F_1 *BBB x BNR*		5.57±0.03	0.10	1.80
E_3: F_1 *AA x BNR*		5.64±0.11	0.37	6.56
E_4: F_1 *LI x BNR*		5.98±0.06	0.22	3.68
M: BNR		5.90±0.14	0.46	7.80
$F_{critical}$=1.84; $F_{0.05}$ (8; 44)=2.00; $F_{critical} < F_{0.05}$; (p=0.140).				
E_1: F_1 *CH x BNR*	PDW, %	33.80±0.69	1.81	5.36
E_2: F_1 *BBB x BNR*		32.76±0.23	1.36	4.15
E_3: F_1 *AA x BNR*		33.04±0.15	0.50	1.51
E_4: F_1 *LI x BNR*		32.68±0.20	0.65	1.99
M: BNR		33.80±0.40	1.30	3.85
$F_{critical}$=1.78; $F_{0.05}$ (8; 44)=2.00; $F_{critical} < F_{0.05}$; (p=0.151).				

[1]CH=Charolaise, BBB=Blanc Blue Belgique, AA=Aberdeen Angus, LI=Limousine, BNR=Romanian Black Spotted.
[2]PLT=platelets/thrombocytes count, MPV=mean platelet volume, PDWc=platelet distribution width.

There is limited data in the literature regarding the clinical interpretation of MPV and PDW. For PLT, the coefficient of variation was situated between 30.82-53.88% for groups E_1: F_1 CH x BNR, E_2: F_1 BBB x BNR, E_3: F_1 AA x BNR and E_4: F_1 LI x BNR, showed a heterogeneous populations. On the contrary, for PDW, the coefficient of variation was below 5.36%, in this case we had a very homogeneous populations (for E_2: F_1 BBB x BNR the values was 4.15%, for E_3: F_1 AA x BNR the values was 1.51% and for E_4: F_1 LI x BNR the values was 1.99%). The obtained means values for WBC, LY and MO (Table 4) were situated above normal physiological limits, without statistical significant differences ($F_{critical}<F_{0.05}$). The high number of white blood cells may be the sign of a bacterial infection or inflammatory syndrome. According to the specialty literature, we have mild leukocytosis when the white blood cells count is between 9.8-12 thousands/mm^3 (Parvu, 2003).

Table 4. The result of WBC, LY, MO, NE parameters in newborn calves

Groups/Breed[1]	Parameters[2]	$\bar{X}\pm s_x$	sd	V%
E_1: F_1 *CH x BNR*	WBC, thousands /mm3	10.99±0.60	1.91	17.38
E_2: F_1 *BBB x BNR*		12.45±1.99	1.96	15.74
E_3: F_1 *AA x BNR*		10.47±0.72	2.29	21.87
E_4: F_1 *LI x BNR*		10.05±0.12	0.38	3.78
M: BNR		11.92±1.00	3.19	26.76
$F_{critical}=1.93$; $F_{0.05}(8; 44)=2.00$; $F_{critical}< F_{0.05}$; $(p=0.124)$.				
E_1: F_1 *CH x BNR*	LY, %	72.68±3.24	10.32	14.20
E_2: F_1 *BBB x BNR*		66.81±3.84	12.22	18.29
E_3: F_1 *AA x BNR*		67.33±3.83	12.20	18.12
E_4: F_1 *LI x BNR*		61.24±4.62	16.38	26.75
M: BNR		59.60±4.91	6.55	10.99
$F_{critical}=1.72$; $F_{0.05}(8; 44)=2.00$; $F_{critical}< F_{0.05}$; $(p=0.164)$.				
E_1: F_1 *CH x BNR*	MO, %	4.95±0.73	2.34	47.27
E_2: F_1 *BBB x BNR*		8,21±0.27	2.71	33.01
E_3: F_1 *AA x BNR*		4.97±1.14	3.63	73.04
E_4: F_1 *LI x BNR*		4.41±1.11	3.55	80.50
M: BNR		6.17±4.30	4.06	65.80
$F_{critical}=1.89$; $F_{0.05}(8; 44)=2.00$; $F_{critical}< F_{0.05}$; $(p=0.131)$.				
E_1: F_1 *CH x BNR*	NE, %	22.37±3.07	9.78	43.72
E_2: F_1 *BBB x BNR*		23.51±3.58	11.40	48.49
E_3: F_1 *AA x BNR*		27.70±4.45	14.16	51.12
E_4: F_1 *LI x BNR*		34.32±5.58	17.77	51.78
M: BNR		30.31±1.30	4.10	13.53
$F_{critical}=1.43$; $F_{0.05}(8; 44)=2.00$; $F_{critical}< F_{0.05}$; $(p=0.241)$.				

[1]CH=Charolaise, BBB=Blanc Blue Belgique, AA=Aberdeen Angus, LI=Limousine, BNR=Romanian Black Spotted.
[2]WBC=leukocytes/white blood cells count, LY=lymphocytes percentage, MO=monocytes percentage, NE=neutrophil percentage.

The lymphocyte leukocytosis can be detected when lymphocytes are more than 60%, found in hematosis, septicaemia and virosis. In cattle, the total number of WBC decreases with age. The total WBC count were higher in newborn calves than adult reference intervals in some studies, but in others reports, the mean of WBC counts in newborn calves were within adult reference intervals (Mohri, 2007; Knowles, 2000). The difference may be attributed variability between individual calves. Calves have a NE:LY ratio greater than 1.0 at birth, with a rapid decrease in NE and increase in LY resulting in an NE:LY ratio similar to that of adult cattles at seven days of age (Jones, 2007). Neutrophils provide the first line of defense against any inflammatory process-microorganisms, tissue trauma, etc. (Appelberg, 2006). The recorded average values for NE were situated in normal physiological limits (means from 21.42%±4.11% to 33.02%±7.31%) with a coefficient of variation between 14.49%-55.39% for all groups. In the present study, we observed a congenital anemia in calves at birth

which are in agreement with results observed in other studies (Parvu, 2003).

CONCLUSIONS

The haematological examination used for health status assessment in newborn calves revealed a slight anemia of these. The incidence of subclinical anemia is, often, found in newborn calves. The obtained results showed no statistical differences between experimental studied groups. However, in case of mean corpuscular hemoglobin concentration, significant differences between the experimental groups were observed. To explore the haematological profile in newborn calves, larger scale studies with more animal's are needed to correlate the results with clinical data.

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EFFECTS OF DIFFERENT ROUGHAGE HARVESTING SYSTEMS ON YIELD AND DRY MATTER LOSSES

Yasemin VURARAK[1], Ahmet İNCE[2], M. Emin BİLGİLİ[1]

[1]Eastern Mediterranean Agricultural Research Institute, 01321, Yüreğir, Adana, TURKEY
[2]Department of Agricultural Machinery and Technologies Engineering, Faculty of Agriculture, Cukurova University, 01330, Sarıçam, Adana, TURKEY

Corresponding author email: aince@cu.edu.tr

Abstract

The objective of this study was to investigate the effects of different roughage harvesting systems in terms of yield and dry matter (DM) losses. Also, some quality parameters such as pH, acid detergent fiber (ADF), neutral detergent fiber (NDF) and relative feed value (RFV) were evaluated. To this aim, four harvesting systems (S1: mower + round baler, S2: disc mower with conditioner + round baler, S3: mower + round baler + wrapping machine, S4: disc mower with conditioner + round baler + wrapping machine) were tested according to randomized block design with three replications. As plant material, vetch + triticale (Vicia sativa L. + Triticasecale wittmack) mixture was used.It was found that the bale silages have lower pH content comparing with dried forage. Also the best harvesting systems was S4 in terms of RFV. While the highest DM losses occurred in S1 system, S4 system gave the highest yield. These results indicate that the choice of method and machine is very important in the roughage harvest.

Keywords: *roughage, bale silage, harvesting machines, yield, dry matter losses.*

INTRODUCTION

In the livestock sector, feed costs take first place in all inputs. Yılmaz et al. (2005) reported that feed production costs accounted for about 88% of total production costs. From this point of view, decreasing feed production costs can be considered as a prerequisite for reducing animal product prices. This condition can be achieved by obtaining a high quality and high yield roughage. The priority of this requirement is to reduce dry matter losses that occur during harvest and storage. Harvesting and storage are the main steps that determine the method of roughage production and vary according to climatic conditions, size of farm, plant variety and mechanization infrastructure.

Roughages with high moisture content are dried by natural drying on the field after the harvesting. However, with this method, dropping the humidity from 85% to 20% depends on the number of sunny days and may not always be possible. DM losses during harvest and storage vary depending on the moisture content of the crop. During drying, the loss rate of DM increases and low quality feed is obtained (Schroder, 2004). The roughage preparation is very difficult in rainy and moist regions.High rainfall rate and moisture reduce drying speed and roughage quality.Because, the plants start to deteriorate in a short period of time (Kılıç, 2010).Nutrient losses such as carotene increase in roughage that are exposed to excessive rain and sunlight. Baytekin and Gül (2009) reported that the digestibility ratio decreased by 12% for alfalfa remains in the rain and by 6% in the wheatgrass.

These negativities have forced producers into alternative methods of producing roughage. Bale silage which is also known haylage is one of these methods. The bale silage, which can be prepared in large and small sizes, allows the farmers to manage the roughage in good quality. In this way, especially small farms that want to expand their herd can be get quality feeds with low cost.

However, DM losses are a matter that needs to be investigated in bale silage as well as in traditional methods.The objective of this study was to investigate the effects of different roughage harvesting systems in terms of yield and dry matter (DM) losses. Also, some quality parameters such as pH, acid detergent fiber

(ADF), neutral detergent fiber (NDF) and relative feed value (RFV) were evaluated. Thus, it is targeted to determine the most suitable method for preparing high quality and low DM loss roughage in Mediterranean climate conditions.

MATERIALS AND METHODS

The study was conducted in Adana province, Turkey which is located in the Mediterranean Region. Researches were done between November-2014 and June-2015. The average climaticvalues during this period were presented in Table 1. The soil characteristics of the experimental area were sandy-loam and alkaline (pH: 7.86-7.75). Organic matter, lime content, potassium and phosphorus in the experimental area were 2.28-2.41%, 15.9-16.3%, 109.2-123.3 kg da^{-1}, 4.32-5.16 kg da^{-1}, respectively.

Table 1. Average Climate Data of Research Area (Anonymous, 2016)

Months	Temp. (°C)	R. Humidity (%)	Rainfall (mm)
November 2014	15.1	57.4	36.06
December 2014	13.4	71.6	50.05
January 2015	9.7	66.9	72.39
February 2015	11.3	70.1	90.68
March 2015	14.0	64.9	148.80
April 2015	16.9	62.5	7.80
May 2015	22.5	64.4	81.02
June 2015	24.2	69.1	0.00
Total rainfall and average of temperature values-R.Humidity	15.8	65.5	486.8

Mixture of vetch + triticale (*Vicia sativa* L.+*Triticasecale wittmack*) was used as plant material. Mixture rate was 70% and 30% for vetch and triticale, respectively. The average yield of the material was calculated as 5000.6 kg da^{-1}. The properties of the mixture are given in Table 2.

Table 2. The properties of the mixture and plant length

Content of mixture	The properties of the mixture		Plant length (cm)
	Kg m^{-2}	Botanical composition (%)	
Vetch	1498	29	155.0
Triticale	3508	71	154.2
Total	5006	100	-

Roughages were harvested at the end of flowering stage. The harvesting and storage systems investigated in the research were given in Table 3.

Table 3. Harvesting and storage systems

System code	Machines used in harvesting	Storage technique
S1	Mower+round baler	Dry hay
S2	Disc mower with conditioner+round baler	Dry hay
S3	Mower+round baler+wrapping machine	Haylage
S4	Disc mower with conditioner +round baler+wrapping machine	Haylage

The technical characteristics of machines were also tabulated in Table 4. For haylage bales were wrapped by using PE material with 25 µ thickness in white color as four layers. The bales weight varied in between 15-20 kg for hay and 30-40 kg for haylage.

The quality of the material was evaluated in terms of DM, pH, ADF and NDF. The DM content of roughage was determined by drying to constant weight at 105°C according to the ASAE standards (AOAC, 1990). The pH values of samples were obtained as reported by Chen et al. (1997). ADF and NDF were determined as suggested Van Soesst et al. (1991) by using ANKOM fiber analyzer. The RFV was calculated according to Mayouf and Arbouche (2014). The systems were compared according to standards assigned by Hay Market Task Force of American Forage and Grassland Council (Mayouf and Arbouche, 2014).

Table 4. Some technical characteristics of machines

Harvesting machines	Disc mower with conditioner	Number of disc	5
		Working width (cm)	240
		Tractor power (Hp)	50-80
		PTO (min)	540
		Weight (kg)	500
	Mower	Number of drums	2
		Working width (cm)	165
		Number of cutting blade	6
		PTO (min)	540
		Weight (kg)	360
Other machines	Wrapper machine	Electrical requirements (V)	12
		Film width (mm)	200/250
		Film stretching ratio (%)	70
		Dimensions LxWxH (cm)	123x158x164
		Weight (kg)	350
	Small round baler machine	Feed Circle (mm)	500x700
		Weight (kg)	540
		Width (mm)	1520
		Length (mm)	2408
		Height (mm)	1550
		Bale weight of dried forage (kg)	25-30
		Bale weight of green forage (kg)	35-40
		Hay baleWeight of hay (kg)	15-20
		Bale production (bale h^{-1})	50-70

The yield was calculated by multiplying the average weight of bales by the number of bales. The DM yield was found by multiplying the parcel yield with DM%. A frame was used to determine the DM losses. The size of frame was equal to the working width of the harvesting machine (m) x 1 (m). The rest of plant material in this frame (in square meters) was weighed in five replication and the obtained data were transformed as decares (da). DM losses were found according to systems.

The experiments were carried out according to randomized blocks design with three replications. The effect of systems on DM, pH, ADF, NDF, RFV, DM losses were analyzed by using a statically program. Duncan's multiple range tests were used to compare the means (Yurtsever 1984; Kalaycı 2005).

RESULTS AND DISCUSSIONS

The systems were found to be statically significant on bale weight and bale density at 1% probability level while there is no significant effect on bale twine weight. When the dry hay and haylage preparation systems are assessed in themselves, the bales weight and density in both are higher in the systems used conditioner. Because the conditioner leaves the material more smooth and fluffy on the field surface and allows the bale machine to pick the material up from the surface and compress it more effectively. This difference was found to be 6.4% and 10.1% higher for bale weight and density in the S2 system than S1 system, respectively. Also, the values in the S4 system was 18.8% and 22.7% higher than the S3 system for bale weight and density, respectively (Table 5).

A review of Table 6 shows that all systems have significant effect on pH, ADF and NDF values at 1% probability level. Bale silages have lower pH content comparing dried roughage. Relative feed value (RFV) is one of the most important criteria in terms of quality. This value is calculated by using ADF and NDF values. Although systems have no effect statistically on RFV, S4 was the best system according to Quality Standards for Wheat and Legume Forage Crops (Mayouf and Arbouche, 2014).

Table 5. Changing of bale weight, density and twine weight according to the systems

Parameters	Bale weight (Kg bale^{-1})	Bale density (Kg m^{-3})	Twine of bales (g bale^{-1})
S1	16.26^{c}	117.97^{b}	26.26
S2	17.30^{c}	129.89^{b}	26.93
S3	30.31^{b}	239.88^{a}	27.20
S4	35.88^{a}	294.44^{a}	29.25
p (%)	<.0001**	0.0008**	00.51^{ns}
$LSD_{(0.05)}$	31.04	58.63	-
CV (%)	8.7	15.0	8.8

$^{*,**,\ ns}$: Significant at the levels of 5%, 1%, and not significant respectively.
Different letters fallowing means in the same column indicate statistical significance from each other (p <0.05)

This result indicates that more nutrients were preserved in bale silage. Schroeder (2004) reported that making haylage has several advantages than hay. However, greater ability to harvest the crop at ideal maturity, as less rain-free weather is needed for harvesting haylage.

Table 6. Changing quality parameters according to the systems

Parameters	pH	ADF(%)	NDF(%)	RFV
S1	6.06^{a}	45.63^{ab}	63.29^{a}	78.40$^{(4)}$
S2	5.56^{b}	42.02^{c}	60.66^{ab}	86.12$^{(4)}$
S3	5.43b^{c}	47.17^{a}	57.56^{b}	84.70$^{(4)}$
S4	5.03^{c}	44.16^{bc}	57.29^{b}	88.56$^{(3)}$
p (%)	0.0086**	0.0070**	12.09**	0.1940^{ns}
$LSD_{(0.05)}$	0.49	2.25	5.22	-
CV (%)	4.5	2.5	4.4	6.0

$^{*,**,\ ns}$: Significant at the levels of 5%, 1%, and not significant respectively;
Different letters fallowing means in the same column indicate statistical significance from each other (p <0.05).
1, 2, 3, 4, 5: Quality Standards for Wheat and Legume Forage Crops.
RFV= ((88.9 - (0.779x%ADF))x(120/%NDF)/1.29)

The systems were found to be statistically significant on DM content, yield of mixture and DM loss at 1% probability level, while theyhave effect statistically on DM yield at 5% probability level. Average DM content was approximately 90% and 48% for dried roughage and haylage respectively. According to the variance analysis, it was determined that S4 has highest DM yield value. In addition, DM losses were found highest for S1system which mower and round baler were used (Table 7). This was thought to be related to the DM content and the effect of the harvester type on the plant. While the mower cuts the plant near the surface and leaves it in the form of a low swaths, the disc mower with conditioner cuts the plant from a few places and leaves it in the form of a high swaths. The bale machine used after mower cannot pick up the material which is in the form of low swaths easily. This situation causes to increase DM losses. Moreover, the quality of the bale silage was found higher than dried roughage.

Table 7. DM, DM losses of yield and analysis of variance and grouping

Parameters	DM (%)	Yield of mixture (kg da^{-1})	DM yield (kg da^{-1})	DM losses (kg da^{-1})
S1	90.00^{a}	788.68^{b}	710.19^{B}	251.31
S2	92.10^{a}	865.00^{b}	796.68^{b}	105.78^{c}
S3	48.18^{b}	1837.37^{a}	888.95^{ab}	160.00^{b}
S4	49.06^{b}	2093.19^{a}	1027.40^{a}	70.66^{c}
p (%)	<.0001**	<.0001**	0.02*	<.0001**
$LSD_{(0.05)}$	6.10	256.20	189.22	38.96
CV (%)	4.3	9.2	11.1	13.3

$^{*,**,\ ns}$: Significant at the levels of 5%, 1%, and not significant respectively;
Different letters fallowing means in the same column indicate statistical significance from each other (p <0.05).

Yaman and Sönmezler (2011), also compared different roughages and found the nourishment of bale silage was better. The DM losses for S1 and S2 were determined as 251.31 kg da^{-1} and 105.78 kg da^{-1}, respectively.

These values were found to be 160.0 kg da^{-1} and 70.66 kg da^{-1} for S3 and S4 respectively. In other words, DM losses are about twice as high in systems where mowers are used.

Russell and Johnson (2014) reported that the amount of digestible protein and carbohydrate in the leaves are higher than in other parts of the plant.

For this reason, leaf losses must be reduced in order to save nutrition value of plant.

In this case, the use of harvesting machines with conditioners to reduce leaf losses would be a more correct approach.

CONCLUSIONS

Bale silage is an alternative option for the storage and processing of roughage obtained from legumes and grasses.

However, the harvester type is an important factor for the quality of bale silage.

Whether bale silage or hay bale, they must be harvested and storaged with protection of nutrient elements.

Since, the losses are quite high in dried roughage, recently, the bale silage is recommended by expert for ruminant feeding.

As a result of this study, it was determined that the mechanization chain used for making roughage affect the quality, yield and DM losses.

In terms of getting good quality roughage and lower DM losses, for small-size farms, "disc mower with conditioner + round baler + wrapping machine" can be recommended in rainy and humid regions like Cukurova region.

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CHARACTERISTICS AND NUTRITIONAL CONTENT OF THE NON-TRADITIONAL FODDER PLANT *Polygonum sachalinense* CULTIVATED UNDER THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

Sergiu Coșman[1], Iulian Iurcu[1], Victor Țiței[2], Valentina Coșman[1]

[1]Scientific and Practical Institute of Biotechnologies in Zootechny and Veterinary Medicine, Republic of Moldova
[2]Botanical Garden (Institute) of the Academy of Sciences of Moldova

Corresponding author email: sergiu-cosman@mail.ru

Abstract:

The study on the non-traditional fodder plant for the Republic of Moldova Hrișca de Sahalin (Polygonum sachalinense) was included the content analysis of the main nutrients, the appreciation of the nutritional value of the green mass and the appreciation of the silos prepared from this plant. It was found out, that the green mass of the plant during the pre-branching and branching period contained 84.77-75.0% moisture, 16.06-11.25% crude protein, 2.87-2.42% crude fat, 27.81-36.12% crude cellulose, from 43.47 to 40.78% of extractive substance doesn't contain nitrite and it has an energy value of 0,19 to 0,2 nutrients units. Hrișca de Sahalin can be successfully ensiled. The obtained silage has an olive color with pleasant smell of pickled vegetables and the lactic acid content is about 78-80% of the total organic acids. This plant starts its primary intensive growth in the spring. In the second decade of April it can be used as a supplement to the basic ration for farm animals throughout the green season. Polygonum sachalinense is a multiannual plant. Its exploitation within 10-15 years and under the conditions of the Republic of Moldova can ensures a harvest of 800-1000 quintals of green mass per 1 ha.

Keywords: *experiment, non-traditional fodder plant, Polygonum Sachalinense, green mass, silage.*

INTRODUCTION

In recent years research has been carried out in many countries on the implementation of new species of new fodder plants, non-traditional that are used as sources rich in protein, essential amino acids, other biologically active substances for the purpose of better balancing the rations, increasing the productivity of animals as well as increasing soil fertility, improving the quality of degraded soils, etc. (Teleuta, 2010; Petukhov et al., 1989; Zabarinsky, 1992).

The introduction into the Republic of Moldova of new fodder plants rich in nutritional and biologically active substances is one of the possibilities to expand the spectrum of fodder resources and to diversify animal feed in order to increase the quality of ratites used in animal feed (Bulanenkova, 1970; Zabarinsky, 1992).

Many of the non-traditional fodder plants have a high resistance to drought, various diseases, are tolerant to soil quality (Bogomaz et al., 1970; Mugnieva, 2000).

From the new fodder plants we highlight multi-year species like *Galega oriental*, Silphia, *Polygonum sachalinense*, which for 10-12 years possesses high biomass productivity, about 800-1000 quintals per hectare and offers the perspective of their implementation in the forage crops sector (Danilenko, 2013).

Studying several non-traditional plants for growing as fodder plants under the conditions of the republic, we have decided to subject the non-traditional plant to our *Polygonum sachalinense*, or the Hrișca de Sahalin, to more detailed research.

MATERIALS AND METHODS

In the central area of the Republic of Moldova (Botanical Garden, Chisinau) were conducted research on the growth, development and chemical composition of the plant *Polygonum sachalinense*, in various phases of vegetation - before buttoning, branching, butonization.

The monitoring of the growth and development of the *Polygonum sachalinense* has been carried out since March and has continued throughout the growing season.

During this period were made various measurements, weighing, plant samples were

taken at different stages of growth, prepared silo parties in various capacities (barrels, trenches) and carried out multiple chemical analyzes in the institute's laboratory.

RESULTS AND DISCUSSIONS

The *Polygonum sachalinense* begins its growth periodearly in spring, has a high growth intensity and a long vegetation period. Accelerated growth and accumulation of a large amount of green mass in a relatively short time, prolongs the periods between the vegetation phases that are much longer than in plants of high intensity of traditional fodder. Thus, under the conditions of the Republic of Moldova, the plant reaches to have the height of 1.0 - 1.5 m on April 19, when it is still very young, green and mustard, and is in the intensive period of growth.

During the growth of plants, samples were taken when the plants were of 98-155 cm high, then in the pre- branching phase, the branching phase and the phase before the buttoning.

During the green season the process of growth of plants of *Polygonum sachalinense* was monitored (Table 1).

Table 1. Dynamics of growth of the *Polygonum sachalinense*

Date	Height of stem,cm
25.03.2017	10-12
05.04.2017	50-60
12.04.2017	60-98
19.04.2017	98-155
11.05.2017	205
18.05.2017	270
29.05.2017	357
13.06.2017	400

On April 5, the plants were of 50-60 cm high. After on April 12, the intensive plant growth period began and within 1 month, namely on May 11, the plants had the height of 205 cm. On May 11, 2017, were picked herbs of *Polygonum sachalinense* from the 1 sow, when the height of the plant was 181-239 cm. Data Table 2 represents the content of basic nutrients that were evaluated following the chemical analyzes performed in the Institute's laboratory.

In the period when the plants were already of 98-155 cm high, the total humidity was high at the level of 87.01%, in the pre-branching stage it fell to 84.77%, during the branching phase it constituted 75.0% and in the pre-butonization phase it dropped to 68.53%. So, there is a steady decrease in humidity with the growth and maturation of plants. At the same time, crude protein content decreases from 19.94% in the early phase, 16.06% in the pre-branching phase, and 11.25% in the branching phase, to 9.88% in the pre-butonization phase. Also, the crude fat decreases from 3.35% in the early phase to 1.93% in the pre-buttoning phase. A rather vertiginous increase manifests in crude cellulose, which from 14.91% in the early phase, rises to 27.81% in the pre-branching phase and stops at 36.12-38.53% in the phases branching and before buttoning.

Table 2. Chemical composition of the *Polygonum sachalinense* depending on the vegetation phase (% in dry substance)

Indices	Young plant, intensive growth 19.04.17	Befo-re rami-tion 18.05. 17	Ramific ation 13.06. 17	Before the butonizati on- 22.06.17
Humidity,%	87.01	84.77	75.0	68.53
Dry sub-stance ,%	12.99	15.23	25.0	31.47
Azote ,%	3.19	2.57	1.8	1.58
Crude protein ,%	19.94	16.06	11.25	9.88
Crude fat ,%	3.35	2.87	2.42	1.93
Crude cellulose,%	14.91	27.81	36.12	38.53
Crude ash,%	9.01	7.14	6.73	6.38
SEN,%	44.72	41.44	38.92	38.31
Carotene, mg/kg	30.35	40.0	63.75	66.57
Nutritional units	0.11	0.13	0.20	0.22

Humidity of the plant. An important role in the use of plants in animal feed, both fresh and in various forms of preparation, conservation and preservation, has the moisture content in plants. It is known that both excess water and low humidity have a negative impact on the conservation and subsequent preservation of fodder. In our research, during the vegetation period, plant moisture was very high at the beginning of vegetation and decreased with growth, maturation and aging. Thus, the total humidity in plants in the early growth period (April) is high and ranges between 84.77 and

87.01%. Gradually, as the plant grows, moisture decreases, and in the period when plants are at the harvest stage (June, July, August) gradually decreases to 72.17% and even to 69.11%. Analyzing the moisture content differentiated in stems and leaves, the stems were found to be very musty and contain up to 90.13% water, whereas the leaves have only 81.6%. Due to excessive humidity in the early intensive growing season, the previously made attempts to mow and dry the hay plants were quite difficult and even problematic. Later, in the maturation stages, when the plants reach the thickness of the ground stemof 1.5 cm, the drying of the hay is unacceptable due to the wooding of the stem and the inability of the animals to consume them.

Crude protein. The crude protein content of the dry substance differs greatly depending on the vegetation phase, the age of the plant, the height of the stubble at which the plant was mown. Analyzes made from our research showed that the crude protein in the plant at the beginning of the vegetation is 21.35%, the next stage of growth decreases to 16.68% and the phase before the botonization when the plant is quite high, reaching 4 m, crude protein continues to decrease to 10.2%. At the maturing stage, the crude protein content stabilizes at 10.0% and does not change essentially until the end of vegetation. Following the analysis performed differently in stems and in leavesa rather significant difference in the amounts of crude protein was also found. The crude protein content in the leaves is nearly twice as high as in the stems and is correspondingly 23.78 and 13.54%.

Plants grown on a more impoverished, unprocessed soil throughout the year had a minimum protein content of 9.81% when plants on other parcels contained 16.68%.

The fodder plant corn, which served in our research as a control plant in the spiking vegetation phase, had 7.06% and in the grain milk phase - 8.06% crude protein.

Crude cellulose. As the plants grow and develop, the crude cellulose content also increases considerably. Thus, with the intensive growth and maturation of plants, the crude cellulose content increased considerably from 17.0% in the early growth stage to 29.79% in the pre-branching phase, 35.41 in the branching phase, and 37.51% in the pre-butonization phase. Gross cellulose content is relatively small in *Polygonum sachalinense* - 21.85% compared to green maize - 26.78%. Analyzing the crude cellulose content differentiated in stems and leaves, crude cellulose was found to be higher in the stem - 28.54% and much less in the leaves - 11.81%.

Regarding the vegetation stages, the higher the stage of vegetation, the more the cellulose increases (from 17.0% in April to 29.79% in May) and the protein decreases (from 21.35% in April to 16.68% in May).

Carotene. Carotene is an important feed nutrient. It varies considerably depending on the vegetation phase and depending on the age of the Polygonum Sachalinense plant. During the time as the age of the plants is increased, the carotene content is increased as well from 30.35 mg/kg in the early growth phase to 66.57 mg/kg in the vegetation phase before a botonization. According to our observations and research, this increase is due to the ramification and the leaf grows. Since carotene is concentrated in leaves and the new ramification of branches have a large amount of leaves, it is logical that the total content of carotene in the plant is increased. An astonishingly large carotene content was found out in different parts of the plant such as in stems and in leaves. In our research the leaf carotene have reached 58 mg/kg, and in stems it was only 4.0 mg/kg.

Nutritional units. Following chemical analyzes, it has been found that the energy value of the *Polygonum sachalinense* plant varies depending on the period and the vegetation phase and increases as the plants mature. In the early growth period of plants, the plant's energy value was around 0.11 UN, in the next stage of growth, before branching-0.13, and from the vegetation phase branching stabilized at 0.2 - 0.22 UN/kg.

Table 3. Nutrient content in various parts of the *Polygonum sachalinense* plant (% in dry substance)

Parts of the plant	Total humi-dity	Crude protein	Crude cellu-lose	Carotene mg/kg
the entire plant	85.2	18.20	25.54	41.98
leaves	81.6	23.78	11.81	58.0
stems	90.13	13.54	28.54	4.0

The chemical analysis of the main nutrients in various parts of the *Polygonum sachalinense* plant, found a fairly convincing difference between stems and leaves. Thus, the stems contain a large amount of water – 90.13% and the leaves - 81.6%, the crude protein in the leaves is 23.78% and in the stems only 13.54%, the crude cellulose in the leaves constitutes only 11.81 % and in stems 28.54, carotene is concentrated in leaves at the level of 58%, and in stems only 4.0%.

As a control plant to highlight the similarity of the Polygonum Sachalinenseplant with a traditional culture cultivated in R Moldova, was selected the maize.

Polygonum sachalinense and Maize plants were taken to assess the amount of nutrients in the optimal harvesting stages for feeding the animals as a green meal. Thus, the *Polygonum sachalinense* was harvested in the vegetation phase prior to butonization, and the corn in the grain milk phase.

From the data of Table 4 it appears that the *Polygonum sachalinense* in the pre-butonization phase contains 10.63% crude protein as opposed to 7.06% in maize.

Table 4. Nutrient content determined in *Polygonum sachalinense* and Maize plants (% dry substance)

Plant	Humidity	Crude protein	Crude Cellulose	Crude fat	SEN	Carotene	Nutrient contents
Polygonum Sachalinense 13.06.017	76.81	10.63	36.89	2.19	38.36	63.75	0.16
Maize 9.08.017	77.55	7.06	25.37	1.84	54.99	55.17	0.22

A fairly large difference is in crude cellulose content, 36.89% in *Polygonum sachalinense* and 25.37% in maize. Carotene is not much higher in *Polygonum sachalinense* - 63.85% than in maize - 55.19%. Crude fat 2.19% in *Polygonum sachalinense* and 1.84% in corn, SEN 38.36% in *Polygonum sachalinense* and 54.99% in corn.

As a result, these differences in nutrient content did not essentially influence the energy value of both plants, which is 0.2 UN in the *Polygonum sachalinense* and 0.22 UN in maize. There are also quite large differences between these two plants in terms of exploitation periods, both in favor of one and the other. *Polygonum sachalinense*, being a plant that grows intensely during the early spring, can be used in animal feed starting with the second decade of April and continuing throughout the spring, summer and early autumn 2-3 mows per season.

Another chapter of the research was the study of the capacity of pretation of green massof *Polygonum sachalinense* to preserve by silage.Thus, the green mass of this plant was chopped and ensiled in small volumes under laboratory and in bulk volumes (barrels 75-150 kg, mini slides 400-500 kg) under semi-production conditions. After 60 days (sufficient time to complete biological preservation processes), were taken samples of silage and chemical analyzes were carried out on the nutrient content (Table 5).

Table 5. Nutrient content in the silage of *Polygonum sachalinense* (% of DS)

Total humidity	Crude protein	Crude Cellulose	Crude fat	Carotene,mg	Ca	P	UN
83.31	13.69	27.56	2.89	19.5	0.83	0.32	0.19

In the silage samples taken from the semi-production capacities, the moisture content was found to be 83.31%, the crude protein at the level of 13.69% in the dry substance, the crude cellulose 27.56%, the carotene 19.5 mg/kg, the calcium 0.83% and 0.32% phosphorus.

Table 6. Content and correlation of organic acids in the silage of *Polygonum sachalinense* (%)

Acetic acid		Butyric acid		Lactic acid		The sum of acids	Correlation of acids,%		
free	fixed	free	fixed	free	fixed		acetic	butyric	lactic
0-0.4	0.05-0.12	0	0-0.02	0.09-0.35	0.3-0.92	0.73-1.22	5.42-22.88	0-2.3	76.68-93.95

Regarding the organic acid content a very small amount of acid was found acetic acid (0.02-0.03%), complete absence ofbutyric acid in a free form and a relatively high amount of lactic acid (0.30-0.92%).
The correlation of organic acids in the obtained silage where the lactic acid level is 76.68-93.65% is a desirable for quality ofsilage. The small amount of acetic acid, the
sufficient amount of lactic acid, has led to a good conservation of the green grass of *Polygonum sachalinense* and to the production of a good quality silage
The organoleptic analysis of the silo found that the consistency, appearance, smell, color, correspond to the quality requirements of the silage prepared from herbs

CONCLUSIONS

In the Republic of Moldova the *Polygonum sachalinense* plant is experimentally cultivated in the plots on different lands and for 15 years
it has adapted well enough to the local climatic conditions.
The *Polygonum sachalinense* has manifested an amazing growth intensity from the early spring, that in the second half of April reached 98-155 cm high making it possible to use it as a green mass in the spring ration of the early animals.
The content of nutrients in *Polygonum sachalinense* is approaching the traditional green maize plant, with the exception of the crude cellulose that at a certain stage of vegetation as a result of maturing the plant, is high - 32.84-38.62% in dry substance.
The green grass of the plant *Polygonum sachalinense* is well suited to silage in the stages of vegetation before branching, and before butonization.

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THE INFLUENCE OF IMPROVED FEED ON THE PRODUCTIVE PERFORMANCES OF PREGNANT AND LACTATING SOWS

Monica MARIN, Georgeta DINIȚĂ, Carmen NICOLAE, Camelia HODOȘAN, Lucica NISTOR

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: marin_monica_zoo@yahoo.com

Abstract

The profitability of breeding sows is determined by their rational feeding, knowing that in the pig cost price of the delivered feed represents approx. 70-80%. Consequently, achieving efficient and consistent productions in pig farms depends on the quantitative and qualitative assurance of feeds, which are used both to ensure their own vital functions and to increase their production. For efficient feeding of pregnant and lactating sows, the morpho-physiological features of this species should be taken into account, testing the use of improved herbaceous species such as Turda maize, 21-1G barley, Tudor peas. The weight of pregnant sows and the consumption of compound feeds were influenced by the digestibility of nutrients, mainly by protein. During the lactation period, the consumption of compound feeds of sows varied according to the number of piglets and the body weight of the sows.

Key words*: sows, nutritive substances, hybrids of maize, barley, peas*

INTRODUCTION

Ensuring a balanced diet for sows is a complex problem because in a relatively short time the sow goes through different physiological stages in which its metabolism is profoundly altered.
Since gestation is an extremely delicate stage due to hormonal transformations in the sows that prepare the uterus to ensure the development and protection of fetuses, the maintenance and feeding system must be adapted to meet these requirements and to prevent factors which may influence gestation negatively (Cuc et al., 2006).
The application of quantitative and qualitative nutritional deficiency is harmful, negatively affecting the growth and development of embryos and fetuses as well as the production of milk from the future lactation (Rekiel et al., 2015).
The abundant diet that leads to the fattening of the sows is also detrimental, resulting in a dystocia calving, low number of piglet during birth, low birth weight, and low milk production in the future lactation that negatively impacts the development of piglets.
The quality of colostrum milk, which provides the antimicrobial protection of newborn piglets, as well as milk itself, plays an important role in the survival and development of piglets.
Sow milk is characterized by a high content of dry substance 19.4%, fat 7.2%, protein 6.1% and moderate in lactose 4.8% (Cuc et al., 2006). It also contains important amounts of minerals and vitamins.
During breastfeeding the sows will lose weight due to the fact that by the production of milk they are exported a great amount of energy and nutrients that cannot be provided only by food (Nel, 2017). Therefore, it is indispensable to apply a correct diet to limit these weight losses to only 13-15% of the weight after calving in the case of an 8-week lactation.
The aim of the paper is to test the improved hybrid plants to increase the production of milk for the weaning of a large number of piglets on the sow, to develop them well, and to keep the sow in a better state of maintenance.

MATERIALS AND METHODS

The biological material was represented by sows of the Great White breed, respectively 40 gestating sows in the second month, which were divided into 4 batches. The batches were made of sows with the closest sowing data, age

and body weight, being uniform as much as possible.

The testing on the sows took place during the gestation and lactation period.

The sows have been maintained in the same box from sowing to breeding. In this way it can be established a behavior adapted to each sow and to prevent accidents and mechanical abortions.

In order to formulate recipes for compound feeds intended for the feeding of pregnant and lactating sows, energetic raw materials, vegetable proteins, animal proteins, mineral ingredients, vitamin-mineral premixes (Table 1 and Table 2) were used. In a previous research iron and copper were used in the young swine organism (Marin et al., 2013).

The content of the compound feed can be tested at different environmental conditions using molecular dynamics in order to adapt to the needs (Marin et al., 2017) using the graphics processing unit and multi-core systems (Marin et al., 2016).

The improved tested plants were Turda corn, 21-1G barley and Tudor peas.

For the prevention of piglets loss during the gestation period, particular attention was paid to the rational feeding of pregnant sows.

For this purpose pregnant sows should be fed with rations containing all the nutrients and recommended amounts for the physiological state of gestation. Thus, the ratio of the pregnant sows should contain enough amounts of digestible protein, mineral salts and vitamins, to ensure a sufficient volume for the animal to feel full and to be made of tasty and varied fodder (Drăgotoiu et al., 2014).

For pregnant sows combined feed was used at which the crude protein was of 14.11-14.17% and the metabolizable energy 2882-2945 kcal/kg.

The structure of the compound feed recipes for pregnant sows included cereals (corn, barley) 60-70%, vegetable fodder feeds (soybean and sunflower meal, wheat bran, fodder peas) 23.50 - 35.50%, synthetic lysine 0.04 -0.18%, mineral feed (salt, dicalcium phosphate, calcium carbonate) 3.56-3.82 %% and vitamin-mineral premix 0.5%.

For feeding of lactating sows, a compound feed made of cereals (corn, barley) in the proportion of 65.50-72.00%, vegetable fodder feed (soybean meal, wheat bran, fodder peas) 18.50-20.50%, protein fodder animal origin (fish meal) 6%, synthetic lysine 0.09-0.18%, mineral fodder (salt, dicalcium phosphate, calcium carbonate) 2.82-3.12% and vitamin-mineral premix 0.5%.

The compound feed used in the alimentation of sows with piglets had a calorific value of 3062-3080 kcal EM/kg.

Concerning the protein level of the compound feed for lactating sows, it was 17.31-17.43% crude protein with a good biological value, providing all essential amino acids at the optimal level (1% lysine and 0.58% methionine and cystine).

Not ensuring this protein level can cause a decrease in milk production that will negatively influence the weight gain of piglets, as well as the evolution of the sows' body weight.

A special role in the production of milk belongs to the mineral substances such as calcium and phosphorus, their share in sour milk being of 0.25% Ca and 0.16% P. Considering this, as well as the requirements for the maintenance of the maternal body, the calcium level was 1.12-1.14% and the phosphorus level was 0.79-0.81%.

The feeding of sows during lactation is influenced by the milk production, which is implicit in the number of breast-fed piglets. The amount of food administered daily will increase progressively to 7-10 days, after which it remains at a relatively constant level, and 3-4 days before weaning it gradually decreases, so that on the day of weaning it is not given feed.

During the gestation period the weights of sows were recorded in the 2nd and 4th months of gestation, as well as the daily average consumption (Table 1).

Also, the daily average consumption of sows in the lactation period, the number of born piglets and the mortality rates when giving birth, the weight of born piglets, the number of weaned piglet and their weight were recorded.

The piglets were weaned at the age of 21 days, because after 3 weeks since the birth the sow's milk production begins to decline.

Table 1. Experimental scheme

Experimental batch	Number of sows (heads)	Followed objectives
Pregnant sows		- Weight of the sows in the second month and the fourth month of gestation, the average daily consumption; - Daily average consumption of sows during lactation, number of piglets and mortality, weight of weaned piglets, number of weaned piglets and their weight
Control batch	10	
Experimental batch with Turda maize in feed (E1)	10	
Experimental batch with barley 21-1G in feed (E2)	10	
Experimental batch with Tudor pea in feed (E3)	10	
Lactating sows		
Control batch	10	
Experimental batch with Turda maize in feed (E1)	10	
Experimental batch with barley 21-1G in feed (E2)	10	
Experimental batch with Tudor pea in feed (E3)	10	

The obtained results were statistically tested using the Student test to highlight the differences between the environments.

RESULTS AND DISCUSSIONS

In the gestation period there was an increase in saliva weight, physiologically normal (Cuc et al., 2006), which varied between 29.98 kg/head in the sows of the E1 batch, which Turda corn was used in the compound feed, and 32.16 kg/head of batch E2, which barley 21-1G was used in compound feed (Table 3).

This variation can be explained by the fact that the use of barley 21-1G improved the apparent digestibility of crude protein compared to other recipes, these results being obtained in a previous research (Marin et al., 2017).

The amount of compound feed consumed by the sows in the second month of gestation varied on average between 2.12 kg/head/day in the control batch and 2.20 kg/head/day in the experimental batch in which the Tudor pea was introduced (Table 4).

Table 2.Recipes of compound feed for sows used during the experimental period

Specification	Compound feed recipes for pregnant sows				Compound feed recipesfor lactating sows			
	C	E1	E2	E3	C	E1	E2	E3
Maize	50.00	0.00	50.00	40.40	62.00	0.00	62.00	58.00
Turda maize	**0.00**	**50.00**	0.00	0.00	**0.00**	**62.00**	0.00	0.00
Barley	20.00	10.00	0.00	20.20	10.00	8.00	0.00	7.50
Barley 21-1G	0.00	0.00	**20.00**	0.00	0.00	0.00	**10.00**	0.00
Tudor peas	0.00	0.00	0.00	**12.00**	0.00	0.00	0.00	**12.00**
Soya meal	0,00	3.00	0.00	0.00	15.50	17.50	15.50	13.00
Sunflower meal	17.50	14.50	16.50	13.00	0.00	0.00	0.00	0.00
Wheat bran	8.00	18.00	9.00	10.50	3.00	3.00	3.00	0.00
Fish meal	0.00	0.00	0.00	0.00	6.00	6.00	6.00	6.00
L-lysine	0.18	0.14	0.18	0.04	0.18	0.13	0.13	0.09
Carbonate calcium	1.82	1.80	1.82	1.61	0.80	0.80	0.80	0.81
Dicalcium phosphate	1.50	1.52	1.50	1.45	1.52	1.57	1.57	1.81
Vitamino-mineral premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Recipes parameters								
Metabolisable energy (EM kcal/kg)	2944	2883	2945	2913	3078	3062	3080	3076
Crude protein (%)	14.12	14.13	14.11	14.17	17.31	17.34	17.43	17.39
Lysine (%)	0.60	0.62	0.59	0.60	1.01	1.00	1.01	1.01
Methionine+cystine (%)	0.54	0.52	0.53	0.51	0.58	0.58	0.58	0.57
Calcium (%)	1.17	1.17	1.17	1.08	1.13	1.14	1.13	1.12
Phosphorus (%)	0.72	0.78	0.72	0.70	0.79	0.80	0.79	0.81
Brute cellulose (%)	5.87	6.54	6.28	5.86	3.11	3.60	3.34	3.26

Table 3. Variation in body weight of pregnant sows during the experimental period

Specification	Batch			
	C	E1	E2	E3
Initial weight of sows (kg)	428.14 ±17.23	410.23 ±22.57	435.67 ±15.67	409.19 ±19.95
Weight of sows in 4th month of gestation (kg)	458.59 ±22.09	440.21 ±24.74	467.83 ±18.22	437.76 ±14.84
Difference (kg)	30.45	29.98	32.16	28.57

In the third month of gestation sows consumed 2.19 kg/head/day in the 21-1G barley batch in the recipe and 2.22 kg/head/day in the Tudor pea batch. In the fourth month, with the exception of the last 2-3 days of gestation, the compound feed consumption was 2.69 kg/head/day in the 21-1G barley batch in the recipe and 2.80 kg/head/day in the experimental batch in the food to which Tudor peas was used (Table 4).

Table 4. Compound feed consumption of pregnant sows during the experimental period

Specification	Batch			
	C	E1	E2	E3
The average daily consumption of sows in the second month of gestation (kg/head/day)	2.12 ±0.01	2.19 ±0.07	2..18 ±0.07	2.20 ±0.05
The average daily consumption of sows in the third month of gestation (kg/head/day)	2.20 ±0.03	2.21 ±0.05	2..19 ±0.08	2.22 ±0.06
The average daily consumption of sows in the fourth month of gestation (kg/head/day)	2.71 ±0.08	2.75 ±0.06	2..69 ±0.05	2.80 ±0.04

During lactation, sows must receive compound feed to cover the energy and nutrients needed for maintenance and milk secretion (Table 5). As a result, during the period between sowing and weaning (21 days) the sows consumed between 6.35 kg of combined feed/head/day (control batch) and 6.85 kg/head/day in experimental batch E2. The results are similar to those obtained by Sulabo et al. (2007); Kruse et al. (2011), who observed that the sows' compound feed consumption varies according to the number of piglets and body weight of the sows.

The number of piglets per sow ranged between 10.50 in the control batch and 12 in the experimental batch E2. The mortality while giving birth was of 2.50 in the experimental batch 2 and 4.76 in the control batch.

Table 5. Influence of nutrition on the performance of sows in lactation and piglets during the experimental period

Specification	Batch			
	C	E1	E2	E3
Average number of born piglets (heads)	10.50 ±0.09	11.00 ±0.15	12.00 ±0.11	11.50 ±0.07
Average number of alive piglets (heads)	10.00 ±0.07	10.70 ±0.09	11.70 ±0.12	11.10 ±0.04
Average weight of born pigs (kg/head)	1.55 ±0.01	1.42 ±0.02	1.35 ±0.01	1.38 ±0.03
Average weight of weaned pigs (kg/head)	6.18 ±0.02	6.35 ±0.08	6.88 ±0.06	5.99 ±0.08
Average number of weaned piglets (heads)	9.60 ±0.09	10.00 ±0.11	11.20 ±0.14	10.70 ±0.08
Daily average intake of sows in the period from giving birth to weaning (kg/head/day)	6.35 ±0.26	6.45 ±0.17	6.85 ±0.30	6.59 ±0.21

The weight of the piglets was affected by the number of piglets, 1.35 kg/head in the batch E2 and 1.55 kg/head in the control batch.

At the age of 21 days of weaning, the piglets had an average weight of 5.99 kg/head in batch E3 and 6.88 kg/head in batch E2, where the greatest number of piglets were weaned (11.20 heads).

CONCLUSIONS

During the gestation period, the weight of the sows registered an ascending trend, ranging from 29.98 kg/head to 32.16 kg/head, the highest value being obtained by the sows in the experimental batch, which barley 21-1G was used in the compound feed, which improved apparent digestibility of crude protein.

Compound feed consumption of pregnant sows had the lowest value in the third and fourth months of gestation in experimental batch E2.

During the lactation period, the consumption of compound feed of sows varied according to the number of piglets and the body weight of the sows.

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RURAL-URBAN DIFFERENCES IN MEAT CONSUMPTION IN ROMANIA

Elena Narcisa POGURSCHI[1], Melania MUNTEANU[2], Carmen Georgeta NICOLAE[1], Monica Paula MARIN[1], Corina Aurelia ZUGRAVU[3]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Vasile Goldis Western University of Arad, 94 Revolutiei Blvd, Arad, Romania
[3]University of Medicine and Pharmacy Carol Davila, 37 Dionisie Lupu Street, Bucharest, Romania

Corresponding author email: corina.zugravu@umf.ro

Abstract

In the last three decades the perceptions of Romanian consumer's food has evolved quickly in response to socio-economic changes. Because Romania is crossing a transitional phase, both economically and socially, the socio economic status (SES) and the settlement type distribution of population generated particular food consumption patterns. Approximately 45% of Romanian population live in rural areas, most of them being either land owners or growing potential food stuffs around the household. Therefore, the food consumption pattern of the rural population is greatly dependant on the household purchasing power and their own food production capacity. On the other hand, the urban population (approximately 55% of total Romanian population) is strictly dependant on the household purchasing power, which in this case is significantly higher than the rural inhabitants (Gfk, 2016), and on whether they still have relatives living in rural areas and the amount of food they receive from them. The overall aim of this study is to show the factors that may affect consumer's attitude towards quantity and origin of food consumed.

Key words*: consumer preferences, food origin, meat consumption, Romania.*

INTRODUCTION

Agriculture is an important branch of the economy in Romania, providing sufficient amounts of food from both animal and plant origin. In our days consumer's choice of food is a more complex matter than producers expecting (Conte, 2014). Consumers are becoming more demanding about the type of food they buy and consume (Corcoran, 2001; Pogurschi, 2009). Even though the consumer behaviour in a "western" style society has changed in relation to the type of food consumed, it still subscribes, to some extent, to the family income levels and food price paradigm. The two before mention factors play a major role in the type of food consumers choose to buy, although we feel that the new era of education through widespread information access could tip the scale towards a more balanced diet even in the ranks of less fortunate population groups. The food group of meat and meat products remains an important part of our nutritional plans. Meat brings proteins and minerals, especially iron, and offers a wide range of alternatives, some of them low in fat and calories. On the other hand, meat products are more and more popular, but their composition is rarely as nutritious as meat per se.

In Romania, an important part of the population is still rural and food often originates in one`s own household production capacities. In the present study, we focused on finding out if any differences can be spotted between meat and meat products consumption in urban versus rural area and if meat intake is in accordance with food pyramid `s indications.

MATERIALS AND METHODS

The food product consumption data has been compiled following the analysis of a questionnaire conducted on a national representative sample of 1495 Romanians in 2014. The survey has been designed to assess the frequency of food consumption over a one year period by inquiring about the type and portion size of food consumed on a daily basis during a week, by means of a validated food

frequency questionnaire. Anthropometric, demographic and socio-economic data has also been collected in relation to the survey respondents. Fresh meat, fresh meat products and processed meat products, were included. We quantified one portion of meat as having 85 g, as stated by many nutritional boards around the world (http://www.heart.org/What is a serving_UCM-301838_Article.jsp).
Descriptive statistics, correlative and chi square tests and graphics were carried out by SPSS 13.0, and p for statistical significance was considered below 0.05

RESULTS AND DISCUSSIONS

Approximately 45% of Romanian population live in rural areas, most of them being either land owners or growing potential food stuffs around the household. Therefore, the food consumption pattern of the rural population is greatly dependant on the household purchasing power and their own food production capacity. On the other hand, the urban population (approximately 55% of total Romanian population) is strictly dependant on the household purchasing power, which in this case is significantly higher than the rural inhabitants (Gfk, 2016), and on whether they still have relatives living in rural areas and the amount of food they receive from them. In Tables 1 and 2 we have summarised the result of the survey, establishing meat consumption by gender, age, settlement type. According to the survey performed, the average total meat consumption (TMC) of the Romanian population, including fresh meat, fresh meat products and processed meat products is situated at 1.010 Kg/week or 54.2 Kg/year. Average distribution of consumption on age and gender is represented in figure 1.

THE YOUNG ADULT POPULATION GROUP (18-24/25-34)

The results of the meat frequency questionnaire show that the young adult males (18 – 24 years old) from rural areas, register the highest total meat product consumption values (1.324 kg/week) when compared to other groups maybe due to higher caloric requirements and possibly motivated by the nature of the labour they perform.

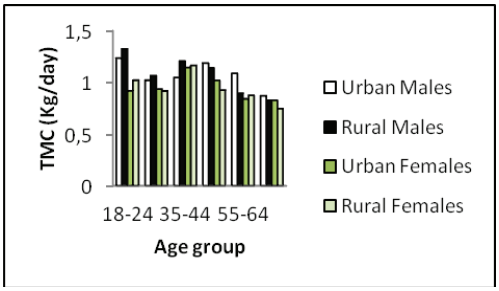

Figure 1.Total meat consumption (TMC) of Romanian males and females from rural and urban areas

The smallest quantity of meat consumption is registered by the females living in the urban areas with values lower than the national average by approximately 10%. Although young adult males (18 – 24) from urban areas consume 20% more meat than the national average this might be an advantage, since at this age males are still growing and have higher protein necessities.

THE MIDDLE AGED POPULATION GROUP

The total meat consumption of the middle aged population group follows the distribution of the national average, exception from this being registered by the male population from rural areas, with a maximum situated at 1.206 Kg/week, and by the female counterparts from the same habitual environment registering the minimum value with 0.926 Kg/week.

THE OLDER PEOPLE POPULATION GROUP

The average total meat consumption values for the older population group drop to figures situated well beneath the national average. Consumption pattern partially motivated by the sedentary lifestyle, possible dentition problems and lower total household purchasing power. The nutritional requirements of elderly adults are different than those of young growing individuals or compared to adults that still engage in high energy consuming activities. However, we do not know anything of the quality of meat consumed and might assume that lower meat consumption might be an advantage at this age, since some cheap and popular meat products are frequently a source of unhealthy fats and cholesterol.

Table 1. Meat and meat products consumption profile of males by age and socioeconomic status

Males $n = 717$		n (%)	Consumption of fresh meat, kg/week (SD)	Consumption of fresh meat products, kg/week (SD)	Consumption of processed meat products, kg/week (SD)
18-24	U	40 (5.57)	0.934 (0.504)	0.195 (0.194)	0.105 (0.077)
	R	45 (6.27)	0.919 (0.479)	0.292 (0.400)	0.113 (0.140)
25-34	U	78 (10.87)	0.779 (0.436)	0.173 (0.222)	0.069 (0.075)
	R	55 (7.67)	0.819 (0.436)	0.179 (0.197)	0.068 (0.066)
35-44	U	79 (11.01)	0.813 (0.467)	0.170 (0.168)	0.065 (0.060)
	R	77 (10.73)	0.989 (0.681)	0.162 (0.182)	0.055 (0.064)
45-54	U	66 (9.20)	0.920 (0.620)	0.199 (0.300)	0.068 (0.066)
	R	43 (5.99)	0.888 (0.501)	0.184 (0.194)	0.069 (0.080)
55-64	U	62 (8.64)	0.850 (1.089)	0.195 (0.330)	0.044 (0.054)
	R	54 (7.53)	0.730 (0.396)	0.130 (0.181)	0.036 (0.045)
65+	U	57 (7.94)	0.716 (0.537)	0.114 (0.176)	0.040 (0.052)
	R	61 (8.50)	0.667 (0.384)	0.129 (0.209)	0.032 (0.046)

Table 2. Meat and meat products consumption profileof females by age and socioeconomic status

Females $n = 778$		n (%)	Consumption of fresh meat, kg/week (SD)	Consumption of fresh meat products, kg/week (SD)	Consumption of processed meat products, kg/week (SD)
18-24	U	52 (6.68)	0.674 (0.422)	0.137 (0.155)	0.108 (0.138)
	R	29 (3.72)	0.796 (0.343)	0.136 (0.215)	0.089 (0.125)
25-34	U	78 (10.02)	0.771 (0.513)	0.120 (0.154)	0.045 (0.055)
	R	51 (6.55)	0.749 (0.598)	0.117 (0.175)	0.051 (0.060)
35-44	U	74 (9.51)	0.909 (0.665)	0.169 (0.238)	0.065 (0.093)
	R	72 (9.25)	0.937 (0.598)	0.156 (0.196)	0.071 (0.125)
45-54	U	66 (8.48)	0.818 (0.480)	0.156 (0.301)	0.046 (0.071)
	R	45 (5.78)	0.754 (0.477)	0.124 (0.166)	0.048 (0.061)
55-64	U	71 (9.12)	0.702 (0.424)	0.097 (0.169)	0.042 (0.059)
	R	60 (7.71)	0.744 (0.428)	0.099 (0.130)	0.032 (0.041)
65+	U	107 (13.75)	0.687 (0.412)	0.109 (0.225)	0.031 (0.044)
	R	73 (9.38)	0.647 (0.472)	0.081 (0.156)	0.017 (0.027)

Meat and meat products consumption did correlate significantly with gender ($P<0.05$), probably due to differences in caloric intake between men and women.

This might come as a negative finding, since women have greater needs of iron, than men and meat is an excellent source of iron. Men eat more meat and meat products in every settlement and every age group.

However, differences in consumption between rural and urban areas were minimal and non-significant.

Men in rural and urban settings eat exactly the same amount of meat per week (fresh meet: 0.835 g), as for women, urban ones eat 0.769 g and rural, 0.771. We expected more meat consumption in urban people, since rural dwellers rely, in theory, mainly on what is produced in the household. Urban/rural differences have been found in other developing or third world countries (Shawel, 2009; Yldirim, 2008).We presume that the absence in differences show an improvement in the economic power in Romanian rural households and it also might be a consequence of subventions offered to farmers, encouraging them to grow more meat animals.

Regarding fresh and processed meat products, again no differences have been found between rural and urban consumers.

With a weekly intake of 0.239 g in rural areas and 0.244 g in urban ones for men, and 0.187 g for urban women and 0.169 g, for rural ones, one can notice that the daily intake of these products is steady, but small.

When converted in daily grams, the average value of meat products consumption is around 30 g, which is the weight of a slice of bologna, salami or a small sausage.

However, the consumption pattern shows that meat products are commonly present in the Romanians daily diets and not an occasional snack. Nutritional value of meat products is generally low, because recipes include always lard or other type of fats, providing too many saturated fats and calories. They also have some preserving ingredients, which are by no means healthy, like high levels of salt and nitrites. The presence of meat products in the daily diet is not a sanogenic habit, but research has shown that consumers know it and limit the intake, in spite of the sensorial attractiveness of this category of foods (Schmid, 2017). In Romania, most of the meat products are industrial ones and only small quantities originate in the household, were they are mainly seasonally produced, like around Christmas. Industrial products have generally a more complex yet unhealthy composition, especially the most popular and cheap ones.

The synchronicity between the indigenous livestock production and the localized meat product industry has suffered greatly during the past 28 transitional years and, at present, the high volume meat product processing industry is heavily relying on frozen raw materials (frozen meat). From a food technology point of view this is translated in higher levels of additives introduced to the recipes in order to better stabilize the frozen raw materials, as opposed to lower levels of the same additives to be added, if refrigerated raw materials are used instead. The consumption of meat products with high levels of additives would also raise consumer health concerns, if consumption frequency and quantity surpasses certain levels. However, Romanians seem to have low level of consumption.

Summing up meat intake both from fresh meat and from products, the average daily consumption is of 0.153 g, for urban men, 0.154 g for rural men, 0.136 g for urban women and 0.134 g, for rural women. Taking into consideration that the Romanian Nutrition Society's indications of healthy eating, a healthy adult has to eat approximately 2-3 portions of meat, eggs and other protein sources (like beans) per day, in order to acquire the due level of nutrients. Meat remains the best source of iron, and when excluded from diets, iron deficiency might be a health threat. Taking into account the above recommend-dations and the fact that one portion of lean meat has around 85 g, we notice that all groups of ages, genders and settlements eat below indications. Women, especially, tend to have far lower intake of meat than ideal, the presumed consequences being serious health problems. It is well known that iron deficiency and anaemia are public health topics for certain population groups and especially for women at fertile ages (Coad, 2011). Since vegetarianism is not a popular trend in Romania, we might infer that lower meat intake has nothing to do with on-purpose avoidance of meat, but probably a consequence of poor purchasing power and of low nutritional knowledge.

LIMITATIONS

Our study is, as far as we know, the most recent one that evaluates directly, by means of a food frequency questionnaire, the meat intake on a representative sample of Romanians. The National Institute of Statistics does similar evaluations, but the method used (Household

Budget Survey) misses frequently products that originate in the household and that do not require special expanses. Our data gives an accurate perspective of meat consumption in all Romanian counties, for rural and urban settlements alike. However, some inaccuracies might be present, since figures have been obtained by interview and not by direct measurements on meat consumption. Food Frequency Questionnaires have, however, been recognised as good tools for food intake evaluation and are used currently in nutrition investigations.

CONCLUSIONS

Our study concluded that there is no significant difference between meat and meat products consumption in rural and urban areas of Romania. However, the level of meat intake is marginally insufficient especially for women, and this might lead to different nutrition problems, among which iron deficiency is the most common. A better nutrition training of interested groups and subventions for meat industry might correct and prevent in time deficiencies arising from insufficient meat consumption.

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POSSIBILITIES OF INCREASING THE FUNCTIONAL ACTIVITY OF THE ROOSTER DEFROSTED GAMETES

Gheorghe BORONCIUC, Ion BALAN, Vladimir BUZAN, Nicolae ROŞCA, Iulia CAZACOV, Melania BUCARCIUC, Ion MEREUŢA, Nicolai FIODOROV

Institute of Physiology and Sanocreatology, 1 Academiei Street, MD 2028, Chişinău, Republic of Moldova, Phone: +373.22.73.96.07,
Email: vladimirbuzan@yahoo.com

Corresponding author email: vladimirbuzan@yahoo.com

Abstract

As a result of experimental researches it has been established that the optimization of parameters at the development of new mediums helps to reduce the cryogenic risks in the preservation of the genetic resources of the rooster. The best results in the restoring of gametes physiological parameters of after cryopreservation in maltose-petumozid-arginine-glycerin medium and defrozen in isotonic sodium citrate solution are achieved at the dilution of semen 1:3, refrigeration at 2-4°C during 15 minutes and freezing for a duration of 2 minutes. The use of such cryobiological parameters allowed us to obtain similar results when using maltose-arginine-glycerol medium as in the control group. At the same time, the additional use of petumozide in the composition of experimental medium increases the mobility of thawed gametes of cock by 11.4%. The effectiveness of the developed medium was tested and in the production conditions. As a result, it was established that the hatchability of chicks reaches 68.8 ± 6.69 against 41.7 ± 7.12%, which is 27.1% higher in favor of the experimental variant. The results of laboratory and industrial experiments allow to recommend developed medium for cryopreservation of semen of the rooster and use in breeding practice.

Key words*: reproductive cells, rooster, cryopreservation.*

INTRODUCTION

The problems of anabiosis and cryopreservation occupy an important place in modern biology of animal reproduction. Their solution allows to develop various technologies for preservation of gametes, including new cryoprotective mediums and technological methods. It is important to note that the method of long-term storage of semen in a deeply frozen state has found wide application in the field of cattle breeding (Наук, 1991). However, the possibilities of this method are far from exhausted, as about half of gametes does not restore functional activity after thawing.

As for other industries such as sheep, pig, poultry and fish farming, this method has not found wide practical application. This is due to the insufficiently of research to identify the specific features of gametes' cryobiology and technological methods of reproduction of the studied species of animals. At the same time, it should be noted that significant progress has been achieved in solving these problems thanks to the fundamental work of a number of researchers (Нарубина, 1998; Сахацкий, 1990; Kopeika et al., 2000).

Advances in the field of cryobiology made it possible to experiment with other species of domestic and wild animals, and at the beginning of the nineties the list of species on which such experiments were based included more than a hundred species of mammals, birds, reptiles, amphibians, fish and invertebrates-echinoderms and molluscs.

For Cryobiology, the main problem is the preservation of biological objects in a viable state in conditions when active vital activity is turned off, the biological metabolism of substances, energy and information are minimized and at the same time the ability to return after defrozen to polyfunctional activity is provided (Грищенкоет al., 2004).

Research of the mechanisms of cryo-damages, the search of optimal cryoprotection conditions at different levels of the biological organization of gametes, besides theoretical, acquires also practical value.

Thus, at present, there are certain successes in solving a number of fundamental and applied

problems of reproduction of animals using cryopreserved material. However, further trends in the development of research in the field of cryopreservation of semen should be directed to a deeper study of the mechanisms of cryopreservation and cryoprotection of reproductive cells; continuation of research to identify the relationship between the chemical structure, physico-chemical properties and toxicity of cryoprotectants; creation of new more effective cryoprotective mediums. Further increase of the efficiency of the method of storage of semen of animals at liquid nitrogen temperature is currently carried out by creating new cryoprotective mediums, using both penetrating and non-penetrating cryoprotectants in the medium, excluding from the mediums of toxic components (Линникет al., 2010).

Increasing the effectiveness of cryopreservation of genetic resources is possible by carrying out fundamental researches with involvement of the modern equipment, mathematical methods for planning experiments and processing of the obtained digital material (Valcarcel et al., 1994). Thus, the solution of the problems of cryopreservation of the genome has a general biological significance and can be achieved on the basis of an interdisciplinary approach.

It should be noted that in the process of cryopreservation of genetic resources, there are a number of physico-chemical processes such as: temperature shock, changes in osmotic pressure, crystallization and recrystallization, phase transitions of proteins and lipids, morphological changes, condensation and decondensation of chromatin, destruction of nucleoprotein complexes. Therefore, the purpose of the research was to find ways to reduce cryogenic damage during preservation of the rooster sperm.

MATERIALS AND METHODS

As an experimental material used the sperm of roosters of the Rhode Island breed contained in the vivarium conditions of the Institute of Physiology and Sanocreatology.

Physiological indicators of gametes were determined according to general accepted methods using the "Ampleval" microscope of the manufacturer Carl Zeiss, at 200-fold magnification. The optimal concentrations of the used medium components were determined by the method of counter series by Милованов (1962). Semen freezing was carried out in liquid nitrogen vapors on the surface of the fluoroplastic plate in the form of open granules with a volume of 0.1-0.2 ml, thawing was carried out in an isotonic solution of sodium citrate at a temperature of 40-42°C.

Statistical processing of digital material was done using the Student's t-test.

RESULTS AND DISCUSSIONS

Having studied the peculiarities of the spermatozoa metabolism of different species of animals in the process of cryopreservation and having tested in laboratory conditions various substances as possible stabilizers of intermolecular interactions and functional activity of gametes at technological processing, there was arose a question about the development of ways to increase the cryoresistance of animals semen and their reproductive ability.

At developing methods for increasing cryoresistance, not only the specific results of our own research were taken into account, but also proceeded from the concept developed by us, in accordance with which the preservation of structural and functional homeostasis at the initial stages of cryopreservation is predetermined by specific protective reactions, and the occurrence of cryo-damages during freezing and thawing, although in many respects it has a specific character, is caused mainly by non-specific changes in the structural-biochemical homeostasis.

To improve the efficiency of cryogenic risks reduction we have developed a new cryoprotective medium for conservation of rooster semen.

Taking into account the influence of carbohydrates and antioxidants on the quality of the stored seed, which suggested the relative regulation of the non-electrolyte composition, the inhibition of lipid peroxidation processes, the intensification of the water vitrification process and maintenance of the charge of plasma membranes in the process of gamete cryoconservation, we performed research on the development of a new medium and the determination of optimal parameters cryopreservation of rooster sperm.

The components of the medium and their content are presented in Table 1.

Table 1.Composition of the medium for cryopreservation of the rooster sperm

Component name	Quantity
Maltose, g	11.5
Petumozide, mg	0.05
Glycerol, ml	7.0
Water, ml	100
Arginine hydrochloride, to pH 6.8-6.9	

Using this medium in subsequent experiments, was determined the effect of the degree of dilution on the quality of the defrozen rooster semen (Table 2) and investigated the optimal parameters of its cooling (Tables 3 and 4).

Table 2. Restoration of gamete mobility in dependence on the degree of dilution of the rooster sperm

Experimental variants	Degree of dilution	Mobility of gametes, point
		M±m
1	1:1	4.3 ± 0.14
2	1:2	4.1 ± 0.21
3	1:3	4.6 ± 0.11
4	1:4	4.4 ± 0.11
5	1:5	4.3 ± 0.14
6	1:6	4.1 ± 0.11*
7	1:7	4.0 ± 0.18*
8	1:8	3.2 ± 0.14*
9	1:9	3.0 ± 0.18*
10	1:10	3.0 ± 0.18*

Note: * Differences are statistically authentic in comparison with the third variant of the experiment

From Table 2 it follows that the best results on restoring the mobility of gametes after their thawing were obtained in the third variant, in case of dilution of the semen in a ratio of 1:3 with the elaborated medium.

The increase of the degree of dilution successively reduces the mobility of the gametes, which can be explained by a change in the buffer capacity in the system of the medium-semen of the cock (Balan, 2012).

The presented data in Table 3 show that the best results in restoring the mobility of gametes after their thawing were obtained in the third variant of the experiment, when the studied indicator reached 5.1±0.27 points.

Reducing or increasing of the duration of cooling does not improve the life longevity of gametes.

The reasons for this may be insufficient cooling time at which the entire volume did not reach 2-4°C or too long a time during which the products of metabolic processes can accumulate or a temperature shock may occur (Ostashko et. al., 2004).

Table 3.Restoration of gamete mobility in dependence on the duration of cooling of the rooster sperm

Experimental variants	Cooling of sperm, min	Mobility of thawed gametes, point
		M±m
1	5	4.1 ± 0.21
2	10	4.8 ± 0.29
3	15	5.1 ± 0.27
4	20	4.9 ± 0.33
5	25	4.5 ± 0.18
6	30	4.1 ± 0.21
7	35	3.8 ± 0.33
8	40	3.4 ± 0.21
9	45	3.2 ± 0.14*
10	60	2.7 ± 0.32*

Note: * Differences are statistically authentic in comparison with the third variant of the experiment

Table 4.Restoration of gamete mobility depending on the duration of freezing of the cock sperm

Experimental variants	Freezing of sperm, min	Mobility of thawed gametes, point
		M±m
1	1.0	2.4 ± 0.21*
2	1.5	3.4 ± 0.11*
3	2.0	5.3 ± 0.28
4	2.5	4.8 ± 0.22
5	3.0	4.4 ± 0.27*
6	4.0	4.0 ± 0.18*

Note: * Differences are statistically authentic in comparison with the third variant of the experiment

The presented digital material allows us to answer that the optimal duration of freezing is 2 minutes. Deviation from this parameter reduces the physiological parameters of thawed gametes, which may be due by crystallization or recrystallization processes (Crister et. al., 2004).

From the data of Tables 2, 3, 4 it follows that the best results on the recovery of thawed sperm motility are achieved by diluting it with ratio of 1:3, cooling at 2°C for 15 minutes and freezing for 2 minutes.

In order to determine the effectiveness of the new medium and the optimal technological parameters, a comparative experiment was conducted, as a control - the Watanabe medium (Table 5).

Table 5. Physiological indicator of rooster gametes cryopreserved in various mediums

Cryoprotectant medium	Mobility of thawed gametes, point
	M±m
1. Watanabe medium (control)	6.1 ± 0.11
2. Maltose-arginine-glycerin	6.3 ± 0.13
3. Maltose-arginine-glycerin-petumozide	6.8 ± 0.14*

Note: * Differences are statistically authentic

The data presented in the table show that the mobility of thawed gametes after cryopreservation using the maltose-arginine-glycerin medium is not significantly different from that in the control variant.

At the same time, the use of petumozide in the experimental medium increases the mobility of the thawed gametes of the cock.

Thus, for example, the mobility of gametes cryopreserved in the control medium reached 6.1 ± 0.11, and in a medium with petumozide – 6.8 ± 0.14 points.

Based on the above results obtained in the laboratory conditions, the experience was performed in production conditions.

The research was conducted using the semen of roosters belonging to the "Chetrosu" breeding center of the Criuleni district of the Republic of Moldova. The results are presented in Table 6.

Table 6. Results of incubation of eggs obtained from hens inseminated with cryopreserved rooster sperm in the form of granules

Indicators of experience	Name of mediums			
	Maltose-arginine-glycerin	Maltose-arginine-glycerin with petumozide	Watanabe medium (control)	Watanabe medium (control) with petumozide
Incubated eggs, pieces	48	48	48	48
Fertilized eggs, pieces	19	34	23	31
Fertilization of eggs, %	39.6 ± 7.06	70.8 ± 6.56*	47.9 ± 7.21	64.6 ± 6.90
Chickens were hatched, pieces	19	33	20	31
Hatchability of chickens, %	39.6 ± 7.06	68.8 ± 6.69*	41.7 ± 7.12	64.6 ± 6.90

Note: * Differences are statistically authentic

From the data of Table 6, it can be seen that the use of maltose-arginine-glycerin medium with petumozide makes it possible to achieve egg fecundity equal to 70.8±6.5%.

In the control variant, the investigated indicator is 23% lower. It should be noted that the introduction of petumozide in the composition of control medium Watanabe also increased the fertilization of eggs. However, the studied indicator was still higher in the case of application of developed by us maltose-arginine-glycerine-petumozide medium. A similar conclusion can be made on the hatchability of chickens.

Thus, the results of laboratory and production experiments allow us to recommend the medium which we developed for cryopreservation of the rooster sperm and use it in breeding practice.

CONCLUSIONS

The researches allow making the following conclusions:

1. Optimization of technological parameters at the development of new mediums allows reduce cryogenic damage in the process of preserving the genetic resources of the rooster.

2. The composition of synthetic mediums determines the degree of preservation of the structural and functional state, and the technological parameters contribute to strengthening of their cryoprotective properties at the conservation of rooster sperm.
3. The best results in restoring of the physiological indicators of gametes after their cryopreservation in maltose-arginine-glycerin-petumozide medium and deconservation in isotonic solution of sodium citrate are achieved at the dilution of semen 1:3, cooling at 2-4°C for 15 minutes and freezing for a duration of 2 minutes.

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EFFICIENCY OF BEE QUEEN REARING DEPENDING ON ORGANIZATION WAY OF THE NEST IN CELL STARTER COLONIES

Valentina CEBOTARI, I. BUZU, Olga POSTOLACHI, N. GRANCIUC

Institute of Zoology of Academy of Sciences of Moldova (ASM)
1, Academiei str., MD 2028, Chișinău, Republic of Moldova

Corresponding author email: valentinaceb@yahoo.com

Abstract

The purpose of this research was to reveal the effective methods of directed (artificial) queens rearing depending on organization way of the nest in cell starter colonies. The study object were the honeybee colonies from the experimental apiary of the Institute of Zoology of the Academy of Sciences of Moldova. To reveal the efficient methods of organizing the cell starter colonies nest for the queens rearing, a special experiment was carried out with 4 similar batches of bee families, with 5 families in each batch, which were different by way of nest structure. The bee families of the I batch served as a control and their nest was organized according to the standard method: in the queenless nest, populated in abundance by bees, 2 food combs (honey+bee bread) and the queen-cells bar frame with 55-65 grafted larvae at the age of 1 day, depending on the rearing series. In addition to the food combs and the queen-cells bar frame, 2 brood combs of all ages in the starter colonies of the the batch II were introduced, 2 sealed brood combs were introduces in the starter colonies of batch III and in the starter families of batch IV - 2 unsealed brood combs were added. The analysis of the research results allowed us to conclude that the presence of the brood in the combs introduced into the cell starter families has a beneficial effect on the final efficiency on the directed queens rearing. The introduction into the cell starter colonies of brood combs of all ages contributes to the obvious increase of the larval acceptance level of 2.5 times (t_d=25.5; P<0.001) and of the yield of obtained queens by 2.9 times (t_d=28.6; P<0.001), compared to the colonies in the control batch, where no brood combs were introduced. The introduction into the cell starter colonies of unsealed brood combs contributes to the obvious increase of the larval acceptance level of 2.3 times (t_d=20.7; P<0.001) and of the yield of obtained queens by 2.7 times (t_d=30.6; P<0.001), compared to the colonies in the control batch, where no brood combs were introduced. The introduction into the cell starter colonies only of the sealed brood combs contributes to no significant growth of the larval acceptance level by 4.3% (t_d=1.8; P<0.1) and of the yield of obtained queens by 5,3% (t_d=3.0; P<0.01), compared to the colonies in the control batch, where no brood combs were introduced.

Key words*: rearing, queens, cell starter colonies, combsi, brood, larval acceptance, yield.*

INTRODUCTION

Over the centuries, beekeepers, both amateur and professional ones, have been constantly concerned with the problem of directed queen rearing, both for setting up new families and for replacing of old queens. In 1565 Jacob Nickel was the first in Europe to describe how honey bees can raise queens from worker eggs or very young larvae (Fert, 2011; Büchler et al., 2013). Based on this postulate, beekeepers have further developed a number of methods and proceedings for the directed rearing of queen bees. Already in 1853, the great beekeeper Lorraine Langstroth (quoted by Fert G., 2011) has developed the method of replacing the queen through the process of transferring a natural queen cell to an queenless swarm. In 1883 beekeeper Henry Alley et al. (quoted by Fert G., 2011) has invented the technique for initiating the directed queens rearing using the "closed starter" method, which is used even today. The development of modern queen rearing techniques started in the 19th Century. Gilbert Doolittle (1889) in the USA developed the technique of grafting one-day larvae to start the rearing of future queens. This technique is used until present under the name of the method that bears his name (Fert, 2011; Büchler et al., 2013).

In the scientific literature of the last decades a wide range of biological and technical issues have been approached, regarding the directed (artificial) rearing of *Apis mellifera* queens (Nuru et al., 1999; Kruk et al., 2002; Genc et al., 2005; Kaftanoglu et al., 2011; Büchler et al., 2013; Wubie, 2014; Alber, 2015; Pătruică et al., 2016; Parvu et al., 2017).

A remarkable contribution to the science of artificial rearing of bee queens was brought by renowned german researchers Ruttner H. (1980) and Ruttner F. (1980), who developed the theory and practice in this field. Along with these researchers may be noted and their contemporary Weiss K. (1980), who described in detail the influence of growth factors on the development of queens.

Among the many methods known in the field, as a standard method for enterprises specialized in the queens rearing, it has become the method of grafting one day larvae and their growth from the beginning, during 24 hours in queenless cell starter colony, with subsequent passage of queen-cells bar frame in complete and quite powerful cell raising colony ("cell finishers") (Siceanu, 2012). The success of larvae acceptance, the efficiency of using cell starter and raising colonies ("cell finishers") depends on a number of important technological factors, including the way of brood comb organization is very important. In the standard technology of organizing the cell starter colony, honeycombs with bee bread and honey, before to be introduced into the empty hive body, are pre-stored in the apiary storehouse, so that any potentially competing larvae or eggs may die, so there is no possibility that bees will grow random queen cells. All the bees, left after removing the swarm with the queen, are shaken over this beehive structure, being left instead of the initial family. In this case, the amount of bee in the cell starter family should be as high as possible, at least 0.5 kg on comb. Ensuring such an abundant amount of bee in the starter family, where the brood is totally absent, is a matter not so much technological as biological.

Famous researcher Hans Ruttner (1980) has mentioned: „It would seem that all the royal jelly is given only to the larvae of the queen-cells,and the larvae of workers (which dosn't exist) don't receive it, but it is not so. Production of royal jelly to a colony with unsealed brood decreases. Thus, the first influx of royal jelly to wild queen-cells is lost". For these reasons, the author recommends Bessonet's proceeding, which consists of the following: a Langstroth hive body whose bottom is made of jute mesh is provided with enough bees, from a very strong colony, and with honeycombs and pollen. In this space two unsealed brood combs, are introduced "because it is more natural" (after Bessonet, quoted by Ruttner H., 1980). This corresponds to Laidlaw's recommendation (quoted by Ruttner H, 1980), that on one side of the growing frame there is a unsealed brood comb and on the other side a pollen comb. After 5 hours of formation of this unit, a frame with 28 wet grafted queen-cells is placed in two free beeways between combs. After 24 hours the queen-cells bar frame with the accepted larvae is removed and introduced into the cell raising colonies ("cell finishers"). Each cell starter colony must have at their disposal two cell raising colonies.

In the same paper, the author (H. Ruttner, 1980) describes another proceeding for the growth into the cell starter colonies of grafted larvae, using the Laidlaw-Eckert method, wich involves the use of 1 box (body) and 5 frames, that has a large ventilation space, but without an entrance. In this body are put 3 unsealed honey combs, pollen and some water. Two beeways between combs remain free for queen-cells bar growth frames. In this brood box starter, 2-3 kg of nurce bees are brushed from the brood comb. First, the box, without queen cells and with liquid food, is kept for 3-5 hours in coolness and in the dark. Some beekeepers are putting an unsealed brood combs in this two beeways during repose, which then are removed out.

The larvae are grafted in 60-90 artificial queen cells, the queen-cells bar frames are introduced and left for 24 hours, after that are transfered in the "cell finishers" colonies.

Thus, from the bibliographic analysis of the organization methods of the cell starter colonies, for the growth of the artificial queen cells with grafted larvae, we have found that in some cases in the starter nest, in addition to the queen-cells bar frame, combs with unsealed brood are introduced, whereas in the standard technology of the starter family nest formation, introducing of the brood combs is not provided, according to the Zootechnical norme regarding breeding of bee families, the growth and certification of genitor beekeeping material, approved by Government Decision no. 306 of 28.04.2011.

We can suppose that both, the above-mentioned methods and the standard organization

technology of the starter families includes both, positive aspects (advantages) and negative aspects (disadvantages).
In this context, the purpose of this research was to reveal the effective methods of directed (artificial) queens rearing depending on organisation way of the nest in cell starter colonies.

MATERIALS AND METHODS

The study object were the honeybee families from the experimental apiary of the Institute of Zoology of the Academy of Sciences of Moldova.

To reveal the efficient methods of organizing the cell starter colonies nest for the queens rearing, a special experiment was carried out with 4 similar batches of bee families, with 5 families in each batch, which were different by way of nest structure, according to the following scheme (Table 1).
The bee families of the first batch served as a control and their nest was organized according to the method elaborated by us (Cebotari V. et al., 2010) for the Zootechnical norme regarding breeding of bee families, the growth and certification of genitor beekeeping material, approved by GD no. 306 of 28.04.2011.

Table 1. Scheme of the nest organization of cell starter colonies for the rearing of queens

Batch number	Nr. of colonies (N)	The order of organization of the combs introduced into the cell starter colonies
Batch I (control)	5	1 food comb (honey+bee bread), queen-cells bar frame, 1 food comb
Batch II	5	1 food comb, 1 brood comb of all ages, queen-cells bar frame, 1 brood comb of all ages, 1 food comb
Batch III	5	1 food comb, 1 sealed brood comb, queen-cells bar frame, 1 sealed brood comb, 1 food comb
Batch IV	5	1 food comb, 1 unsealed brood comb, queen-cells bar frame, 1 unsealed brood comb, 1 food comb

For the organization of the cell starter colonies, the healthy, strong families and with a lot of young bees has been chosen. From these, the queens (queenless) with 1-2 brood combs and bees were removed to make another swarm. The bees of the rest of the combs, from each separate family, were shaken in separated bodies of the hives.
At least after3 hours, 2 food combs (honey+bee bread) and 1 queen-cells bar frame, with grafted larvae were introduced in these hives. The amount of bee per beeway frame (including the queencells bar frame) for each family was at least double and was for Dadant frame not less than 500 g. Thus, in the starter families of the batch I - control were no combs with brood.
Unlike the control group, in the cell starter colonies of the II, III and IV batches, 2 brood combs, different by age, were introduced. Thus, in the starter families of batch II were introduced 2 brood combs of all ages, in the batch III there were introduced 2 sealed brood combs and in the starter families of batch IV 2 unsealed brood combs were introduced. 3 series of grafted larvae per cell starter family were initiated for growing.
The larvae growth time in queen-cells bar in the starter family was 24 hours and the number of accepted larvae and the acceptance rate (%) were then appreciated.
After that the queen-cells bar were transfered in the "cell finishers" colony formed in similar batches, according to the above-mentioned (GD RM no. 306 of 28.04.2011).
In each starter family in all experimental groups, in each rearing series the same number of one-day larvae was grafted.
In the first series of rearing (on 24.04.2017) a queen-cells bar frame with 60 grafted larvae was introduced, in the second series (on 04.05.2017) on queen-cells bar frame were grafted 65 larvae, in the third series (14.05.2017) - 55 larvae were grafted on the queen-cells bar frame.
After the queens emerged in the cell raising colonies ("cell finishers"), the final result was appreciated - the number and percentage (yield) of queens obtained from the grafted larvae in each cell starter family and average on the starter families in each experimental batch.
The obtained in experience data were statistically processed using computer software "STATISTICA - 12" and evaluated their

certainty, according to variation biometric statistics, by methods of Plohinskiy (1989).

RESULTS AND DISCUSSIONS

The research results have shown that the efficiency of queen rearing is determined, in whole, by the way of organization of the combs in the nest of the cell starter colony. It is obvious that the rate of acceptance of the grafted larvae into artificial queen cells from the queen-cells bar frame is primarily influenced by the organization technology, of specificity and type of combs introduced into the starter family (Table 2).

Table 2. Results of acceptance of grafted larvae into artificial queen cells and introduced into starter families, in profile on experimental batches

Experimental batches wih starter colonies	Number of larvae			Acceptance difference versus control,%	The criterion of certainty of difference (t_d)
	grafted	accepted	% acceptance		
Series I (24.04 – 03.05)					
Batch I (control)	60 ± 0.0	21.0 ± 1.0	35.0 ± 1.7	-	-
Batch II	60 ± 0.0	54.2 ± 0.7	90.3 ± 1.1	+55.3	27.3***
Batch III	60 ± 0.0	24.8 ± 1.7	41.3 ± 2.8	+6.3	1.9*
Batch IV	60 ± 0.0	50.2 ± 0.6	83.7 ± 1.0	+48.7	24.7***
Series II (04.05 – 13.05)					
Batch I (control)	65 ± 0.0	27.8 ± 1.1	42.8 ± 1.8	-	-
Batch II	65 ± 0.0	59.4 ± 0.7	91.4 ± 1.0	+48.6	23.6***
Batch III	65 ± 0.0	27.0 ± 0.9	41.5 ± 1.5	-1.3	0.5
Batch IV	65 ± 0.0	55.0 ± 1.6	84.6 ± 2.4	+41.8	13.9***
Series III (14.05 – 23.05)					
Batch I (control)	55 ± 0.0	22.2 ± 1.6	40.4 ± 2.8	-	-
Batch II	55 ± 0.0	51.6 ± 0.7	93.8 ± 1.6	+53.4	16.6***
Batch III	55 ± 0.0	22.0 ± 0.7	40.0 ± 1.3	-0.4	0.1
Batch IV	55 ± 0.0	44.4 ± 1.2	80.7 ± 2.1	+40.3	11.5***
Total in all series					
Batch I (control)	180 ± 0.0	66.0 ± 3.7	36.7 ± 2.1	-	-
Batch II	180 ± 0.0	165.2 ± 0.8	91.8 ± 0.5	+55.1	25.5***
Batch III	180 ± 0.0	73.8 ± 2.0	41.0 ± 1.1	+4.3	1.8*
Batch IV	180 ± 0.0	149.6 ± 1.5	83.1 ± 0.8	+46.4	20.7***

*Remark: * - P < 0.1; *** - P < 0.001.*

The obtained data denotes the fact that, in all growth series, the highest larval acceptance rate was recorded in experimental batches II and IV, where, in addition to the two food combs (honey+bee bread) and the queen cell bar frame there were added 2 brood combs of all ages inclusiv with unsealed larvae. At the same time, in all growth series, the lowest rate of larval acceptance was recorded in the I and III groups, in which besides the 2 food combs (honey + bee bread) and the queen-cells bar frame, no brood combs were introduced or only sealed brood comb have been added.

Thus, in the first series of rearing of grafted larvae into the starter families of batch II, in which two brood combs of all ages were introduced, of the 60 larvae introduced into each family, several larvae were accepted, comparative with families in the control group, averaging 33.2 larvae. In this case, the rate of larval acceptance was biger than 2.6 times (t_d = 27.3; P<0.001). Also in the starter families of batc IV, where 2 unsealed brood combs were introduced, the larvae acceptance rate was higher compared to that of the control group families, 2.4 times (t_d = 24.7; P<0.001). At the same time, in the starter families of batch III, in which in addition 2 sealed brood combs were introduced, the acceptance rate of the larvae tended to be higher compared to the control group by 6.3% (t_d=1.9; P<0.1).

In the second and third series the same regularity of the larvae acceptance in the starter bee families of different batches was recorded. Thus, the starter families of batch II, in which 2 brood combs of all ages were introduced, both in the second and third series, had a higher larval acceptance rate, compared to the control

group of 2.1 and 2.3 times, respectively (t_d=23.6 and 16.6; P<0.001). In starter colonies of batch IV, in which 2 unsealed brood combs were introduced, the larvae acceptance rate was higher than in the families of the control group, both in the second series of rearing, and in the third, 1.98 and 1.99 times, respectively (t_d=13.9 and 11.5; P<0.001).

In total, on all rearing series, the larval acceptance rate in the starter families of batch II, in which 2 brood combs of all ages were additionally introduced, was quite high, averaging 91.8±0.5% being higher than in the control group families (36.7±2.1%), by 2.5 times (t_d=25.5; P<0.001). In bee families of batch IV, where 2 unsealed brood combs were added, the larval acceptance rate was also high, on average on all growth series 83.1±0.8%, being higher than in the control batch on 2.3 times (t_d=20,7; P<0,001). At the same time, in the starter families of batch III, where 2 sealed brood combs were added, the larval acceptance rate had on average on all the rearing series only a tendency to be higher compared to the control group with 4.3% (td = 1.8; P<0.1).

Thus, generalizing data on larval acceptance rate in cell starter families, depending on the tipe of combs organization in the nest, we can conclude that the introduction of the brood combs of all ages, as well as of the unsealed brood combs, leads to a significant increase of the larval acceptance rate, respectively to the increase of the efficiency of using the cell starter colonies. After us, the presence of all age brood, especially of the unsealed one, causes an instinctive attraction of young bees in the starter's nest, being activated by the brood care instinct of the nursing bees. In the absence of the brood, a big number of bees leave the starter nest. According to H. Ruttner (1980), the existence of brood in the starter colony, contributes to the increase of royal jelly amount, produced by nurses. According to Билаш Г. (1991), "the less unsealed brood have the family , the sooner it begins to build queen cells randomly, after the queen has been emerged the sooner it begins to build queen cells randomly, after the queen has been emerged". This author affirm that "beyond any doubt, the presence of the brood in the starter family, positively influences their food supply. The brood from the starter colony combats the phenomenon of bee desertion and maintains the nest's integrity. The nurse bees are concentrated on unsealed brood combs, that's why these combs are placed next to the queen-cells bar frame, so that the nursing bees can feed the queen larvae in abundance. In addition, the optimal air temperature can be maintained only in the presence of the brood in the bee nest". The last sentence of the quoted text seems to clarify the role of the sealed brood in favor of the larval acceptance rate in the cell starter colony. If it is clear that the unsealed brood stimulates the production of the royal jelly by the nurses, then it is concludent that the sealed brood attracts the bees to keep the optimum temperature in the nest. This explains why the highest rate of larval acceptance in our experiment was recorded in the starter colonies with brood combs of all ages. Although, the role of sealed brood in the starter family is lower compared to unsealed brood, yet this (influence) is often significant.

Thus, in the first series of rearing, the acceptance rate of the grafted larvae into the starter families of batch III, in which were introduced sealed brood combs, was higher compared to that of the control batches families by 6.3% (t_d=1.9; P<0.1). On average on all rearing series, the larvae acceptance rate in the starter families of group III, in which sealed brood combs were introduced, was higher, compared to that in the control group families by 4.3% (t_d=1.8; P<0.1).

The method of the combs organizing in the nest of the starter colony obviously influences the efficiency of queen rearing, expressed in the final results - the percentage (yield) of the raised queens from the total number of grafted and introduced for growth larvae in the starter families (Table 3).

The results demonstrates that in all rearing series the highest yield of emerged queens were recorded in the cell starter families of batches II and IV, in which 2 brood combs were added and the lowest yield was recorded in the starter families of batches I (control) and III, where no brood combs were introduced or, respectively, only sealed brood combs were introduced. We have found out that in the first series of queen rearing (25.04-03.05) the yield of obtained queens from the bee families of batch II, in which 2 brood combs of all ages were added,

was higher, compared to the one in the families of the control group, in which no brood combs were introduced, by 3.05 times (t_d=19.2; P<0.001). Also the same regularity was recorded in the second and third series of rearing.

Table 3. Final results of queen rearing from grafted larvae, introduced into the starter colonies, in profile on the experimental batches

Experimental batches wih starter colonies	The amount of larvae introduced into the starter family	Number of emerged queens	The yield of obtained queens, %	Difference of obtained queens from the control, %	The criterion of certainty of difference, (t_d)
Series I (25.04 – 03.05)					
Batch I (control)	60 ± 0.0	15.6 ± 0.9	26.0 ± 1.4	-	-
Batch II	60 ± 0.0	47.6 ± 1.4	79.3 ± 2.4	53.3	19.2***
Batch III	60 ± 0.0	20.0 ± 1.7	33.3 ± 2.8	7.3	2.3*
Batch IV	60 ± 0.0	47.2 ± 0.9	78.7 ± 1.4	52.7	26.6***
Series II (04.05 – 13.05)					
Batch I (control)	65 ± 0.0	21.0 ± 1.8	32.3 ± 2.7	-	-
Batch II	65 ± 0.0	55.4 ± 0.7	85.2 ± 1.0	52.9	18.3***
Batch III	65 ± 0.0	22.6 ± 0.7	34.8 ± 1.1	2.5	0.8
Batch IV	65 ± 0.0	51.8 ± 1.9	79.7 ± 2.1	47.4	13.8***
Series III (14.05 – 23.05)					
Batch I (control)	55 ± 0.0	15.2 ± 1.0	27.6 ± 1.8	-	-
Batch II	55 ± 0.0	47.6 ± 0.9	86.5 ± 1.6	58.9	24.4***
Batch III	55 ± 0.0	18.8 ± 0.7	34.2 ± 1.3	6.6	3.0**
Batch IV	55 ± 0.0	42.2 ± 1.1	76.7 ± 2.1	49.1	17.8***
Total in all series					
Batch I (control)	180 ± 0.0	51.8 ± 2.1	28.8 ± 1.2	-	-
Batch II	180 ± 0.0	150.6 ± 2.7	83.7 ± 1.5	54.9	28.6***
Batch III	180 ± 0.0	61.4 ± 2.4	34.1 ± 1.3	5.3	3.0**
Batch IV	180 ± 0.0	141.2 ± 1.9	78.4 ± 1.1	49.6	30.6***

*Remark: * - P < 0.05; ** - P < 0.01; *** - P < 0.001.*

The yield of obtained queens in batch II, in which 2 brood combs of all ages were introduced, was higher than in the families of the control group, where no brood combs were introduced, on 2.64 and 3.13 times, respectively (t_d=18.3 and 24.4; P<0.001).

High efficiency, with an increased degree of significance according to the Student criterion, of the final results of the queens rearing, was also noted in the bee families of batch IV. Thus, in the first series of rearing, the yield of obtained queens in bee colonies of batch IV, in which two unsealed brood combs were added, was higher compared to that in the families of the control group, in which no brood combs were introduced, by 3.03 times (t_d=26.6; P<0.001). Also the same regularity was manifested in the second and third series of growth. The yield of queens rearing in the starter colonies of batch IV, in which two unsealed brood combs were added, was higher than in the families of the control group, in which no brood combs was added, of 2.47 and 2.78 times, respectively (t_d=13.8 and 17.8; P<0.001).

A much lesser tendency to increase the efficiency of rearing of queens, depending on the way of combs organizatin in starter families, also was recorded in the families of batch III. Thus, in the first (24.04-03.05) and third (15.05-23.05) rearing series, the yield of obtained queens from the starter colonies of batch III, in which additionaly 2 combs of brood were added, was significantly more higher than in the families of the control group, where no brood were introduced, with 7.3% and 6.6%, respectively (t_d=2.3 and 3.0; P<0.05 and P<0.01).

On average, on all rearing series, the yield of obtained queens in starter families of batch II, in which two brood combs of all ages were introduced, was higher than in the families of

the control group, in which there were no brood combs introduced, by 2.91 (t_d=28.6; P<0.001). The yield of queen rearing in the starter colonies of batch IV, in which two unsealed brood combs were added, was higher than in the families of the control group where no brood combs were introduced, on average by 2.72 times (t_d=30.6; P<0.001). Similarly, in the bee families of batch III, in which 2 brood combs were introduced in addition, the queen rearing yield was significantly higher than in the families of the control group, in which they were not introduced combs with brood, by 5.3% (t_d= 3.0; P<0.01).

Finally, generalizing the results of experimental testing of different ways of organization of the combs in the nest of cell starter colonies, we can say that adding of the combs with brood in starter families has a beneficial effect on the ultimate efficiency of directed (artificial) queens rearing.

CONCLUSIONS

The presence of the brood in the combs introduced into the cell starter families has a beneficial effect on the final efficiency on the directed queens rearing.

The introduction into the cell starter colonies of brood combs of all ages contributes to the obvious increase of the larval acceptance level of 2.5 times (t_d=25.5; P<0.001) and of the yield of obtained queens by 2.9 times (t_d=28.6; P<0.001), compared to the colonies in the control batch, where no brood combs were introduced.

The introduction into the cell starter colonies of unsealed brood combs contributes to the obvious increase of the larval acceptance level of 2.3 times (t_d=20.7; P<0.001) and of the yield of obtained queens by 2.7 times (t_d=30.6; P<0.001), compared to the colonies in the control batch, where no brood combs were introduced.

The introduction into the cell starter colonies only of the sealed brood combs contributes to no significant growth of the larval acceptance level by 4.3% (t_d=1.8; P<0.1) and of the yield of obtained queens by 5.3% (t_d=3.0; P<0.01), compared to the colonies in the control batch, where no brood combs were introduced.

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RADIOISOTOPE STUDY OF SKELETAL AND CARDIAC MUSCLE METABOLISM AND IONIC FLUXES IN RATS WITH EXPERIMENTAL HYPERTHYROIDISM. THE IMPACT OF GEROVITAL H3

Cristian Romeo REVNIC[1], Flory REVNIC[2], Cosmin SONEA[3], Silviu VOINEA[4]

[1]Pierre et Marie Curie University, Paris VI, France
[3]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]"Ana Aslan" National Institute of Gerontology and Geriatrics, Bucharest, Romania
[4]Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

Corresponding author email: f_revnic@yahoo.com

Abstract

The aim of this study was to see the effect of Thyroxine administrated in excess upon striated and cardiac muscle metabolism as well as upon $^{45}CaCl_2$ transport in aging rats. Another objective was related with investigation of Gerovital H3 effect upon ^{3}H Tryptophane uptake. Our studies have been pointed out that Thyroxine administrated in excess has an influence upon proteic metabolism and membrane permeability. Our data regarding the effect of in vitro incubation with Gerovital H3 upon ^{3}H Tryptophane uptake. The rats treated with different doses of Thyroxine may have a clinical significance regarding Gerovital H3 therapy in the case of patients with hyperthyroidism, conducting to an improvement in metabolic function of cardiac muscle.

Key words*: Gerovital H3, cardiac muscle, striated muscle, hyperthyroidisnm.*

INTRODUCTION

It is well known that thyroid hormones modify the muscle metabolism and ionic content of muscle fibre as well as blood supply of skeletal and cardiac muscle. Literature data (Davis, 1993) pointed out that thyroid hormones administrated in excess have a direct cardiostimulatory action by increasing cAMP (cyclic adenosine monophosphate) content in myocardium with a positive effect on Ca^{++} dependent ATP-ase in myocyte`s sarcolemma (Agostini et al., 1987). On the other hand, thyroid hormones have the ability of increasing the number of beta adrenergic receptors by intensifying the direct action of cathecolamines upon myocardium and to stimulate adenylat cyclase action, which can explain the increase in myocardial sensitivity to catecholamines (Nwoye, 1981). Thyroid hormones do not affect directly cAMP concentration, but they may modulate the target cell response to stimulatory and inhibitory of cAMP, respectively catecholamine, glucagon and parathormon favouring the action upon adrenergic receptors.

The molecular mechanism implicated was related with a change in sensitivity of beta adrenergic receptor unit, but this fact has not been completely confirmed (Celsing, 1986). Even, the answer to beta adrenergic agents is increased in hyperthyroidism and decrease in hypothyroidism.

It is possible that thyroid hormones to produce a change in regulatory unit of beta adrenergic receptor, respectively of membrane proteins (Gs stimulatory, Gi inhibitory). The excess of thyroid hormones intensify the lipolysis by a modulatory effect of intracellular answer of cAMP, on G regulatory protein action, resulting in sensitization of adipose tissue to catecholamine, growth hormone and glucagon.

At the level of striated muscle, the excess of thyroid hormones induce changes in muscle fibres because of the liberation of lysosomal enzymes, resulting in in muscle atrophy and an increased catabolism of muscle proteins (Koreney, 1981).

Our previous studies (Revnic, 1990) pointed out that during ageing, there is a progressive decline in the active shortening capacity of sarcomere from papillary muscle of left

ventricle from white Wistar rats. This fact may be the result of a diminished protein synhesis at cellular level and of a reduction in the ability of myosin ATP-ase to liberate Pi due to a decrase in total number of SH groups at the level of enzyme situs, due to a progresive decline in thyroxine secretion.

The aim of study was related with evaluation of thyroxine treatment in different dose on skeletal and cardiac muscle metabolism and on $^{45}Ca^{++}$ transport, as well as evaluation of Gerovital H3 effect on 3H tryptophane uptake in the rat heart.

MATERIALS AND METHODS

Thyroxine treatment was performed using the following dosage: 0.25 μm/kg body weight, 0.50 μm/kg body weight, and 1.0 μm/kg b.wt. for 12 days according to the published method (Revnic et al., 1990). Our study was performed on 28 white Wistar rats aged 27 month old divided into 4 groups: 7 rats in control group, 7 rats treated with 25 μm thyroxine/kg b.wt. (experimental group 1), 7 rats treated with 0.50 μm thyroxine/kg b.wt. (experimental group 2) and 7 rats treated with 1 μm thyroxine/kg b.wt. (experimental group 3). The animals were weighted before and after finishing the tretment.

The animals were sacrificed by cervical dislocation, the heart and fragments from Sartorius muscle have been removed and washed in in physiological salt solution on ice bath, the heart has been weighted then placed again on ice bath in physiological salt solution.

Experimental protocol:

For radioisotope experiments of 3H Tryptophane incorporation and for investigation of $^{45}CaCl_2$ transport, were used left ventricle and Sartorius muscle tissue fragments between 50-100 mg each.

After weighting, tissue fragments were reintroduced in Hanks (1 ml for each test tube) and set for preincubation for ½ hour at 37°C.

In each test tube were placed 10 μl 3H Tryptofane from 500 mCi/ml solution, the specific activity was 26 mCi/mg. Incubation of biological samples with radioisotopes was done for 1½ hour at 37°C.The next step was concerned with extraction with HCl 2N. Biological samples were removed from the incubation solution and placed in test tubes containing 1 ml HCl 2N each. The samples were kept in extraction solution for 24 hours. The next day, biological samples were processed for radioactive uptake measurement in incubation and extraction solution. Radioactivity has been assessed in vials with scintillation liquid and incubation solution and extraction solution, respectively by means of Beta Berthold Scintillation Counter with 3 channels for 3H Tryptofane.

The same procedure was used also for the uptake experiments of $^{45}CaCl_2$.

A set of biological samples for each radioisotope was prepared using Gerovital H3, 10 mg/ml in incubation medium.

RESULTS AND DISCUSSIONS

A significant increase in ATP-ase activity in treated rats with Throxine ($P<0.05$) for concentration of 0.5 mM and 1mM and very significant ($P<0.001$) for concentration of 2mM ATP were observed (Table 1).

The experimental data pointed out an increase in 3H Tryptophane uptake in treated rats versus control group (Table 2). The optimum dose for the highest increase in 3H Tryptophane uptake was 0.25 μg/ml thyroxine. 3H Tryptophane uptake is inversely proportional with Thyroxine concentration used (Table 3).

It can be concluded that 0.25 μg Thyroxine has the highest stimulatory effect upon protein synthesis.

It can be observed the stimulation effect of GH3 of protein synthesis in rat heart treated with different concentrations of Thyroxine (Table 4).

A significant increase in quantity of myosin SH groups in treated rats with Thyroxine was recorded in rat heart (Table 5).

Literature data (Florini, 1985) pointed out that protein metabolism is stimulated by thyroide hormones, stimulation of protein synthesis being partialy in relation with calorigenic effect and with an increase in synthesis of other enzymes which may explain these effects.

The effects of thyroid hormones upon protein metabolism depends on metabolic state of the organism and on the administated dosage at thyroidectomised rats, and that higher concentrations of hormone administrated in rats

inhibit protein synthesis and increase the aminoacid concentration in plasma, liver and muscle.

It can be observed a reduction in the uptake of 3H Tryptophane in treated rats with different concentrations of Thyroxine in comparison with control group (Table 6) which are in accordance with literature data (Florini, 1985) who pointed out that thyroid hormones administrated in excess have an inhibitory effect on protein synthesis.

A characteristic discovery is that florid hyperthyroid condition incudes a significant increase in final dyastolic volume of left ventricle and an increase in heart index and in beat volume. As it was expected, thyroxine treatment resulted in heart hypertrophy (50% increase in HW/BW - heart weight/body weight).

Another objective of this study was related with investigation of changes in $^{45}Ca^{++}$ transport in rat heart treated with different concentrations of thyroxine (Table 7). It is well known that Ca^{++} together with other ions are implicated in regulation of neuromuscular excitability and in transmision of nerve influx, for triggering muscle contraction (skeletal, heart and smooth muscle).

Hyperthyroid condition is associated with abnormal metabolism of Ca^{++} (i.e. hypercalcemia and hyperphosphatemia).

The reduction of $^{45}Ca^{++}$ uptake is inversely proportional with Thyroxine concentration used for treatment; this can be correlated with the fact that the experimental hyperthyroidism induced with thyroxine in excess leads to an increase of $^{45}Ca^{++}$ uptake in rat heart in such a way that all sites for $^{45}Ca^{++}$ are occupied by the existent Ca^{++} from heart tissue.

Table 1. The values of body and heart weight in controls and thyroxin treated rats

Age (months)	No	Thyroid status	BW (g)	RV (mg)	RV/BW (mg/g)	LV (mg)	LV/BW (mg/g)
27	7	Control	52.1±20*	62±3	0.120±0.08*	274±0.2	0.158±0.04**
27	21	Treated	479±20*	94±15	0.18±0.03	323±5*	0.687±0.08

BW = body weight, RV = right ventricle, LV = left ventricle
*p<0.01 Control/Treated (BW)
**p<0.001 Control/Treated (RV/BW)

Table 2. Activity of myosin ATPase of Pi release in relation with age and Thyroxin treatment (mM Pi/100mg) in 27 months old rat heart

Group	[ATP] 0.5 mM	[ATP] 1 mM	[ATP] 2 mM
Control	5	8	38.5
Treated	21*	27*	87**

*p<0.05; **p<0.001

Table 3. The mean values of 3H Tryptophan uptake in heart of Thyroxin treated rats

Age (months)	RA (cpm/g tissue) in control group	RA (cpm/g tissue) in treated rats		
		Thyroxine [0.25ug]	Thyroxine [0.50ug]	Thyroxine [1.0ug]
27	348	454	410	373

RA = radioactive uptake (cpm/g tissue)

Table 4. Mean values of 3H Tryptophan uptake in control rat heart and in rat heart treated with Thyroxin and incubated with GH3

Age (months)	RA (cpm/g tissue) in control group	RA (cpm/g tissue) in Thyroxin treated rats (incubated with GH3)		
		Thyroxine [0.25ug]	Thyroxine [0.50ug]	Thyroxine [1.0ug]
27	679	830	975	1070

Table 5. Mean values of SH myosin content in relation with Thyroxin treatment (mM/g tissue)

Age (months)	SH myosin (mM/g tissue) in control group	SH myosin (mM/g tissue) in treated rats
27	94±1	178±2*

Table 6. Mean values of 3H Tryiptophan (cpm/g tissue) uptake in skeletal muscle from rats treated with different concentrations of Thyroxin

Age (months)	RA (cpm/g tissue) in control group	RA (cpm/g tissue) in treated rats		
		Thyroxine [0.25ug]	Thyroxine [0.50ug]	Thyroxine [1.0ug]
27	679	418	366	298

RA = radioactive uptake (cpm/g tissue)

Table 7. The uptake of 45Ca++ in heart from Control and thyroxin treated rats with different concentrations

Age (months)	RA (cpm/g tissue) in control group	RA (cpm/g tissue) in treated rats		
		Thyroxine [0.25ug]	Thyroxine [0.50ug]	Thyroxine [1.0ug]
27	324	276	238	179

RA = radioactive uptake (cpm/g tissue)

CONCLUSIONS

Thyroxine administrated in excess in rat influences protein metabolism and ATP-ase activity as well as membrane permeability.
The effect of Gerovital H3 from the incubation media of heart fragments from rats treated with thyroxine in excess may have a clinical relevance concerning the utilization of Gerovital H3 therapy in the patients with hyperthyroidism in order to improve the heart metabolical functions.

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THE IMPACT OF TREATMENT WITH INSULIN ON INTERMEDIATE METABOLISM IN BROILER'S BODY

Florentina Claudia DUMITRACHE, Monica MARIN, Bálint CSÓK

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania, Phone: +4021.318.25.64, Fax: + 4021.318.25.67

Corresponding author email: mihalache75@yahoo.com

Abstract

The experiment was performed on seven weeks old broiler chickens ant they are divided in experimental and control groups. Once a day, the insulin was administrated in a dose of 4.00 IU/kg body weight. In the experiment, the effect of insulin hormone experimental treatments on growth was pursued, in parallel with its effect on the main metabolic pathways: protein, carbohydrate, lipids. The treatment was performed for 8 day and the chickens were periodically weighted. At the end of the treatment capillary and venous blood was harvested. The results were statistically processed and the means and standard error of means were calculated. The statistical significance differences of the means between control and experimental group was searched by Student test. Following the experimental treatment of insulin on chickens, the insulin treatment had a significant decrease in blood glucose ($P<0.05$), the level being 124 mg/dl, compared to 152 mg/dl in the control. Plasma lipid levels, however, underwent changes by decreasing from 639 mg/ml in the control to 361 mg/ml in the insulin-treated group.

Key words*: chickens, insulin, protein, lipid, carbohydrate, metabolism.*

INTRODUCTION

Priority for many institutions and researchers is the increase in productive capacities of animals, to a large extent the decrease in animal costs. One way to achieve these goals is to stimulate growth based on hormonal treatments. However, the use of hormones in animal breeding should not alter their health status. For this, the effects of hormones used to stimulate different productions on different metabolic processes must be fully understood.

Insulin controls the carbohydrate metabolism and lipid and protein metabolism. It is important to note that the liver is the main target organ of insulin, in part because pancreatic venous flow enters directly into the liver (Mihalache, 2004).

The net effect of insulin action is lowering blood levels of glucose, fatty acids and amino acids to promote intracellular transformation of these compounds in their forms of storage: glycogen, triglycerides and proteins (Serban et al., 1993).

MATERIALS AND METHODS

Biological material was represented by chickens aged 7 weeks (Broiler hybrid). The avian youth was provided by S.C. Vis Campi S.R.L., Ciorani Commune, Prahova County.

It were set up two groups of chickens: a control group and a group treated with insulin. Both groups were fed *ad libitum* with food compound recipes for the stage and physiological status (growing youth) and benefited from a program conducted by artificial lighting (8 hours per day), according to technology growth.

At the end of the treatment capillary and venous blood was harvested. The venous blood was harvested without anticoagulant. From this blood, the serum was used to determine the concentration in lipids, total protein, albumin, globulin and carbohydrate.

The 18 chickens from the two groups were fed pelleted feed of prescription industrial code 21-3 (Table 1).

A batch consists of nine chickens were treated with insulin, which was administered at a dose of 4.00 IU daily, once a day. Treatment duration was eight days. The control group remained untreated.

In this category of birds were also determined the effects of insulin on the main biochemical blood parameters.

Determination of serum lipid concentration was done by the vanillin method, blood glucose dosing by colorimetric method with antron and

protein levels was determined by the method of Gornall. The results were processed statistically and the significance of difference between groups was performed based on t test (Student test) (Tacu, 1968).

Table 1. Food compound recipe 21-3 used to feed chickens

No.	Ingredients	Quantity (kg)	Metabolizable energy (kcal)	Crude protein (%)
1	Corn	36.0	1212. 3	3.13
2	Barley	14.0	378.0	1.44
3	Wheat	16.0	476.6	1.90
4	Soybean cake	15.0	345.0	6.60
5	Sunflower cake	7.0	105.0	2.24
6	Meat flour	2.0	57.3	1.14
7	Oil	4.5	396.0	-
8	Premix methionine	0.9	18.8	0.50
9	Dicalcium phosphate	1.8	-	-
10	Calcium carbonate	1.0	-	-
11	Salt	0.3	-	-
12	Premix (MVP)	1.5	30.0	0.10
13	Total	100	3019.0	17.05

Legend: MVP = mineral-vitamin premix

RESULTS AND DISCUSSIONS

Every day, during treatment, the chickens of both groups were weighted (Table 2 and Figure 1).

Table 3 presents the results of determinations regarding the experimental group treated with insulin compared with controls.

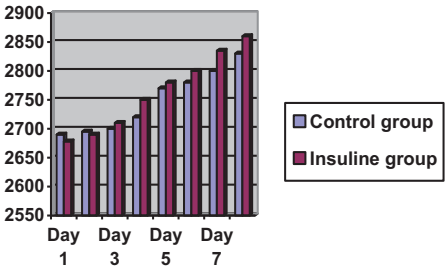

Figure 1. Evolution of chickens weight during the treatment with insulin

Table 2. Evolution of chicken weight (g) during the treatment with insulin

Group	Experimental period (day)							
	1	2	3	4	5	6	7	8
Control group	2690	2695	2700	2720	2770	2780	2800	2830
Experimental group	2678	2690	2710	2750	2780	2800	2835	2860

Insulin treatment had a significant decrease in blood glucose ($P<0.05$), the level being 124 mg/dl, compared to 152 mg/dl in the control. Plasma proteins were not significantly affected by this hormone.

Thus, total plasma protein concentration was 56.9 mg/dl in the control and 43.4 mg/dl respectively in the insulin-treated group.

Table 3. Determination of concentration in lipids, total proteins, albumin, globulins and carbohydrates in chicken

Group	Analysed parameter	Maxim level (mg/dl)	Minim level (mg/dl)	Average (mg/dl)	Standard error
Control group	Glucose	162.0	142.0	152.0	10
Experimental group		133.0	115.0	124.0	9
Control group	Albumin	42.4	42.4	42.4	0
Experimental group		32.0	32.0	32.0	0
Control group	Globulin	15.3	15.3	15.3	0
Experimental group		12.3	12.3	12.3	0
Control group	Total protein	67.9	45.9	56.9	11
Experimental group		56.4	30.4	43.4	13
Control group	Lipids	815.0	463.0	639.0	176
Experimental group		425.0	297.0	361.0	64

Plasma lipid levels, however, have been changed by decreasing from 639 mg/ml in the control to 361 mg/ml in the insulin-treated group (Figure 2).

This is also found in the literature, according to which hyperlipidemia occurs in mammals in diabetes mellitus (diabetes mellitus).

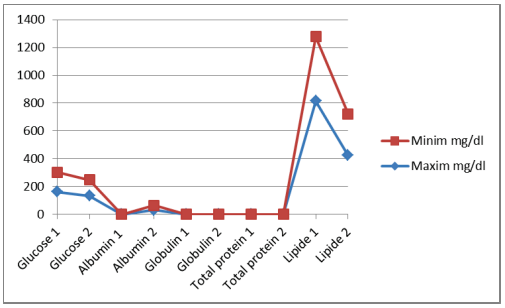

Figure 2. Plasma biochemical parameters measured in control and experimental groups

The total feed intake of the injected lot was 60 g higher than in the control group (Table 4).

Table 4. Evolution of feed consumption of broilers

Period	Feed consumption (g)	
	Control group	Experimental group
Total period	2424	2484
Day 8	325	330
Day 7	321	326
Day 6	304	315
Day 5	309	314
Day 4	303	311
Day 3	296	300
Day 2	279	299
Day 1	287	289

This weight gain has been observed to be late, because the anabolic effects of insulin are delayed, insulin being a protein, lipid and carbohydrate anabolic.

A side effect of therapy with this hormone is weight gain. If exogenous insulin is administered, it will first get into the systemic circulation and then pass into the liver.

Thus, insulin preferentially favors the penetration of blood glucose into adipose or muscle tissue, lowering blood glucose, causing subsequent hepatic glycogen mobilization to make the necessary blood corrections.

CONCLUSIONS

The body weight of broiler chickens treated with insulin evolved after a witness superior curve, although in other species, commonly, hipoglicemy cause conversely, a lower weight curve.

So, the average weight of 2678 g at the beginning of insulin administration, after 8 days of treatment it was 2860 g (an average daily gain of 22.75 g per day, compared to 20.00 g in controls).

This beneficial effect appears to be due to anabolic protein action of insulin, which sometimes go beyond what was catabolised. Since it is not fat deposits, means that insulin stimulates the growth itself.

The administered insulin to chickens Broiler induced a significant decrease in their blood glucose at 124 mg/dl, compared to 152 mg/dl in the control group;

Plasma protein levels have changed insignificantly in the experimental group

A significant decrease in plasma lipid levels from 639 mg/dl to 361 mg/dl was seen in controls, thus demonstrating that diabetes mellitus is specific for hyperlipidemia.

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RESEARCH ON THE APPRECIATION OF CONFORMATION TRAITS FOR THE HOLSTEIN BREED IN ROMANIA

Marius Cristian Pașcu, Gheorghe Emil MĂRGINEAN, Livia VIDU

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: cristi.pascu31@yahoo.com

Abstract

This paper aims to present the ratings obtained by a group of animals divided by the lactation rank and the differences between them and the breed standard. The tool that helps us to have an overview of the herd that we are working with is the type classification action. It is a very important element in the exploitation of dairy cows because it helps us to establish a hierarchy of animals in terms of conformation. Most of the results obtained from type classification of the animal group, have values close to those of the Holstein international standard. In some farms, there is now no emphasis on proper animal breeding technology nor is there a clear direction of genetic improvement in cows. In order to achieve higher economic performance, it is recommended to establish the long-term objective of development of the farm and then use of all existing means in the field to reach it.

Key words*: type classification, Holstein, lactation rank.*

INTRODUCTION

Type classification is used to appreciate the exterior of animals from a long time ago. Although this was done empirically, it was the only method of assessing the animals. Over time, with the evolution of mankind and a growing demand for consumption, and because of the farmers desire to have the most productive livestock, the assessment of the outside of the animals has evolved (Acatincăi, 2004). Type classification is now a fairly accurate cow assessment method and an extremely important activity for obtaining breeding values for bulls for sex limited characters (VP/05.0393, 2005).

Given the particular importance of cow's milk in human life and well-being, dairy cow husbandry occupies an important place in the global livestock production economy, representing a core sector in modern agriculture. Cows are the main raw material source for milk processors (Georgescu et al., 1995).

The tool that helps us to have an overview of the herd that we are working with is the type classification action. It is a very important element in the exploitation of dairy cows because it helps us to establish a hierarchy of animals in terms of conformation. After the animals are valued, we can objectively observe the qualities and defects of the herd and we can make comparisons between the animals (Pantelic, 2013).

It should also be borne in mind that type classification is the basis for bull selection, these being tested on descendants.

This paper aims to present the ratings obtained by a group of animals divided by the lactation rank and the differences between them and the breed standard (Alexoiu, 2002; Hamoen, 2016).

MATERIALS AND METHODS

The material analyzed and used for the writing of this paperwork is represented by 8026 Holstein Frisian cows. The working method used is represented by the analysis of the animal exterior using the linear description method. By this method, 18 traits were analyzed for each animal, grades from 1 to 9 were awarded for each trait except for “Stature” which is appreciated in centimetres, according to biological extremes in agreement to the methodology proposed by ICAR (Holstein Foundation Dairy Judging Workbook, 2016). A cow classification was then made according to their total score. A number of 8026 Holstein Friesian cows were analyzed, found in 13 farms

with different exploitation systems which were divided into groups according to the lactation rank.

RESULTS AND DISCUSSIONS

The deviations of the analyzed cows from the breeds standard are presented in the Table 1.

For stature, we can see a difference from the standard of +2.38 cm for all the classified animals, +2.9 cm for first calving, +2.37 cm for second calving and +1.97 cm for multiparous cows.

Analyzing these differences, one clearly distinguishes a tendency to increase the stature in the first calving versus the multiparous cows.

Table 1. Comparison of averages obtained with the standard of the breed

Parameters	Average total	Average first calving	Average second calving	Average multiparous	Breed standard
Stature (cm)	147.38	147.90	147.37	146.97	145.00
Chest width	5.295	4.775	5.169	5.809	5
Body depth	5.859	5.526	5.841	6.146	5
Angularity	4.709	4.423	4.700	4.949	5
Condition	5.087	4.857	5.194	5.199	5
Rump angle	4.872	5.026	4.897	4.728	6
Rump width	5.626	5.651	5.629	5.606	5
Rear legs rear view	4.782	4.985	4.761	4.625	9
Rear legs side view	6.073	6.094	5.897	6.182	5
Foot angle	4.116	4.286	4.074	4.001	5
Locomotion	4.222	4.160	4.291	4.217	9
Fore udder attachment	4.187	4.288	4.344	3.993	9
Front teat placement	4.705	4.791	4.890	4.507	5
Teat length	5.166	4.896	5.130	5.413	5
Udder depth	3.690	4.570	3.819	2.879	8
Rear udder height	5.421	5.624	5.461	5.228	9
Ligament	5.142	5.410	5.180	4.901	6
Rear teat placement	5.735	6.081	5.897	5.348	5
Frame	80.812	80.644	80.834	80.928	
Dairy strength	80.511	80.068	80.425	80.933	
Udder	79.482	80.222	79.620	78.791	
Feet and legs	79.077	79.199	79.304	78.811	
Total score	79.446	79.594	79.661	79.275	

In Table 1 we can see the differences in the average of the characters in the analyzed cows and the international standard of the Holstein breed.

The greatest difference from the breed standard was recorded for “fore udder attachment”. All the studied animal groups had a roughly equal score of around 4.2, the optimum of the breed being 9. Thus there was a difference of 4.8 points.

Another difference from the standard is recorded for the "udder depth" character. The standard is 8 points and the results obtained were 4.6 points for first calving, 3.8 points for second calving and 2.8 points for multiparous cows. At first, we can see the smallest difference between the credit rating of 3.4 points. However, the first calving is superior to the other groups of animals analyzed for this character, which were assessed by 1.8 points above the multiparous average and by 0.8 points above the second calving average, mainly by noticeable improvement of this character.

Table 2. Calculated statistical parameters for all trait

Parameters	Average (X)	Standard deviation (S)	Variance (S^2)	Variability (V%)
Stature	147.384	4.613	21.279	3.130
Chest width	5.295	1.478	2.185	27.917
Body depth	5.859	1.185	1.404	20.225
Angularity	4.709	1.358	1.844	28.838
Condition	5.087	1.659	2.753	32.616
Rump angle	4.872	1.838	3.380	37.732
Rump width	5.626	1.579	2.494	28.068
Rear legs rear view	4.782	1.966	3.864	41.104
Rear legs side view	6.073	1.683	2.832	27.708
Foot angle	4.116	1.684	2.837	40.928
Locomotion	4.222	1.879	3.531	44.510
Fore udder attachment	4.187	1.889	3.570	45.124
Front teat placement	4.705	1.656	2.744	35.207
Teat length	5.166	1.600	2.560	30.971
Udder depth	3.690	1.681	2.826	45.557
Rear udder height	5.421	1.347	1.815	24.853
Ligament	5.142	1.749	3.059	34.014
Rear teat placement	5.735	1.845	3.403	32.166
Frame	80.812	3.083	9.506	3.815
Dairy strength	80.511	3.449	11.895	4.284
Udder	79.482	3.265	10.663	4.108
Feet and legs	79.077	3.543	12.555	4.481
Total score	79.446	3.949	15.595	4.971

As we can see in Table 2, the most homogeneous analysed character is represented by stature with a variability of only 3.1%.

For the traits udder depth, fore udder attachment, locomotion, rear legs rear view and foot angle the value for variability is over 40% and we can say that the animals are very heterogeneous.

This heterogenity comes from a faulty management of the farm, from the lack of use of modern breeding techniques such as mating programs and from a poor choice of bulls used for artificial insemination.

In some farms, there is now no emphasis on proper animal breeding technology nor is there a clear direction of genetic improvement in cows. In order to achieve higher economic performance, it is recommended to establish the long-term objective of development of the farm and then use of all existing means in the field to reach it.

In order to obtain productive and healthy animals with a good longevity and conformation it is advisable to carry out a rigorous selection of the Holstein cows in Romania, a selection that can be made after classifying the animals because we will have a clear picture of the qualities and defects of the animals. It is also recommended to use a mating program which is very helpful in avoiding inbreeding and combined with type classification, can increase the precision of getting very good animals in future generations.

CONCLUSIONS

Most of the results obtained from type classification of the animal group, have values

close to those of the Holstein international standard. The exceptions are "udder depth", "rear udder height", "locomotion" and "rear legs rear view" that are far below standard in all the groups of analyzed animals.

From the analysis we can see a tendency to increase the stature of the animals, the first calvers having higher values than the multiparous cows.

Given the above-mentioned characters as inferior to the breed standard, we can conclude that the main problems encountered in the Holstein cows farms in our country are related to limbs and udder.

Limb problems like rear legs rear view could, in time, lead to walking disorders, different hoof diseases, pain when the cows are standing and all these, to a smaller milk yield.

Udder traits, especially udder depth, are very important in maintaining a good udder hygiene thus limiting the incidence of diseases like mastitis, abscesses. The higher the udder, the better it is for trying to avoid these inconveniences.

The amount of milk obtained in some Romanian farms could increase significantly if the technological conditions of operation and hygiene were improved.

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AMMONIA TRAP FOR DECREASING AMMONIA LEVEL IN BROILER HOUSE – A PATENTED SYSTEM DESIGN

Ali COSKAN[1], Atilgan ATILGAN[2]

[1]Süleyman Demirel University, Faculty of Agriculture, Soil Science & Plant Nutrition Department, 32260 Isparta, Turkey
[2]Süleyman Demirel University, Faculty of Agriculture, Agricultural Structure and Irrigation Department, 32260 Isparta, Turkey

Corresponding author email: atilganatilgan@sdu.edu.tr

Abstract

The urea existing in broiler manure is converted into ammonia gas by urease enzyme and consequently the concentration of ammonia rising in the poultry house which results decline on broiler productivity. To reduce in-barn ammonia level a patented ammonia trap system is developed. System is looking like a miniature cooling tower. This so-called cooling tower is filled by the polyethylene balls to increase surface area and balls are wetted continuously by 5 M of phosphoric acid solution. In-barn air forced to pass through within the balls, ambient ammonia reacts by phosphoric acid and ammonium phosphate obtained as a by-product which can be used as a mineral fertilizer. Initial results revealed that the system is effective on ammonia reduction in barn and it was patented by Turkish Patent Institute with the patent Nr: TR 2012 13292 Y. System is under development stage; however, meanwhile we did not maintain patent protection. Thus, we would like to encourage farmers to use this system to reduce ammonia levels in their facilities to improve their benefit as well as reduce atmospheric pollution.

Key words*: Ammonia trap, broiler house, In-barn gases, poultry.*

INTRODUCTION

In order to provide better living conditions for animals, animal barns should be designed by considering not only the thermal conditions but also a wide range of environmental factors. These environmental conditions cover many issues such as providing adequate living space, growing factors, lighting, feed and water requirements as well as manure management. These conditions closely related by the purpose of facility, animal type and geographical conditions. At the present time, intensive production techniques are being followed; therefore, animals are staying in the poultry house throughout their lifetimes. Thus, environmental conditions of barns are as important as growing material and feed for broiler productivity, health and feed utilization rates. Indoor environmental condition term refers adequate temperature, humidity and sanitation conditions in the poultry house, avoiding harmful gases, regulation of enlightenment and animal density in the poultry house (Atilgan, 2000). Common air pollutants in the poultry houses are CO_2, ammonia and CO. These gases can create an uncomfortable environment for both labourer and animals (Alchalabi, 2006).

The most common air pollutant in poultries is ammonia gas (Akbay, 1986). Emission rates should be assessed very well in countries, because the ammonia emission values vary widely according to breeding type and season. These differences in ammonia emission values are sometimes not explained by physical or chemical processes (Groot Koerkamp et al., 1998).

The ammonia concentration in the poultry house is affected by a number of factors. These factors include ventilation, bedding age and type, animal age, inner temperature of poultry house, relative humidity, fan status and water lines (Fairchild, 2006). Ammonia gas is formed as a result of decaying manure which has been accumulating on bedding throughout the growing period (Okuroglu, 1987; Yahav, 2004). Ammonia is a gas which is colourless, pungent-scented, lighter than atmosphere and soluble in water. Ammonia concentration

shows fluctuation depending on the poultry house conditions. In the poultry which ventilation capacity is 1.1 m^3 h^{-1}, the concentration of ammonia at 24°C varies between 15 to 90 ppm. If the ventilation rate is almost doubled to 2.3 m^3 h^{-1}, the concentration of ammonia will increase to a maximum of 50 ppm (Valentine, 1964; Anderson et al., 1964, Hellickson and Walker, 1983).

In poultry houses where ventilation was not well performed, the problems were observed when the ammonia concentration was 50 ppm (Sainsbury, 1981). Ammonia can be easily recognised due to the pungent malodorous where even at 5 ppm human nose can feel the odour. Over 20 ppm it causes tears (Wathes et al., 1997). Between 100 to 200 ppm ammonia causes sneezing, irritation of the respiratory system and reducing appetency.

When it is reached to 1500 ppm, cough and foaming in the mouth appear; it is fatal over 5000 ppm. Ammonia levels in the poultry exceed 25 ppm in winter even in normal ventilation conditions.

The 25 ppm ammonia concentration is considered the limit value for poultries (Carlile, 1984, Gurdil, 1998, Erensayin, 2000, Gurdil et al., 2001, Gurdil, 2003). Ventilation is one of the important parameters to ensure environmental control in poultry houses.

A well-planned ventilation system is essential for adequate ventilation. Ammonia gas level increases in the winter time even if barn equipped with effective ventilation system because the outside temperature in winter is quite low; therefore, low ventilation rates preferred for conserving in-barn heat (Alchalabi, 2006). Thus In the winter months, determined ammonia value in the barn was found to be well above the critical limit of 25 ppm. The most practical way to reduce ammonia is ventilation; but the environmental concern and increasing energy cost are the obstructions of effective ventilation. In this study, it was aimed to design a system that help to reduce ammonia level in poultry without increasing costs.

MATERIALS AND METHODS

The system constructed by polyethylene materials to prevent corrosion. The barrel was used as a main body of the system which capacity is approximately 0.07 m^3 (Figure 1). The volume of the system is not critical, it can be selected any size, but the bigger capacities results the better reducing effects of ammonia.

Figure 1. Designed ammonia trap system

This barrel is filled up by polyethylene balls to increase surface area (Figure 2). Smaller ball size provides higher surface area in same volume; however, salt occurrence may prevent uniform solution and air flow throughout the balls. Thus, the smaller diameter less than 3 cm is not recommended. In the designed system the 80 ball which diameter was 8.5 cm each were placed to barrel and 1.82 m^2 of surface area was achieved.

The solution in the bottom reservoir is transferred to top of the system via pomp and the balls inner the barrel is wetted. For the uniform distribution to be able to wet entire balls, ½" polipropilen water pipe is rounded, bottom face drilled to have evenly spaced 60 hole by 2 mm bit (Figure 3).

As an inlet ports, 18 holes of 3.3 cm in diameter were opened 14 cm above the bottom of the barrel. Eight cm long plastic pipes with an outside diameter of 3.3 cm and an inside diameter of 2.1 cm placed to these holes. On the upper side of the system, a single hole with a diameter of 11.5 cm is opened and a fan with 190 m^3 h^{-1} capacity is placed here (Figure 4).

Figure 2. The balls used in the barrel

Figure 3. Solution distributor

In the preliminary experiment 5 M of phosphoric acid solution was used. Expected reaction in the barrel is presented was:

$$NH_4OH + H_3PO_4 \rightarrow NH_4H_2PO_4 + H_2O$$

Figure 4. Complete system with fan

Although ant type of acid solution even water can be used in the system. In the room temperature more than 500 litre of ammonia gas can be solved in the water; however, acid solutions are more effective and by product can be used in agricultural system as fertilizer.

Briefly system works in following steps:

1. The solution pumping from bottom reservoir to top sprayer/distributor unit.
2. While solution is wetting balls, the barrel air sucking by fan that located the top centre of the barrel.
3. Sucked air forced the barn atmosphere enter the system via inlet ports.
4. While barn atmosphere passed within balls, ammonia is trapped by acid solution. Thus, ammonia concentration in outlet reduced compared the inlet.
5. Ammonium phosphate fertilizer occurs in the solution. Some of them have been precipitated on the surfaces.

RESULTS AND DISCUSSIONS

This system tested in the poultry and bulk of data recorded. The ammonia measurement results will be summarized at prospective manuscript; however the aim of this paper is presenting entire system and potential improvement possibilities. First of all this system is promising options to reduce ammonia level in poultry; but it needs extra labour; therefore, system should be developed for automatization. These developments will also eliminate the disadvantages we meet, which will be explained below. Those disadvantages are:

(1) The acid solution somehow reaches the metal equipment of the barns that stimulate corrosion. Moreover concentrated acid solution is not safe for the workers and any accident would treat animal welfare. Thus lower acid concentration should be used but in this case ammonia fixing capacity is reduced.

(2) Depending on the in-barn ammonia concentration, the neutralization interval is not predictable. Besides the ammonia trap efficiency is related the H^+ ions of the solution which decreased by the time. As a result, acid solution should be continuously controlled which lead to extra workload.

(3) System powered by mains voltage and acid solution is rather conductive. In case of electrical leakage employees may be injured. Although the fan can be replaced by 12 volt brushless version, the 12 volt pomp is not providing adequate circulation of the solution.

(4) The reservoir of the system increases the system weight. When the system located at the floor, around 1 square meter of the area occupied for each. Alternatively, system may be hanged to the roof construction, but in this case extra weight should be considered.

To overcome the above mentioned problems a cascade system is designed (Figure 4).

Cascade system design allows weaker acid solution usage while effective ammonia trapping. The pH of the solution can be easily controlled by on-line pH probe which control the one dosage pomp to keep the pH constant. In this design, high voltage required only for outside located facility which is less risky. Due to the no solution will be located under the each system; it will be lightweight compared to the original design. Moreover, with this cascade design, system may adapt every poultries without considering size and the shape of barn as well as labour requirement.

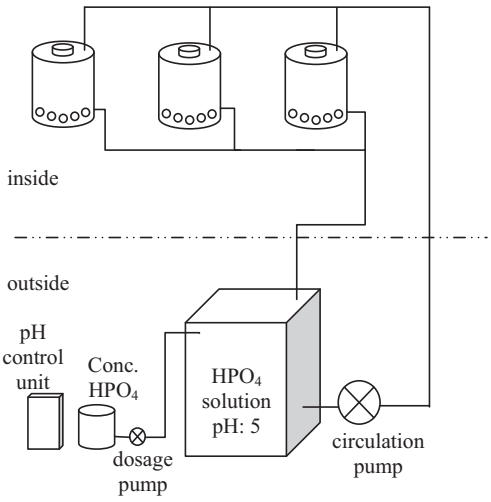

Figure 4. The cascade system design trap systems (the number of the unit can be easily increased as required)

Obtained ammonium phosphate solution can be directly used as a fertilizer or can be dried out via solar drying systems. In our experiment the ammonium (NH^+_4) concentration of solution in the ammonia trap reached up to 1.2% whereas the acquired salt after drying process was contained 10.4% ammonium.

CONCLUSIONS

Based on the overall observations and measurements it is concluded that developed system is decreasing in-barn NH_3 level evidently without treating livestock production system. This system although needs some improvement to increase its efficiency as well as usability, presented version is good start point to mitigate NH_3 emissions. As well-known, ammonia is one of the gas that treat in-barn and surrounding communities.

The system that presented here is considerably promising approach to reduce this huge anthropogenic emission without causing any side effects. On one hand, decreasing in-barn NH_3 concentrations lead welfare improvement for both animals and the workers in livestock facilities. On the other hand each system acts as

a so-called nitrogenous fertilizer production plant.

Expected by-product of the system is ammonium phosphate which is one the most commonly used fertilizer in greenhouse vegetable production in Turkey; therefore, marketing this by-product would provide additional income.

Although organic farming system totally refused mineral fertilization with minor exceptions, limited usage of this by-product may be considered one of the fertilizer sources considering its production techniques which both environmentally safe and human-friendly.

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RESEARCH ON INTRODUCTION OF MODERN FEEDING SOLUTIONS FOR YOUNG FEMALE BOVINE INTENDED FOR REPRODUCTION

Oana Simona DOROBĂȚ[1], Gheorghe MĂRGINEAN[1], Vasile MACIUC[2], Livia VIDU[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd, District 1, Bucharest, Romania
[2]University of Agricultural Sciences and Veterinary Medicine of Iași,
3, Mihail Sadoveanu Alley, Iaşi, 700490, Romania

Corresponding author email: oana.dorobat@yahoo.com

Abstract

The main purpose of the work was to make a comparative research on the feeding of calves. The research was conducted in dairy farm Agroserv Mairut, Ialomita county. The farm has more then 1000 dairy cows that produce 35 litres per day. All there production is register in a program that help employees to manage all dairy process. The animals in the experiment belong to the Holstein breed. It was selected 30 animals and was formed two experimental groups (each group with 15 animals). The first group received a classic feeding scheme, and group 2 a scheme based on super-intensive feeding. From the statistical analysis it was observed that between groups the differences are significant, respectively 15 kg live weight.Monitoring of body weight is very important because the introduction theyoung females of reproduction is done at 70-75% of the average weight of the breed at the age of adult. On the other hand, early introduction to reproduction reduces the non-productive period of the animal.

Key words*:dairy cow, genetic, milk, breed.*

INTRODUCTION

Beside a good feeding program calves need a good genetic support in a farm depende by a good selection of females from first weeks of life. Females are the future of a dairy farm, calves femele need a good mother and a good sire too.Farmers need evaluate the sire after their standardised transmitting abilities and predictability. The sire age is important too, theoretically, the most rapid rate of genetic progress is achieved by using young sires (Tyler and Ensminger, 2006;Schefers and Weigel, 2011).

New super-intensive fedding program is to produce remplecement heifers that will will implement a feeding and breeding program for youngstock, and the dairy breed will excel regularly and without problems. To plan a productive winning life for dairy cows, it is necessary to reduce health problems.Each generation should represent genetic progress over the previous generation and the farmer need thing about starter of all calves and to find good solution that are fitting in dairy

The number of replacement, in the farm, each year to mantain herd size depend on the culling rate because of disease, injury, low production, or poor type. Normal turn over is over 30 percent each year. To meet this need, to allow some opportunity for culling undesirable first calf heifers, it is necesary to raise approximately one third each year. Is better to produce your own heifers than to buy one, is better when you know that your animals are in good conditions.

This are the points that are making a interes for farmers and will improve the genetic part giving better heifers who will give for dairy daughters and year by year the farm will pogress in the direction that the farmer propose it. Most of time dairy farms propose better quality and quantity of milk.

There are many factors that can influnce calves welfare in that first 2 months: calving difficulty; calving environment; quantity and quality of colostrum; how soon after birth is fed; housing; ventilation; clean and dry bedding; incidence and severity of scours and respiratory diases; the amount of milk or milk remplacer; quality and consistency of solids and nutrients; quantity and quality of water; adjustements in feeding program and housing due to temperatures outside calf's zone of

thermal neutrality (Cziszter et al., 2003; Vidu et al., 2015).

First 24 months of age the heifers will gain 780-860 grams per day and heifers on their first calving will weight around 600 kilos and 580 kilos after calving. Cows lose 11% from there weight after calving.

This percent represent the value of fluids tissues associated with calving. Next month following weaning (2 months of age), this period mean a transsion key that the body of young femalesneed be prepared.

In first two months farmers put calves in big groups, here are feedding with a starters feeding program or sometime they are putting on TMR forage, the result is gut fill, which distorts any true body wieght growth and actually often reduce it (Table 1) (Britt, 2017;Wiggans et al., 2017).

Table 1 Correlation of body weight with age

Age (months)	Body weight (kg)	% from mature body weight
0	48.75	6.5
1	72.75	9.7
2	96	12.8
3	123.75	16.5
4	151.5	20.2
5	168	24
6	207.75	27.7
7	235.5	31.4
8	262.5	35.0
9	291.75	38.9
10	318	42.4
11	347.25	46.3
12	374.25	49.9
13	402.75	53.7
14	430.5	57.4
15	458.25	61.1
16	485.25	64.7
17	513.75	68.5
18	541.5	72.2
19	570	76.0
20	597	79.6
21	624.75	83.3
22	653.25	87.1
23	681	90.8
24-precalving	705	94
24-postcalving	637.5	85

MATERIALS AND METHODS

The investigated material consisted of 30 calves (Figures 1 and 2), divided into two groups:

- *Group number 1*:15 calves, which are feeding after clasic program (Table 2);
- *Group number 2*: 15 calves, which are feeding with a super-intensive feeding program (Table 3).

Calves are raisedin the same barn and the same conditions.

The classic feeding program for group number 1 is presented in Table 2.

Table 2.Classic feeding scheme

Age of calf	Feeding scheme
0-5 days (colostrum period)	6 liters/day
6-15 days	6 liters cow milk, water and concentrate ad-libitum
16-40 days	8 liters cow milk, water and concentrate ad-libitum
41-50 days	6 liters cow milk, water and concentrate ad-libitum
51-55 days	4 liters cow milk, water and concentrate ad-libitum
56-67 days	1 liters(in the morning) cow milk, water and concentrate ad-libitum
68-77 days	Only concentrate

For the second group feeding scheme involves the use of large quantities of milk (Table 3).

Table 3.Super-intensive feeding scheme

Age of calf	Feeding scheme
0-7 days (colostrum period)	6 liters colostrum/day, water and concentrate ad-libitum
8-60 days	12 liters cow milk, water and concentrate ad-libitum
61-67 days	6 liters cow milk (in the morning), water and concentrate ad-libitum
68-77 days	only concentrate

The calves were weighed at birth and at the end of feeding milk (weaning).

The data obtained were statistically processed, and we used the Student test to compare the results.

Figure 1. Calves of experimental groups

Figure 2. Calves of experimental groups

RESULTS AND DISCUSSIONS

Applying a scientific feeding requires knowledge of basic elements such as:
- the morpho-functional features of the digestive tract in this category;
- specific feed standards;
- the feedingstuffs indicated for calves,
- the methodology and standard procedures to follow;
- the technique of feeding and watering calves.

In this period the animals are expose to many stress factors as blood test, dehorning, ear tag, and in the 60 day they are forced to eat only concentrate because milk meal is stopped.
Not only weight wasimportant, the same importance had the hight.From birth to first calving the animal need to growth more than 96 cm, this depende by genetic.
Is very important that calves to be measurethe hight and weight at birth, 2 months of age, 6 months of age, a year of age and right prior at fist calving. If the animals did not achieve the weight and the height set, it should be seen where the growth program was wrong.
In Table 4 we can see the evolution of the body weights of the cattle through the two feeding schemes.
From the analysis of the average values of the body weight of the experimental groups, group 2 achieved a higher weight to 15 kg.
The chosen animals for the comparative experiment had equal weights.
From the experiment, we can see that a feeding scheme based on a larger quantity of milk stimulates the weight gain of the calves.
The statistical differences between the two experimental groups are significant ($p<0.05$).
The quality of the youngstock is of particular importance for dairy farms, because the new generation must be genetically superior to the old one - replaced.
The duration of the experimental observations was 77 days. At the end of the period between the two experimental groups there were notable differences.
Group 1, fed with the classic scheme, achieved a mean average weight gain of 32.44 kg.
Group 2, fed with the modern scheme, achieved a mean average weight gain over the entire feeding period of 46.94 kg (Table 4).
The statistical calculation performed for group 1 shows a daily average body weight increase of 421 g and for group 2 an average daily gain of 609 g.
These average values are comparable to those in the literature, so that Georgescu et al. (2006) recommended for calves (0-3 months) with body weight between 40-60 kg, average daily gains of 400-600 g.

CONCLUSIONS

Growing youth for reproduction is an important problem on the farm. Respecting feeding procedures in the first part of life leads to healthy and fit animals to produce significant amounts of calves and quantity of milk.
Our experiment has shown that other feeding schemes for calves can also be practiced in order to ensure a harmonious and quickly growth of young females.

Table 4. Results of experimental observations

Experimental group 1			Experimental group 2		
No.	Birth weight (kg)	Weaning weight (kg)	No.	Birth weight (kg)	Weaning weight (kg)
1	50	89	1	50	102
2	32	90	2	32	75
3	55	91	3	55	105
4	45	78	4	45	92
5	43	75	5	43	90
6	42	74	6	42	91
7	41	75	7	41	84
8	40	76	8	40	82
9	39	79	9	39	81
10	43	78	10	43	87
11	45	77	11	45	107
12	46	75	12	46	110
13	44	72	13	44	91
14	43	71	14	43	93
15	42	68	15	42	99
X		**77.6**	X		**92.6***

* $p<0.05$

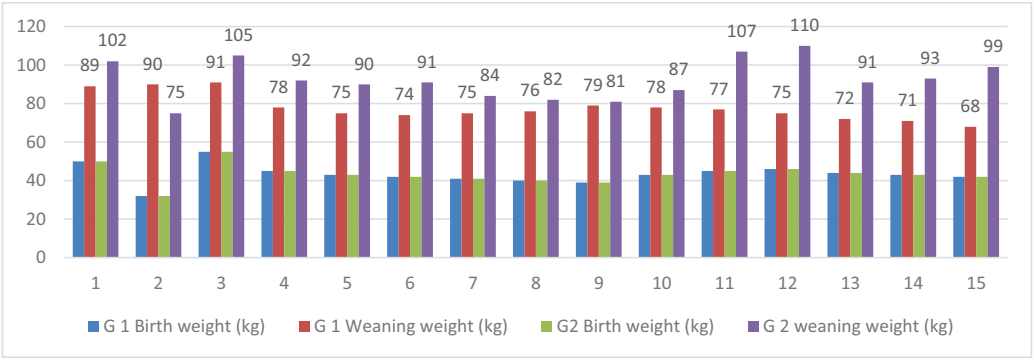

Figure 3. Weight variation between experimental groups

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TRANSITION PERIOD - A NEW CONCEPT USED IN ROMANIAN DAIRY FARMS

Ion Silver MILITARU[1], LiviaVIDU[1], Nicușor ȘERBAN[2], Gheorghe MĂRGINEAN[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 MarastiBlvd, District 1, Bucharest, Romania
[2]Agroserv Mărițța, Ialomițacounty, Romania

Corresponding author email: liviavidu@gmail.com

Abstract

Dairy represents the most complex type of farming, farmers have the opportunity to develop knowledge and to apply it in farms. Received much attention in the research and popular-based literature in recent years due to the recognition of its importance in the productivity and health of cows. The problem that our research scientists are facing is difficulty in gathering enough cows of similar productivity characteristics in one place to conduct the research necessary to help them understand this animal. Because of this difficulty, we have used animals outside of this short time frame and tried to simulate the same conditions that exist in the true transition cow.

Key words: *conditions, productivity, advantage, healthy, research.*

INTRODUCTION

'Transition period' is not a very commonly used term in Romania. The expression describes the first 21 days before and after calving. The term transition describes the most important physiological, metabolic and nutritional changes. It constitutes a turning point in the productive cycle of the cow from one lactation to the next (Jordan, 2005).

The manner in which these changes occur and how they are managed are of great importance as they are closely linked to lactation performance, clinical and subclinical postpartum diseases and reproductive performance that can significantly affect profitability. This period doesn't have a term used in Romanian animal science literature, but is mentioned as being critical for cows (Jordan et al., 1993)

The three weeks before and the three weeks after calving are an important and vulnerable period for the dairy cow. Her metabolic requirements increase dramatically and how she copes with this high-energy transition period will impact how well she performs during the rest of the lactation. This is why having a good transition cow management program is crucial for a successful dairy operation (Grummer, 1995).

This method allowed us to understand the problems faced by these cows, but has also led to some confusion in interpreting data.

Poor transitions also result in milk income losses (Yokoyama et al., 1994; Markusfeld, 1987).

Every pound of milk lost from peak production represents 90 kg of milk for the lactation. Poor transition periods often result in the loss of 4.5 kg to 9 kg of peak milk (Ferguson, 2001), which could represent 900 to 180 kg of unrealized milk yield. Elaborating nutritional strategies to facilitate the passage of the cow through this transition phase; while minimizing health problems and optimizing productivity/ profitability for the remainder of the ensuing lactation (Cziszter et al., 2013; Markusfeld, 1987).

Physiological changes*:* A number of profound physiologic changes occur in the transition cow that modifies her metabolism drastically.

The rapidly increasing demands of the fetus and the development of the synthesis of milk components, are causing these changes (Grummer, 1995).

The concentration of plasma insulin declines in the transition period until calving and that of somatotropin increases rapidly between the end of gestation and the initiation of lactation.

MATERIALS AND METHODS

Transition period also comes with diseases that can negatively affect the next milk production or total production in the case of heifers.
The conditions described above increase the likelihood of health problems during the transition period (Yokoyama, 1996).
The experiment involved 50 cows that were in dry period, 25 of them were fed only with ration for dry cows until the calving.
The other 25 cows, were fed with the same ration until 21 days before calving, after that they received ration for close-up dry cows (Vidu et al., 2004).

RESULTS AND DISCUSSIONS

Both ration contain water to avoid feeding selection during consumption, because cows have a tendency to consume selective first (Dyket al., 1996). A negative DCAD ration (acidic diet) stimulates the process of calcium resorption from the bones. It is also thought that it allows more calcium to be absorbed from the intestine.

Table 1.The fodder timetable during dry period

Days before calving	Group 1	Group 2	Days before calving	Group 1	Group 2
60	Dry cow Ration	Dry cow Ration	29	Dry cow Ration	Dry cow Ration
59	Dry cow Ration	Dry cow Ration	28	Dry cow Ration	Dry cow Ration
58	Dry cow Ration	Dry cow Ration	27	Dry cow Ration	Dry cow Ration
57	Dry cow Ration	Dry cow Ration	26	Dry cow Ration	Dry cow Ration
56	Dry cow Ration	Dry cow Ration	25	Dry cow Ration	Dry cow Ration
55	Dry cow Ration	Dry cow Ration	24	Dry cow Ration	Dry cow Ration
54	Dry cow Ration	Dry cow Ration	23	Dry cow Ration	Dry cow Ration
53	Dry cow Ration	Dry cow Ration	22	Dry cow Ration	Dry cow Ration
52	Dry cow Ration	Dry cow Ration	21	Dry cow Ration	close up ration
51	Dry cow Ration	Dry cow Ration	20	Dry cow Ration	close up ration
50	Dry cow Ration	Dry cow Ration	19	Dry cow Ration	close up ration
49	Dry cow Ration	Dry cow Ration	18	Dry cow Ration	close up ration
48	Dry cow Ration	Dry cow Ration	17	Dry cow Ration	close up ration
47	Dry cow Ration	Dry cow Ration	16	Dry cow Ration	close up ration
46	Dry cow Ration	Dry cow Ration	15	Dry cow Ration	close up ration
45	Dry cow Ration	Dry cow Ration	14	Dry cow Ration	close up ration
44	Dry cow Ration	Dry cow Ration	13	Dry cow Ration	close up ration
43	Dry cow Ration	Dry cow Ration	12	Dry cow Ration	close up ration
42	Dry cow Ration	Dry cow Ration	11	Dry cow Ration	close up ration
41	Dry cow Ration	Dry cow Ration	10	Dry cow Ration	close up ration
40	Dry cow Ration	Dry cow Ration	9	Dry cow Ration	close up ration
39	Dry cow Ration	Dry cow Ration	8	Dry cow Ration	close up ration
38	Dry cow Ration	Dry cow Ration	7	Dry cow Ration	close up ration
37	Dry cow Ration	Dry cow Ration	6	Dry cow Ration	close up ration
36	Dry cow Ration	Dry cow Ration	5	Dry cow Ration	close up ration
35	Dry cow Ration	Dry cow Ration	4	Dry cow Ration	close up ration
34	Dry cow Ration	Dry cow Ration	3	Dry cow Ration	close up ration
33	Dry cow Ration	Dry cow Ration	2	Dry cow Ration	close up ration
32	Dry cow Ration	Dry cow Ration	1	Dry cow Ration	close up ration
31	Dry cow Ration	Dry cow Ration	CALVING		
30	Dry cow Ration	Dry cow Ration	next 21 days both groups will be feed with fresh cows ration		

Cations are positively charged ions such as sodium (Na+) and potassium (K+) and anions are negatively charged ions such as chloride (Cl^-) and sulphur (S^-).

$$DCAD = (Na + K) - (Cl + S)$$

To see how both rations are functioning on the 42 days of dry period when the second group started to be feed different by first group we are doing a urine test and we will measure the pH (Drackley, 1999).

Table 2. Ration for close up dry cows
(21 day before calving)

No.	The structure of the ration	Content (kg)
1	corn silage	17.00
2	straw	2.00
3	soybean meal	1.20
4	rape-seed meal	1.20
5	alfalfa hay	1.00
6	Triticale	0.50
7	corn	0.50
8	propilenglicol	0.25
9	premix	0.20
	Total	23.85

Close up dry cows period represent the 21 days before calving. Nutritionists appreciate that one cow in close up period need to eat 23.5 kilos per day of TMR (Table 2).

Table 3. Ration for dry cows

No.	The structure of the ration	Content (kg)
1	corn silage	11.00
2	brewers grain silage	5.00
3	straw	1.50
4	rape-seed meal	0.70
5	alfalfa hay	5.00
6	triticale	1.00
7	corn	0.80
8	propilenglicol	0.25
9	premix	0.05
	Total	25.30

Dry cows period during 60 days ahead calving will receive the group number 1 (Table 1). Total forage per day appreciate by nutritionist of farm is 25.3 kilos per day (Table 3).
Both rations are made for a single animal, personalized depending on the physiological state in which it is.

After calving cows will be fed with the same ration and they will be housing in the same barn being carefully supervised by vets. The ration that was given to both groups can be seen in the Table 4.

Table 4. Ration for fresh cows
(21 day after calving)

No.	The structure of the ration	Content (kg)
1	corn silage	14.00
2	brewers grain silage	7.00
3	straw	0.15
4	rape-seed meal	1.50
5	alfalfa hay	0.50
6	triticale	2.20
7	corn	2.00
8	propilenglicol	0.25
9	premix	0.45
	Total	28.05

Figure 1. Cows waiting for calving

A urine pH of 6-6.5 indicates that the rations are well suited for a transition cow. As illustrated in the Table 5, group number 2 has an average of 6.17 that shows that the ration with negative DCAD is well formulated for close-up dry cows, far better than group number 1's ration.
Group 2 has the pH urine average 8.13, alkaline pH who belong to positive DCAD. Urine pH is influenced by cows fed.
Although some recommendations suggest for Holstein lactating cows urine pH within a range of 6 to 6.5.Here's why: showed that if cows' pH fall below about 6.0 or rise above about 7.0, it takes a relatively greater change in DCAD to effect a urine pH change. But when they're within the 6.0 to 7.0 range, a small DCAD change causes a bigger pH jump.

Table 5. Testing the urine pH from both experience groups

Tested cow	Group 1 dry period ration	Tested cow	Group 2-negative DCAD ration
1	8.1	1	6.0
2	7.6	2	6.0
3	8.6	3	6.2
4	7.0	4	6.1
5	7.2	5	6.1
6	7.8	6	6.3
7	8.7	7	6.0
8	8.2	8	6.0
9	8.3	9	6.4
10	8.2	10	5.9
11	7.9	11	6.0
12	8.21	12	6.1
13	8.5	13	6.5
14	8.4	14	6.3
15	8.3	15	6.3
16	8.5	16	6
17	8.1	17	6.2
18	8.3	18	6.2
19	8	19	6.4
20	8.8	20	6
21	8.2	21	6.1
22	8.1	22	6.5
23	8	23	6.5
24	8.4	24	6.2
25	8	25	6.1
Avg.	8.1364	Avg.	6.176

CONCLUSIONS

Although some recommendations suggest for Holstein lactating cow urine pH within a range of 6 to 6.5.Here's why: showed that if cows' pH fall below about 6.0 or rise above about 7.0, it takes a relatively greater changing DCAD to effect a urine pH change. But when they're within the 6.0 to 7.0 range, a small DCAD change causes a bigger pH jump.

A well formulated DCAD ration for close-up dry cows results in increased dry matter intake in early lactation, increased milk production, decreased disease incidence, decreased retained placentas and uterine infection, decreased displaced abomasum, decreased udder edema in heifers and improves reproductive performance.

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COMPARATIVE STUDY ON CARCASS TRAITS OF TWO DIFFERENT RABBIT GENOTYPES REARED IN TRADITIONAL AND CONVENTIONAL SYSTEMS IN ALBANIA

Alma LLAMBIRI[1], Lumturi PAPA[1], Kristaq KUME[2]

[1]Agriculture University of Tirana, Albania
[2]ALBAGENE Association.

Corresponding author email: daijaalma@yahoo.it

Abstract

Carcass characteristics of Albanian local rabbit breed, farmed in two different rearing systems traditional family production system and conventional one and commercial hybrid "Local rabbit x Californian breed" farmed only in conventional system, were compared. The carcasses were measured and retailed according to the norms of the World Rabbit Scientific Association. Local rabbits reared in traditional system in family farms (n=36) had lower live weight at slaughter age of 101 days (2040 v. s 2314 g, P<0.05) than the animals (n=36) reared in the conventional production system of commercial farms. Dressing yield, hot carcass (HC %) and reference carcass (RC %) of local breed rabbits were not affected by rearing system. Commercial hybrid had higher live weight at slaughter age (2493 g), dressing yield: HC (62.03%) and RC (50.26%) than local breed rabbits. Rearing system (P<0.01) and the genotype (P<0.01) were the main factors affecting on the carcass characteristics. The Albanian local breed rabbits despite of rearing system had carcass characteristics similar to them of middle rabbit breeds. The F_1 crosses reared in commercial farms could be one of effective ways to improve rabbit meat production in Albania.

Key words*: rabbit, local breed, crosses, carcass traits, production system.*

INTRODUCTION

The rabbits of local breed are reared in general as an alternative production activity of small scale family farms in Albania (Daija et al., 2009). The rearing system is traditional one with low inputs. The conventional system with better feeding and housing conditions is applied in commercial farms producing rabbit meat for market. Rabbits of local breed and (F_1) crosses "Local rabbit x Californian breed" are reared in commercial farms. The farmer's interest to farm rabbits is growing up recently as consequence of increased request of market for rabbit meat. This situation has initiated the studies to evaluate the production capacities of local rabbit breed and finding out the ways to improve rabbit meat production in Albania (Piu and Daija, 2005, Daija et al., 2009, Llambiri et al., 2010, Daija et al., 2009).

In National Action Plan for conservation of animal genetic resources less attention is paid to small animals like as poultries and rabbits. While the interest of market for rabbit meat is growing up. As consequence the farmers interest to farm improved rabbit breeds and commercial hybrids is increasing (Toscano Pagano and Lazzaroni, 2004; Paci et al., 2008. Daija et al., 2009). In such conditions the local rabbit breed is threatened to be extinct and the need for the conservation of this indigenous genetic fund is current. Continuation the farming of local breed rabbits in traditional system could be an effective alternative for the conservation of this genetic fund as well as a real possibility for biological farm development (Paci et al., 2008). The Albanian local rabbit breed is classified in middle breeds group. According to previous studies of (Daija et al.,2009), the rabbits of Albanian local breed have good performances of rearing during fattening period, high feed efficiency and can be successfully farmed in commercial farms. The carcass characteristics are the main criteria's used to evaluate the productive capacities of animals for meat production. Special attention is paid to study these characteristics of local rabbit breeds and their crosses with genetically improved breeds reared in different systems from different authors. (Metzger et al.,2003a, Paci et al., 2008, Bawa et al., 2008, Pinheiro et al., 2008, Ouyed

and Brun, 2008, Elamin et al., 2011a, Elmaghraby, 2011).

MATERIALS AND METHODS

Animals

A total of 116 rabbit, 64 male and 52 female, of local breed farmed in traditional and conventional systems, and commercial hybride (F_1) "Local breed x Californiane" farmed only in conventional system were used in this study. The local breed rabbits kept in traditional system were weaned at age of 37 days. The local breed and commercial hybrid (F_1) rabbits kept in conventional system were weaned at age of 35 days.

The rabbits of local breed farmed in traditional system of family farms, were housed in wooden cages equipped with roof and plastic wired floor placed one meter high from the ground. Sixteen rabbits per cage or eight animals for square meter were kept. The cages were placed in open areas near the farmer's house.

The rabbits farmed in conventional system were housed in metallic cages, in density of 6 animal/m^2.

The cages were placed in closed environment equipped with ventilation system. In traditional system the feed ration during all fattening period was alfalfa hay given ad libido and combined with some fresh alfalfa depending on the season. Some limited quantity (20g/day/head) of grains like as wheat, barley and other farms waste were also provided. Drinking water was available.

The rabbits farmed in conventional system were fed with 35 g/day concentrate feedstuff constituted by rise, oat and wheat (12.5% crude protein, 12.5% ether extract, 7.9% crude fibre). Alfalfa hay was given ad-libido. The feed ration used 2375 kcal/kg ME and 16% crude protein). Good quality drinking water was available continuously from nipples.

Carcass traits

The warm carcass were weighed 30 minutes after sloughtering and stripping. To study the carcass characteristics the measures were done acoording to the methodology decribed by Blasco et al. (1996). In the warm carcass weight were not included the weights of: blood, skin and the terminal portion of the tail, the extremities of the front and hind legs, gastro-intestinal and uro-genital tract. It were included the weights of: head, liver, kidneys, heart, lungs, ezofagues and tracheas.

The carcas was divided in accordance with WRSA normes (Blasco et al., 1996).

The weights of the front porpotion (shoulder and the legs) and the back porpotion (the rear thighs and sacrum part) were expresed as percentage of the reference carcas weight.

The reference carcas weight was calculated as difference between the weight of hot carcas and that of all interior organs.

The technic ratio was estimated as pecentege of hot carcass weight to live weight.

The reference ratio was estimated as percentage of reference carcass weight to live weight

The traits and statistical analysis

The analysis concerned the following variables: WbF-Live weight before fasting (g), LWS-Live weight at slaughter (g), HCW- Hot carcass weight (g), RCW-Reference carcass weight (g), TSH-Two shoulders (g), HL-Hind legs (g), GP-Giblet (%) (Heart, liver, kidneys). Inedible parts and rapport Inedible: edible, were evaluated.

The carcasse traits were estimated by the last squares means and analyses of variance with the fixed effects of genotype, sex, season and rearing system using the procedure of GLM (Statgraf Centurion ver. IX):

$$Y_{ijkln} = \mu + g_i + s_j + c_k + r_l + (gs)_{ij} + (gc)_{ik} + (gr)_{il} + (sr)_{jl} + e_{ijkln}$$

where :

Y_{ijkln} - carcass traits

μ - Theoretical average

g_i - effect of genotype (i=1,2)

s_j - sex effect (l=1,2)

c_k - season effect (k=1,2)

r_l - effect of rearing system (k=1,2)

$(gs)_{ij}$ - interaction effect "genotype x sex"

$(gc)_{ik}$ -interaction effect "genotype x season"

$(gr)_{il}$ - interaction effect "genotype x rearing system"

$(sr)_{jl}$ - interaction effect "sex x rearing system"

e_{ijkln} - residual effect.

RESULTS AND DISCUSSIONS

The ANOVA results, df, residual mean squares, and tests of significance for carcass traits are presented in Table 1. Genotype was significant for all traits (P<0.05, 0.01). These results are in agreement with those reported by Ozimba and Lukefahr, 1991, Ouyed and Brun, 2008. The effect of sex was not significant (P>0.05) for TSH and HL. Male rabbits tended to have a higher WbF, LWS and HCW (+11%) than did female rabbit. It was the same situation with that reported by (Ozimba and Lukefahr, 1991; Yakubu et al., 2007), Gasim-Boubaker et al. 2007). The effect of season of birth was significant for WbF, LWS and HL (P<0.05). The effect of rearing system was significant for all traits and it was the most important factor that affected the variance of carcass traits. This result was in agreement with those reported by Bergoglio et al. 2004, Pinheiro et al., 2008, Paci et al., 2008.

The "genotype x rearing system" interaction affect was significant for all traits (P<0.05, 0.01), meanwhile "genotype x season" was not significant (P>0.05), the "sex x rearing system" interaction was not significant for HL and GL, only (P>0.05) and the "genotype x sex" interaction affect was significant for WbF, LWS, HCW, TSH and HL (P<0.05) (Table 1).

Table 1. Degree of freedom, tests of significance and residual main squares for carcass traits[a]

Item	df	WbF	LWS	HCW	RCW	TSH	HL	GP
Genot.	1	**	**	**	*	*	*	*
Sex	1	*	*	*	*	NS	NS	*
Season	1	*	*	NS	NS	NS	*	NS
Rearing system	1	**	***	**	*	**	**	*
Genot. x Sex	1	*	*	*	NS	*	*	NS
Genot. x Season	1	NS	NS	NS	NS	NS	NS	NS
Genot. x Rearing system	1	**	**	**	*	*	*	*
Sex x rearing system	1	*	*	*	*	*	NS	NS
Residual	109							
Residual mean square		9.672	10.106	8.762	8.659	2.125	3.542	0.09
Model R^2		.71	.74	.63	.66	.58	.61	.65

[a]Trait abbreviations: WbF - Live weight before fasting (g), LWS- Live weight at slaughter (g), HCW- Hot carcass weight (g), RCW- Reference carcass (g), TSH- Two shoulders (g), HL- Hind legs (g), GP- Giblet (%) (heart, liver, kidneys)
NS-non significant, *P<0.05, **P<0.01, *** P<0.001

Least squares means

The least squares means and their standard errors of WbF, LWS and carcass traits are presented in Table 2.

Table 2. The least squares means and their standard errors for WbF, LWS and carcass traits

Item	Local bred		Local breed x Californian breed
	Traditional system	Conventional system	Conventional system
Rabbit (n)	36	36	44
Live weight before fasting, g	2088±189[a]	2361±21[b]	2543±242[c]
Live weight at slaughter (LWS),g	2040±194[a]	2314±207[b]	2493±237[c]
Hot carcass, (HC), g	1213±74[a]	1391±84[a]	1547±89[c]
Reference carcass, (RC), g	1008±69[a]	1140±72[b]	1253±72[c]
Giblet (heart, liver, kidneys), g	100.2±3.6[a]	119.3±3.2[b]	133.3±3.6[c]
% RC	*9.6*	*10.42*	*10.58*
Head, g	115.2±4.1[a]	138.5±4.7[ab]	146.4±5.0[b]
% HC	*8.7*	*9.8*	*9.6*
Two shoulders	164±5.2[a]	195±5.8[b]	246±6.1[c]
% RC	*16.2*	*17.3*	*20.3*
Hind legs	336.2±7.2[a]	398.6±7.9[b]	438±8.6[c]
% RC	*33.4*	*34.2*	*35.3*
Dressing yield			
Hot carcass, %	59.46±0.2[a]	60.12±0.2[a]	62.03±0.3[b]
Reference carcass, %	49.13±0.2[a]	49.28±0.2[a]	50.26±0.2[b]
Total inedible parts	682.3±35[a]	775.1±42[a]	856.2±47[b]
%	*33.4*	*33.5*	*33.9*
Inedible: edible	1:1.78[a]	1:1.79[a]	1:1.81[a]

These results are comparable with those reported by Bergoglio et al., 2004; Pinheiro et al., 2008; Paci et al., 2008; Mehrez and Mousa, 2011, at the end of fattening period (age of 101 days) the rabbits of local breed farmed in conventional system realized around 13% higher WbF that one in traditional system. The same differences were noted and in HC and RC weights. Meanwhile the differences of 13-15 % higher of TSH and HL of local breed rabbits reared in conventional system were almost because of better feeding. The feeding ration

has affected to better musculature development in comparison with rabbits reared in traditional system. The same situation is referred by Paci et al., 2008. Commercial hybrid F_1 rabbits had higher carcass performances in comparison with rabbits of local breed. Their hot carcass and reference carcass weights were 11-25% heavier than them of local breed rabbits and the same phenomena could be noted for TSH and HL that were about 15-33% heavier. The data reported point out the positive effects of industrial crosses and rearing system on the improvement of rabbit meat production. Similar results are reported from Ozimba and Lukefahr, 1991; Raddy and Eady, 2002; Prayaga and Eady, 2003; Larzul et al., 2005; Dimitrova et al., 2008; Llambiri et al., 2010.

The dressing yield indicators had similar values to all rabbits of local breed despite of rearing system. In contrast the commercial hybrid rabbits had higher dressing yield ($P<0.05$). The same situation was for total inedible parts, while the ratio "Inedible: edible" did not have significant difference ($P>0.05$). The least squares means for HC (%) and RC (%) were comparable with them reported by the literature about rabbits of middle breeds group (Metzger et al., 2003b; Paci et al., 2008; Ghosh and Mandal, 2007; Ouyed and Brun, 2008; Yakubu et al., 2007; Ekpo et al., 2009; Elamin et al., 2012). The estimation of means and variation coefficients of internal body organs and the weights of head blood and fur are given in Table 3.

The estimated mean values are comparable with them reported by (Ghosh and Mandal, 2007). The significant differences of these values of rabbits of different genotypes and farmed in two systems are rather low. The same situation is reported by Ghosh and Mandal, 2007, Elamin et al., 2011b.

The values of variation coefficients are within 6.0–11.2% edges being lower than them reported by Ghosh and Mandal (2007).

Table 3 Statistical means of giblets and other carcass traits

	Local breed				Local breed x Californian breed	
	Traditional system		Conventional system		Conventional system	
	Male	Female	Male	Female	Male	Female
Rabbit (n)	20	16	20	16	24	20
Liver	76.7±3.1^{a}	72.1±3.1^{a}	91.4±4.6^{b}	86.5±4.0^{b}	104.2±4.8^{d}	96.7±4.3^{b}
%RC	*7.1*	*7.5*	*7.6*	*7.9*	*7.8*	*8.0*
Kidneys	17.2±0.9^{a}	15.3±0.5^{b}	21.2±0.9^{c}	18.3±0.9^{a}	22.4±1.0^{c}	20.5±0.9^{c}
%RC	*1.58*	*1.63*	*1.79*	*1.67*	*1.71*	*1.74*
Heart	10.0±0.2^{a}	9.2±0.1^{a}	10.5±0.1^{a}	9.5±0.1^{a}	11.2±0.2^{b}	9.8±0.1^{a}
%RC	*0.91*	*0.98*	*0.87*	*0.87*	*0.85*	*0.83*
Lunngs	17.3±0.6^{a}	15.4±0.7^{b}	18.6±0.7^{ac}	16.2±0.6^{b}	20.1±0.5^{c}	18.4±0.6^{ac}
%RC	*1.61*	*1.64*	*1.58*	*1.49*	*1.54*	*1.56*
Blood, g	60.3±3.87^{a}	59.7±4.25^{a}	60.1±5.02^{a}	61.5±5.12^{ab}	67.2±4.87^{c}	66.8±5.11^{c}
% LWS	*2.78*	*3.14*	*2.63*	*2.78*	*2.58*	*2.85*
Pelt, g	284±8.3^{a}	259±8.6^{a}	365±8.1^{b}	312±6.7^{c}	384±5.1^{b}	346±5.9^{b}
% LWS	*13.14*	*11.65*	*15.21*	*14.31*	*14.6*	*14.8*
Feet and tail, g	82±4.1^{a}	78±4.3^{a}	87±4.5^{b}	82±5.2^{a}	92±5.3^{c}	86±5.1^{b}
% LWS	*3.81*	*4.12*	*3.65*	*3.67*	*3.63*	*3.66*
Spleen, g	2.03±0.1^{a}	1.91±0.1^{a}	2.15±0.2^{a}	2.09±0.1^{a}	2.24±0.1^{b}	2.12±0.1^{a}
% LWS	*0.09*	*0.10*	*0.09*	*0.92*	*0.86*	*0.09*
Lungs and trachea, g	22.4±1.6^{a}	20.1±1.3^{a}	24.2±1.1^{b}	23.8±1.4^{b}	28.6±1.3^{c}	26.3±1.3^{c}
% LWS	*1.04*	*1.06*	*1.02*	*1.06*	*1.09*	*1.12*
G.I. tract full.g	264±7.5^{a}	232±6.9^{b}	272±5.3^{c}	246±6.1^{ab}	302±5.3^{d}	284±5.2^{c}
% LWS	*13.61*	*13.8*	*11.4*	*11.6*	*15.6*	*12.3*

Means with different letters on the same row differ significantly.

Figure 1. View from a rabbit Farm in Albania

CONCLUSIONS

Genotype, sex and rearing season effected significantly on the variation of carcass characteristics. Genotype, the farming system and the interaction "genotype x rearing system" had higher effects on these variations.

The rabbits of local breed farmed in conventional system had higher meat production performances. The positive effect of this rearing system did not appear on dressing yield. Carcass characteristics of rabbits of Albanian local breed were comparable with respective ones of middle group breeds despite of rearing system.

The commercial hybrid reared in commercial farms had better carcass performances and higher dressing yield than rabbits of local breed.

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GREEN HOUSE GASES EMISSIONS FROM NONRUMINANTS

Dana POPA, Răzvan POPA, Marius MAFTEI, Monica MARIN, Carmen NICOLAE, Livia VIDU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: poparasvan@yahoo.co.uk

Abstract

The paper aimed to present the evolution of green house gases emissions from entheric fermentation and manure management at nonruminants Romanian livestock during the period 2014 -2017. The emissions are based on the data provided by National Institute for Statistics. The data have been processed into the following indicators: nonruminants livestock, number of: breeding females (sows), swine youth categories as piglets (under 20 kg and 20-50 kg), fattening swine, breeding swine, horses, for entheric fermentation. For manure management were including two poultry categories: broilers and adult laying chickens. All categories included in this study were in accordance with IPCC Good Practice Guidance and Uncertainty Management in National Greenhouse Gas Inventories, 2006, and parameters used in equations have national values (gross energy intake, digestible energy, EF, MS, N_{ex}). The researchproved that the green house gases emission trend from entheric fermentation and manure management were descending, due to the decrease in the number of animals, and due to conversion to sustainable agriculture sustained by government subsidies for environmental measures.

Key words*: greenhouse gas, emissionss, entheric fermentation, manure management, nonruminants*

INTRODUCTION

Methane from enteric fermentation is the product of microbial activity from the animal rumen. The amount of methane produced in the enteric fermentation is positively correlated with the animal live weight, production and thus the quantity and quality of food intake in order to achieve the production concerned. In conditions of normal feed, methane is 15-30% of the total ruminal gas (a mixture of carbon dioxide, methane, hydrogen, nitrogen, etc.). The proportion of these gases varies according to feed nature and the fermentation intensity. The production of ruminal methane is not directly proportional to the consumed feed digestibility. Feed with high digestibility form less methane per unit of caloric energy consumed, than those with lower digestibility (Cristea, 1985) In other words, if the energy intake have higher value, the amount of methane from enteric fermentation will be higher.

On the other path, animal waste is a major source of anthropogenic greenhouse gases emissions, most of which is methane and nitrous oxide. Regarding methane, manure resulting from rearing of economic interest animal species contributes with 5-10% of the total emissions (IPCC, 2006).

The natural degradation of animal waste during storage leads to the release of methane into the atmosphere, as a result of the anaerobic degradation of organic matter. The methane emissions from entheric fermentation and manure management is higher in cold season due to value of food ratio which contains more energy from feed used and more manure quantity kept on platform than in grazing season.

Nitrous oxide (N_2O) accounts for approximately 5% of total greenhouse gases from human activity. This compound is naturally occurring in the atmosphere, as part of the nitrogen global cycle, and it also has a wide variety of natural sources. A number of human activities, such as: agriculture, fossil fuel combustion, waste water management and industrial processes increase the amount of nitrous oxide in the air. These molecules remain in the atmosphere for 114 years until they are removed by rain or destroyed by various chemical reactions at this level. The contribution of nitrous oxide to global warming is about 300 times higher than that of carbon dioxide.

In agriculture, sources of nitrous oxide pollution are represented by the use of synthetic fertilizers and manure management. Soil management is the main source of pollution, accounting a total of approx. 72-74% of the total emissions, while the degradation of animal waste from species of economic interest contributes with approx. 5%.

MATERIALS AND METHODS

The primary data used in this report were provided by the National Institute of Statistics, EUROSTAT and FAOSTAT.

To estimate the methane emissions from entheric fermentation and manure management, livestock'sdata have been corrected with the "days of exploitation" factor that is specific to each subcategory of use within species. This correction factor refers to the number of days in a year, during which the animal is exploited and it is applied to youth categories. The correction of the livestock was made based on the following relation:

$$\text{AAP} = \text{Days of life} * \left(\frac{NAPA}{365}\right) \quad (1)$$

where:

AAP = average annual population;

NAPA = number of animals produced annually.

The methane emission from manure management was calculated using method 2 from the IPCC 2006; national data are available for GE, DE and EF. The methane emission was calculated based on equations 10.19, 10.20, 10.21 of the IPCC Good Practice Guidance and Uncertainty Management in National Greenhouse Gas Inventories, 2006:

$$Emissions = EF_{(T)} * \frac{N_{(T)}}{10^6}$$

where:

Emissions= methane emissions from enteric fermentation, Gg CH_4/year;

$EF_{(T)}$= emission factor for the defined livestock population, kg CH_4 / head/ year;

$N_{(T)}$= the number of head of livestock species / category T in the country;

T= species or category of livestock.

$$\text{Total } CH_{4\ \text{ENTERIC}} = \sum_i E_i$$

where:

Total $CH_{4\text{Enteric}}$= total methane emissions from enteric fermentation, Gg CH_4/year;

E_i= the emissions for the i^{th} livestock categories and subcategories.

$$\text{EF} = \left[\frac{GE * \left(\frac{Ym}{100}\right) * 365}{55.65}\right]$$

where:

EF= emission factor, kg CH_4/head/year;

GE= gross energy intake, MJ/head/year;

Ym= methane conversion factor, per cent of gross energy in feed converted to methane;

55.65 (MJ/kg CH4) = the energy content of methane.

The N_2O emissions from animal waste were calculated according to equation 10.25, of IPCC 2006:

$$N_2O_{D(mm)} = \left[\sum_S \left[\sum_T \left(N_{(T)} * Nex_{(T)} * MS_{(T,S)}\right)\right] * EF_{3(S)}\right] * \frac{44}{28}$$

where:

$N_2O_{D(mm)}$ = direct N_2O emissions from manure management in the country, kg N_2O/year;

$N_{(T)}$ = number of head of livestock species/ category T in the country;

$Nex_{(T)}$= annual average N excretion per head of species/category T in the country, kg N/animal/year;

$MS_{(T,S)}$ = fraction of total annual nitrogen excretion for each livestock species/category T that is managed in manure management system S in the country;

$EF_{3(S)}$ = emission factor for direct N2O emissions from manure management system S in the country, kg N_2O-N/kg N in manure management system S;

S = manure management system;

T = species/category of livestock;

44/28 = conversion of (N_2O-N)(mm) emissions to N_2O (mm) emissions.

The nitrogen excretion (N_{ex}) was calculated according to the equation 10.30 of IPCC 2006 (table 18), using N_{rate} (table 10.19, the IPCC guide) as default value, and national values for animal live weight.

$$\text{Nex}_{(T)} = N_{rate(T)} * \frac{TAM}{1000} * 365$$

where:

$Nex_{(T)}$ = annual N excretion for livestock category T, kg N/animal/year;

$N_{rate(T)}$ = default N excretion rate, kg N/1000 kg animal weight/day (table 10.19 IPCC, 2006);

$TAM_{(T)}$ = typical animal mass for livestock category T, kg/animal.

Table 3 indicates the N_{ex} values after applying equation 10.30.

For $EF_{3(S)}$, the emission factor for direct N_2O emissions from the *S* manure management

system, kg N_2O-N/kg N of the *S* manure management system, were used default values of the IPCC guide, listed below:

- Pasture/paddock for horses – 0.001
- Daily spreading (horses) – 0
- Solid storage (horses, poultry) – 0.005
- Sludge/liquid (all species) – 0.005
- Poultry with/without bedding – 0.001
- Pit storage – 0.002

For the calculation of each GE (gross energy intake) value, based on the exploited species and category, an average ration was considered, both in summer and in winter.

The ration can provide the necessary maintenance (allow normal operation of the animal body, at basal metabolism level, providing vital functions), and the need to develop productions for cattle, buffaloes.

It should be made clear that the data provided by the National Institute of Statistics do not make the difference between exploitation systems (intensive, semi-intensive, extensive, subsistence), between the exploitation (farms or individual households) and their size, as well as between various management types (occurring depending on the farm size, species and categories of animal exploited), and as a result, the values of energy gross intake (GE) have been established linking the nutritional requirements of each exploitation species and category with the nutritional content of the rations and the average recipes that are considered (expert's opinion) to ensure the productions of the official data (NIS).

When calculating the caloricity of the energy gross intake of each recipe or ration, the following equivalences were considered (Stoica, 1997):

1 g crude protein = 5.72 kcal; 1 g crude fat = 9.5 kcal; 1 g crude fiber =4.79; 1 g SEN (non-nitrate extractable substances) = 4.17 kcal.

The GE calculation formula is (Stoica, 1997):

GE (kcal/kg) = 5.72·GP + 9.5·GB + 4.79·CelB + 4.17·SEN, where: GE = gross energy intake; GP = crude protein; GB = crude fat; CelB = crude fibers; SEN = non-nitrate extractable substances.

The rations were established according to the equation above, and the values of crude protein, crude fat, crude fiber and non-nitrate extractable substances were taken from the tables with the feed chemical composition (Stoica, 1997).

The percentage of digestible energy (DE%) of raw energy is calculated by applying the cross-multiplication rule, according to the following relation: DE % = (DE/GE) x 100.

RESULTS AND DISCUSSIONS

The values used for calculation of methane emission from enteric fermentation are presented in Table 1.

Table 1. The values used for calculation of methane emission from enteric fermentation

SPECIFICATION	AAP (thousands head)				GE (MJ/day)	DE (Mj/day)	Ym	EF	Days of life
	2014	2015	2016	2017					
Piglets < 20 kg	133.22	122.20	120.55	114.28	8.18	6.7	13.00	6.97	56
Piglets 20-50 kg	346.84	344.20	324.06	305.70	13.49	11.7	13.00	11.50	75
Fattening pigs	441.60	431.94	412.73	389.35	46.86	40.66	13.00	39.96	100
Boars	7.07	5.600	6.10	5.80	45.32	39.3	13.00	38.64	365
Sows	343.61	374.60	361.20	342.00	45.34	37.7	13.00	38.66	365
Horses	524.74	503.46	541.23	511.19	225.79	121.84	2.50	37.02	365

Table 2. CH_4 emissions from entheric fermentation

SPECIFICATION	Emissions of CH_4 (Gg)			
	2014	2015	2016	2017
Piglets < 20 kg	0.929	0.852	0.841	0.797
Piglets 20-50 kg	3.989	3.959	3.727	3.516
Fattening pigs	17.644	17.259	16.491	15.557
Boars	0.273	0.216	0.236	0.224
Sows	13.284	14.482	13.964	13.221
Horses	19.428	18.640	20.038	18.926

The number of animals has decreased in the analyzed period from 5.041.7thousand pigs in the year 2014 to 4441.1 thousand heads in the year 2017, and the methane emissions following the same descendent trend. The methane emission trend from enteric fermentation is descending due, on the one hand, to the decrease in the number of animals, and on the other hand, due to the technological improvements at farms level and genetic improvements, at animal level. The data used for calculation of N_2O emissions from manure management are presented in Table 3.

Table 3. The values used for calculation of N_2O emissions from manure management

SPECIFICATION	N_{ex} kgN/head/ year	Management system (MS)						
		Pasture	Daily spread	Solid storage	Anaerobic lagoons	Pit storage<1m	Poultry with bedding	Poultry without bedding
Piglets < 20 kg	2.3506			0.2	0.45	0.35		
Piglets 20-50 kg	5.8765			0.3	0.4	0.3		
Fattening pigs	22.0825			0.15	0.45	0.4		
Boars	45.333					1		
Sows	20.9875			0.2	0.44	0.36		
Horses	54.75	0.7		0.3				
Laying hens	0.53874		0.25					0.75
Broilers	0.8833		0.23				0.77	

Table 4. N_2O emissions from manure management

SPECIFICATION	Emissions of N_2O (Gg)			
	2014	2015	2016	2017
Piglets < 20 kg	0.0127	0.0116	0.0115	0.0109
Piglets 20-50 kg	0.0639	0.0634	0.0597	0.0563
Fattening pigs	0.2125	0.2079	0.1986	0.1874
Boars	0.0010	0.0008	0.0009	0.0008
Sows	0.0444	0.0484	0.0467	0.0442
Horses	0.3837	0.3682	0.3958	0.3738
Laying hens	0.0271	0.0277	0.0259	0.0199
Broilers	0.0040	0.0043	0.0043	0.0058

The nitrous oxide emission from poultry manure recorded for the *broilers* subcategory an almost constant trend (although the regression line shows a slight decrease in emissions, due to a better poultry manure management, dissolution of large poultry complexes), but in the last year recordered an increase trend, due to market request for this type of meat. For the *laying chickens* subcategory, direct nitrous oxide emissions from manure management are placed descending trend, due to laying chickens decrease number. The N_2O emission decreased during the analysed period, due to livestock decrease, as well as due to the organization of these livestocks in farms and complexes that practice manure management systems, in accordance with the legislation on environmental protection and on polluting emissions reduction.

CONCLUSIONS

These significant emissions decreases are not only due to the decrease of animal livestock, but also due to cancellation of rearing this species in individual households (there is a trend to give up rearing pigs in the households, but it is also a tradition), on the one hand due to the economy (the price of pork is affordable,

compared to beef price, for example), on the other hand, because of feed which if it's not produced by the same farmer/landowner, may be less affordable when they are purchased from a different producer (there were dry years when the produced feed were preserved for animal feeding).

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COMPARISON OF SOME PROPERTIES OF DIFFERENT SUPER SISTER QUEEN GROUPS IN BUMBLEBEE

Ramazan OZŞAHIN, Ayhan GOSTERIT

Department of Animal Science, Faculty of Agriculture, Süleyman Demirel University, Isparta, Turkey

Corresponding author email: ayhangosterit@sdu.edu.tr

Abstract

Commercial bumblebee colonies have been used for the pollination of a number of crops, mainly of tomatoes (95%). In comparison with other bumblebee species, Bombus terrestris has the most year round rearing. The year round rearing procedure of B. terrestris involves some main stages: colony foundation, obtaining of young queens and males, mating and diapause control. Success of these stage are affected by environmental conditions, physiological properties and genetic structure ofthe queens. Knowledge of the effects of these factors will increase the rearing success. Because of the B. terrestris queens mate once, female individuals in their colonies are supersister. There is a 75% genetic relatedness between the supersister young queens. This study was conducted to compare the different super sister queen groups and to determine the effect of genotype on weight, mating performance and diapause performance of queensin B. terestris. Results showed that genetic differences affected the copulation duration and pre and post diapause weight of queens, but survival ratio during the diapause duration did not affected by genotypic differences.

Key words*: bumblebee, Bombus terrestris, genotype, supersister queen.*

INTRODUCTION

The majority of food consumed by humans are provided from some plant species, and 75% of these plant species are pollinated by bees (Klein et al., 2007). Honeybees *(Apis mellifera* L.*)* are most effective pollinators for both natural and cultivated plants. In addition to honeybees, bumblebees have incontrovertible importance among the pollinator insects because of their some features such as long tongue, large and hairy bodies, and high pollinator capacity. Currently, about 250 species of bumblebees have been determined and five of these (*Bombus terrestris, B. lucorum, B. ignitus, B. occidentalis* and *B. impatiens)* are commercially reared. *B. terrestris* is most commonly reared bumblebee species commercially and used for pollination agent (Velthuis and van Doorn, 2016). This species is indispensable pollinator, especially for greenhouse tomato production, because of its pollination efficiency, which reduces pollination labor costs and improves the quality and quantity of crops (Ahmad et al., 2015).

In bumblebees, the expression of artificial rearing is called in different names such as the mass rearing, the rearing under controlled condition, the laboratory rearing or the year round rearing. This rearing procedure of bumblebee takes place in four main stages: colony foundation from queens, obtaining of young queens and males from colonies, mating of queens and males, and controlling of diapause (Beekman et al., 2000; Velthuis and van Doorn, 2006). These stages should be realized in controlled conditions for sustainability of production. Some losses occur in each of these stages in mass rearing procedure of *B. terrestris*. Especially, losses in colony initiation stage are critical for success of mass rearing. One of the main criteria is how many of 100 queens can establish colonies suitable for pollination in mass rearing. Pollinator colony production ratio is usually ranges from 30% to 40%. This low ratio leads to increasing of the production cost and colony price (Gosterit and Gurel, 2016).

Like many other organisms, two main element affect the phenotypic value of bumblebee in terms of any characteristic: genotype and environment (Vogt et al., 2008). Traits related with colony foundation success and colony development are phenotypic characteristics in *B. terrestris*. There are significant variations in the egg laying performances and colony foundation success of queens, in the total number of workers, males and young queens produced by

colonies, and in the time of production of sexuals (male and young queens) in *B. terrestris* (Duchateau et al., 1988; Gosterit, 2011). While some queens egg lay, some queens do not egg lay after diapause. On the other hand, male production occurs at variable times of colony development, either early in colony development before the start of young queen production or toward the end of the colony life cycle after the start of young queen production. Under controlled conditions, the part of the colonies produce only males or only queens, other colonies produce both males and queens, while other colonies produce neither males nor queens (Duchateau and Velthuis, 1988; Cnaani et al., 2000; Alaux et al., 2005; Lopez-Vaamonde et al., 2009; Gosterit, 2011).

The effects of many factors such as mating success of founder queen, environmental condition, availability and quality of food, diapause duration, parasites and diseases, and nest area on these variations related with colony development were studied by different researchers (Amin et al., 2010; Saglam and Gosterit, 2015; Imran et al., 2017). Colony development traits of different native populations were compared to determine the effects of genetic differences (Gosterit and Gurel, 2005; Gosterit, 2017).

A honeybee colony comprises a superfamily of three worker groups: supersisters, full sisters, and half sisters. The relationship is 75%, between the super sisters, 50% between full sisters and 25% between half sisters (Harbo and Rinderer, 1980). In contrast to honeybees, *B. terrestris* queens mate once, and their colonies only include supersisters (Schmid-Hempel and Schmid-Hempel, 2000; Gosterit, 2016). As a result, female individuals produced by *B. terrestris* queens are super sisters, and relationship between these individuals is 75%. This present study was conducted to compare the different super sister queen groups and finally determine the effect of genetic differences on weight, mating performance and diapause performance of queens were aimed.

MATERIALS AND METHODS

Experimental groups

A total of 8 *B. terrestris* colonies that produced males and young queens were used. To prevent the adverse effect of inbreeding on survival rate in diapause (Gerloff and Schmid-Hempel, 2005; Gosterit, 2016), males were obtained from 4 colonies (M: male colony group) which taken from a bumblebee breeder, while young queens were obtained from other 4 colonies (Q: queen colony group) which provided from our laboratory stocks. Super sister queens produced by same colony (Q1) were mated with males produced by other same colony (M1). Similarly, queens produced in Q2, Q3 and Q4 colonies were mated with males produced in M2, M3 and M4colonies in time of their sexual maturity (7 days for queens and 12 days for males), respectively (Amin et al., 2010). At the end of the mating process, four different super sister queen groups were designed.

Mating and diapause control

Queens and males were mated in mating cage in an illuminated room (23 ± 1°C and 50 ± 5% RH). Copulation duration of queens were determined following the method reported by Gosterit and Gurel (2016). Immediately after the initiation of copulation, the time was noted and each mating pair was separately transferred to a transparent plastic box. As soon as copulation was terminated, the time was noted again and copulation duration was calculated. All mated queens were weighted on an electronic balance (0.001 g sensivity) to obtain pre diapause weight of queens. Then, all mated queens were put into artificial diapause at 2.5^{o}C for 2 months. At the end of the diapause duration, survival ratio of queens was determined and surviving queens were weighted to obtain their post diapause weight.

Statistical analysis

In the experiment, 160 queens (40 queens for each group) were used. Data were square-root transformed and tested for normality before analysis. Survival ratio of queens in diapause were compared two-proportion *z*-tests. One-way analyses of variance were run to determine the effects of genetic differences on weight and copulation duration of queens (Minitab Statistical Software, Version 16.2.4).

RESULTS AND DISCUSSIONS

In *B. terestris*, copulation duration of queens which lasts 20-40 mindoes not affect colony foundation period, but it has significant effects on the number of workers produced in first

brood (Amin et al., 2009; Gosterit and Gurel, 2016). On the other hand, worker number produced in first brood could has directly effect on other colony development patterns, especially for number of individuals produced during the colony life (Gosterit, 2011; 2016). Copulation durations of 160 *B. terrestris* queens (40 queens for each genotype group) were determined in this study (Table 1). According to results, genotypic differences affected the copulation duration. One group was significantly different from other three genotype groups in terms of copulation duration (P<0.01).

Table 1. Copulation duration of queens in different super sister group (a, b: P<0.01)

Groups	N	$\bar{x}$ ±s.e	Min	Max
Q1 x M1	40	38.40±2.46[a]	13.00	77.00
Q2 x M2	40	25.82 ± 1.37 [b]	13.00	49.00
Q3 x M3	40	33.75 ± 1.41 [a]	19.00	57.00
Q4 x M4	40	33.60 ± 1.67 [a]	15.00	62.00

It is clear that quality of the queens directly affects the colony quality in *B. terrestris.* Studies relating to the weight of bumblebee queens have been focused mainly on the effect of the weight on survival during diapause and their colony foundation success. According to Gosterit and Gurel (2007), the weight of the queen after diapause has no effect on variation between the colony development traits.

However, the weight of the queens before diapause affects their survival ratio during diapause. Queens weighing less than 0.6 g before diapause may not survive during diapause according to long duration (Beekmanet al., 1998). Therefore, known of factors affect the weight of young queens is valuable to diapause success. Some factors such as food quality, diseases and social structure of colonies may affect the queen weight. Our results also showed that variation of queen weight was significantly affected by genetic differences (Table 2 and Table 3).

Significant differences were determined between the pre diapause weights of different super sister queens groups (P<0.01). Pre diapause weight of queens changed from 0.520 to 1.118 gram, while post diapause weight of queens changed from 0.571 to 1.069 for al queens used in the experiment.

Table 2. Pre diapause weight of queens in different super sister group (a, b: P<0.01)

Groups	N	$\bar{x}$ ±s.e	Min	Max
Q1 x M1	40	0.984± 0.012[a]	0.705	1.118
Q2 x M2	40	0.852 ± 0.013[b]	0.607	1.010
Q3 x M3	40	0.770 ± 0.018[bc]	0.601	1.019
Q4 x M4	40	0.801 ± 0.019[c]	0.520	1.111

Table 3. Post diapause weight of queens in different super sister group (a, b: P<0.01)

Groups	N	$\bar{x}$ ±s.e	Min	Max
Q1 x M1	39	0.865 ± 0.013[a]	0.648	1.069
Q2 x M2	37	0.799 ± 0.013[b]	0.657	0.943
Q3 x M3	37	0.713 ± 0.018[c]	0.574	0.962
Q4 x M4	35	0.751 ± 0.018[bc]	0.571	1.056

Inbred mating reduces the likelihood that the queen will survive in diapause (Gerloff and Schmid-Hempel, 2005). Survival ratios of queens in different super sister groups were given in Figure 1. In groups, survival ratios were determined as 97.5%, 92.5%, 92.5% and 87.5%, respectively. However, no significant differences were found between the different super sister queen groups in terms of survival ratio during the diapause duration.

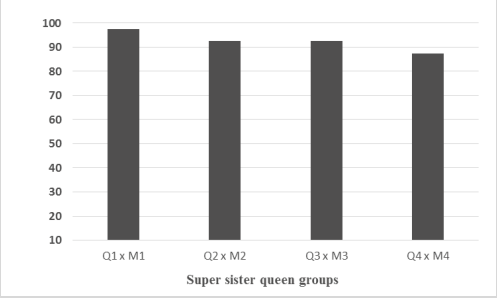

Figure 1. Survival ratio of queens in different super sister group

CONCLUSIONS

Knowledge related to rearing process are needed to sustainability of bumblebee mass rearing. Therefore, it is vital to determine the factors affect the colony development. It is estimated that mating success of queens, environmental conditions, food quality and availability, diseases and parasites, diapause conditions, and genotype of queens and males affect the rearing success. In this study, we investigated the effects of genotype on weight,

mating performance and diapause performance of queens. According to results, significant differences were determined between the different super sister queen groups in terms of mating duration and pre and post diapause weight of queens in *B. terrestris*. However, genetic differences had no effect on survival ratio during the diapause duration.

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STUDY REGARDING MORPHO-PRODUCTIVE TRAITS IN TELEORMAN'S BLACK HEAD SHEEP IN THE SOUTH EASTERN REGION OF ROMANIA

Iulian VLAD[1], Marius MAFTEI[1], Elena POGURSCHI[1], Daniela IANITCHI[1] Mirela CĂRĂTUȘ STANCIU[2]

[1]University of Agricultural Sciences and Veterinary Medicine, 59 Marasti, 1 district, 011464 Bucharest, Romania
[2]„Lucian Blaga"University, 41 Nicolae Teclu, 550200 Sibiu, Sibiu County, Romania

Coresponding author email: vladiul@yahoo.com

Abstract

Current trends for sheep breeders are in a close relation with the european or national financial support. In this case,this species deserve all this support on the characteristic market. In this study we try to put in a better light the performances of an autohtone sheep breed, Teleorman's Black Head sheep, analyzing the body mesurements. We analized all this performances as an average, because only in this way we can have a correct view of population. Ram's body weight was 87.8 ±1.98 kg with an CV%=9.57. Females body weight, in average, was 69.15 ± 0.19 kg with an CV%=5.62. The average performances for wither's height was 72.66±0.59 cm. Croup's height was 74.13±0.47 cm, and average performance for body length was recorded at 82.66±0.62cm. Thoracic perimether was 104.76±0.64 cm, and cannon bone perimeter 8.34±0.06 cm. Chest width had an average performance at 25.24±0.18 cm, and the croup's width at hips was 25.78±0.31 cm The head length was 28.7±0.19 cm, and the head width 17.32±0.10 cm. All this calculated values reveal us an hypermetric conformation with tendencies for brevimorphism.

Key words*: Teleorman's Black Head sheep, milk production, quality, body dimensions.*

INTRODUCTION

The world's sheep population is obviously held by the largest states such as China with over 162 million heads, India with over 75 million head, Australia 67 million head, Sudan 53 million head (Chifres cles ovins 2016, 2017). There is a worldwide trend to increase the sheep population in comparation with the European downward trend recorded after the 1990s with different trends across states.

These trends are based on global and regional market requirements, with some particularities, depending on some general factors such as environmental pollution but also regional restrictive specifics such as sanitation, type of product and its commercial role and type of exploitation, aspects that can make an extensive subject of analysis in the sheep trade but also sheep production.

World sheep meat production has an upward of approx. 1% per annum, especially in Europe and America. In 2016 it exceeded 316 thousand cwe., being bigger in the major producing states such as China and Australia (holding the current world top), as a global vision for 2026 (Perspectives OCDE et FAO, 2017 – 2026).

According to Eurostat (Figure 1) the European sheep flock is owned by; Great Britain (27%), Spain (19%), Romania (12%), followed by Greece (10%), Italy (9%), France (8%), Ireland (4%), Bulgaria (2%), Portugal (2%) and the rest of the countries (4%).

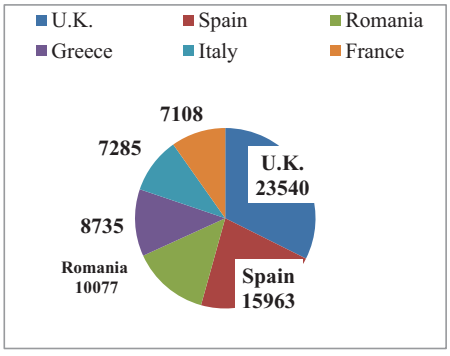

Figure 1. Top six country of sheeps number in EU 28

Considering the competition for milk production between dairy species, meat as a product has become the most important sheep product. In this context, the economic value of sheep meat production is somewhat different; Great Britain with 1,813.46 ths. €, Spain 1099 thousand. €, France 855 thousand €, Greece 642 thousand €, Norway, Ireland and Romania with 215 thousand € (with an annual increasing trend of 0.6% - Eurostat, 2016).
It is considered to be a reinvigoration in sheep breeding, and also a cause for increasing the stock, mainly due to the common and local agricultural policies (Figure 2).

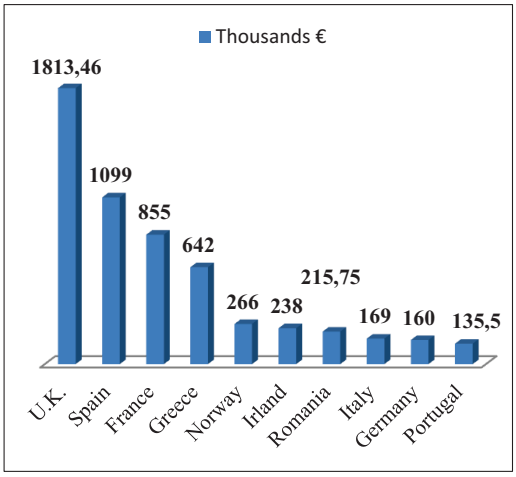

Figure 2. The economic value of sheep and goat meat production in the top 10 EU

In this new condition it seems to be a good opportunity for rustic and mixed breeds, exploited under extensive conditions, such as Teleorman's Black Head Sheep (Draganescu, 1999).

MATERIALS AND METHODS

The biological material was represented by a flock of Teleorman's Black Head Sheep exploited in a farm from Braila County.
The first determination was focused on prolificacy, weight of sheep and rams, average daily gain in lamb and lactation control, respectively the chemical composition of the milk.
Body measurements were performed with the help of specific instrumentation and it was calculated the body indexes.

RESULTS AND DISCUSSIONS

Recorded values, of somatometric measurements, allowed the determination of corporal indexes, in order to confirm the orientation of the population for a particular production. The average body weight was: rams – 87.8 ± 1.98 kg, with a CV% = 9.57%, (min = 72 kg., max = 103 kg), sheeps - 69.15 ± 0.19 kg with a CV% = 5.62% (min = 41 kg, max = 94 kg). The wither's height average performances was 72.66 cm ± 0.59 cm, and for croup's height 74.13 ± 0.47 cm.
The average performance for body length was 82.66 ± 0.62 cm. Thoracic perimeter average performances was 104.76 ± 0.64 cm, and for cannon bone perimeter 8.34 ± 0.06 cm. Average performance for chest width was 25.24 ± 0.18 cm. Crous width at hips was 25.78 ± 0.31 cm. At the head level, head length was 28.7 ± 0.19 cm, and head width 17.32 ± 0.10 cm. By the body indexes point of view we can say that the Teleorman's Black Head Sheep is a population with a strong bone structure, with an antero-posterior ascendant superior line, with and a hypermetric conformation with brevimorfe tendencies. The lateral body format index had a value at 114%. All this characteristics recommend this breed to be used in production of commercial hybrid for meat. Prolificacy is at only 112% (Draganescu et al., 2005). Analyzing Table 1 it is easy to observe the differences between flocks. For measured characters variability was 6 – 8.2%.
Determination for milk production, using Fleischmann method, had reveal a modest average production with 120 kg for 165 lactation days. Lactation curve has a good beggining, starting from the second month, followed by a continous decreasing, with an average daily performance at 0.745 kg (Figure 3). The milk chemical composition has reveal 7.1% fat and 5.54% protein. This values indicate a good efficiency in manufacture of dairy products (Figure 4).
A great variability has recorded also for lactation period. Our oppinion is that all this is due to a weak selection or to the fact that individuals was buyed from different flocks with a small productivity. In this case it is imperious necessary to be applied a continous selection (Figure 5).

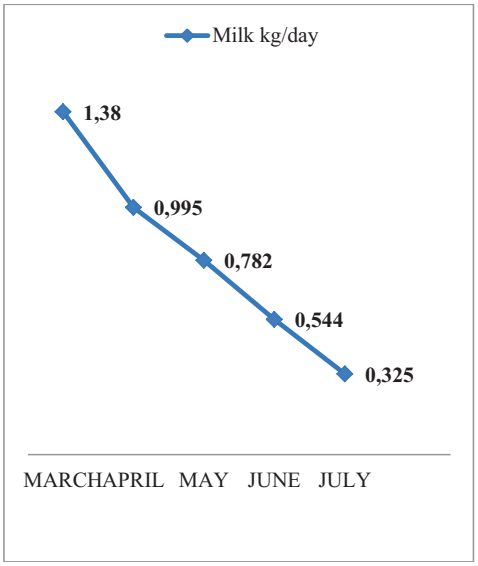

Figure 3. Average dairy production

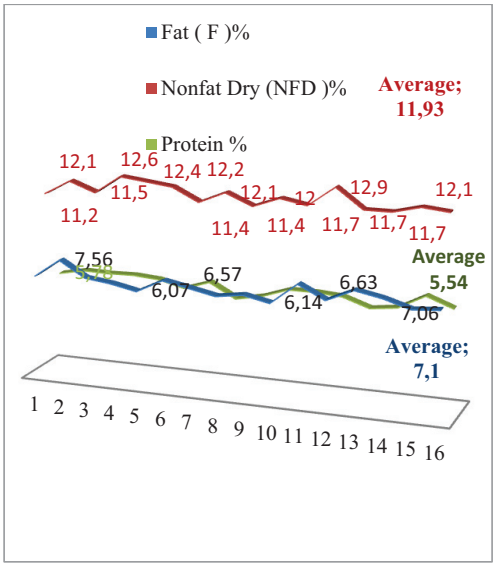

Figure 4. Average milk chemical composition

Table 1. Body development differences between three flocks of Teleorman's Black Head Sheep

Flock	Wither's height	Croup's height	Body length	Thoracic perimeter	Weight
	cm	cm	cm	cm	kg
Braila	**72.60**	**74.13**	**82.60**	**104.76**	**69.15**
Călineşti (Drăgănescu et al.. 2005)	75.74	76.70	88.39	98.08	79.28
Măldăeni 2 (Drăgănescu et al.. 2005)	74.31	76.81	86.81	109.6	76.31
Average value (Drăgănescu et al.. 2005)	75.02	76.75	87.6	103.84	77.8
Differences (%)	*96.77*	*96.59*	*94.29*	*100.9*	*88.88*

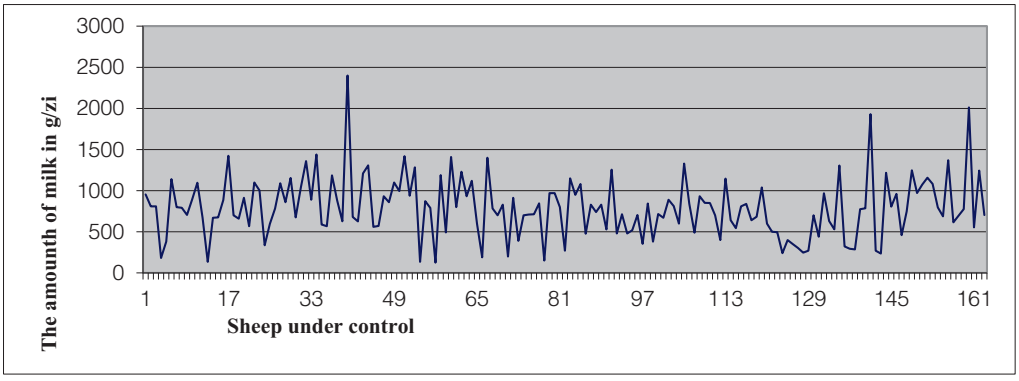

Figure 5. Variation in milk production at first control (g/day)

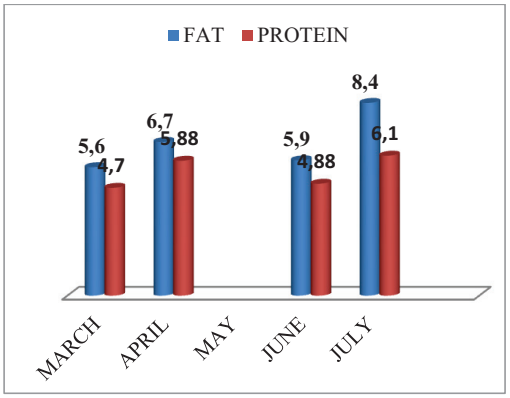

Figure 6. Milk chemical composition in estival period

Fat content had a value at 5.6% in March and 8.4 at the end of lactation. Recorded values, for protein was 4.7% at the beggining of lactation and 6.1 in the last month of lactation (Figure 6). All this are justified by dual feeding. Just in the second part of lactation the feeding is based only on pasture

Analyzing the stages of growth in lambs it was observed different rates. These are due to physiological phenomenon strictly conditioned by dairy consumption in the first period.

We analyse weight at birth, at one week, at 30 days and, for the youth selectionated for reproduction, at 60 days. In these 3 stages we record an average daily gain at 0.275 g for the first week, 0.229 g for the first month and 0.341 g for the next 4 weeks (Figure 7). Modern technologies use weaning at 15 days or maximum one month. in breeds specialized for milk productions (Kukovics, 1998). For mixt and autochthones breeds is not justified such a weaning, without a specific technology for an early feeding (based on enzymatic stimulation and specifical preparation for lambs), especially in the first month.

CONCLUSIONS

Body measurements and body indexes, in Teleorman's Black Head Sheep, highlighted a hypermetric conformation, compared with other autochthones breeds, with a compact bones structure. The body format is rectangular, with brevimorfe tendencies. Regarding the productive parameters, like milk production and milk quality, we must say that the recordered values are weak, being imperious necessary to have a selection for milk production and for udder conformation. The average daily gain in lambs and youth have better values compared with other autohtones breeds. Also for meat production it is necessary to apply an intense selection, especially in that farms who stimulate youth by nutritive point of view. Also it will be a good choice to use the Teleorman's Black Head Sheep in production of commercial hybrids for meat production.

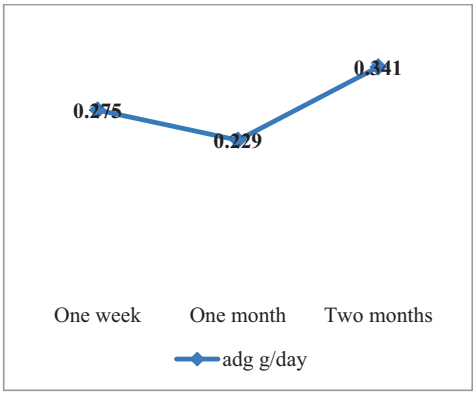

Figure 7. The growth in lambs

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INFLUENCE WATER CONTENT OF BIOGAS SUBSTRATE MADE BY DAIRY COWS FAECES AND RICE STRAW ON THE QUANTITY AND QUALITY OF SLUDGE

Tubagus Benito Achmad KURNANI, Indrani HAMIDAH, SUDIARTO, Yuli Astuti HIDAYATI, Eulis Tanti MARLINA, Ellin HARLIA, Lovita ADRIANI

Faculty of Animal Husbandry, Padjadjaran University, Bandung, Indonesia

Corresponding author email: tb.benito@unpad.ac.id

Abstract

Biogas is an alternative to livestock waste management, however, it leaves a sludge which is contains organic composition that can be used as a solid organic fertilizer. This research aims to know the effect water content of biogas substrate consisting of dairy cow faeces and rice straw to the quantity and quality of sludge. The water content will influence C/N ratio, which is highly dependent on the dry material. Then it can affect the organic materials fermentation. This experimental design using a Completely Randomized Design with three water content treatments, namely, P1 = 60%, P2 = 75%, and P3 = 90. Each treatment has six replicates. The sludge parameters measured were chemicals (N, P, K, Ca, Mg and CEC, heavy metal, microelement) and biological (Salmonella sp. and E.coli). Biological parameters were analysed descriptively. The results showed that the 60% provides higher quantity of sludge compare to 75% and 90%. However, although 60% water content provides chemical parameters concentration similar to 75%, exclude phosphor, but higher count of E. coli and Salmonella sp. than that of 75%. Finally, 90% water content resulted in lower quantity, lower all chemical parameters and higher meters. Therefore, 75% water content provides the best result in quantity and quality of sludge, so it can be used as the best alternative for biogas production in batch system digester.

Key words*: dairy cows faeces, rice straw, sludge, solid organic fertilizer.*

INTRODUCTION

Biogas production was influenced by various factors, such as pH, temperature, dry matter content, oxygen, C/N ratio, agitation, time and activity of microorganisms in a digester. Dry matter is a critical factor for biogas because other factors heavily depend on it. Therefore, the gas production can be measured by the total of dry matter contents in the substrates (Deublein and Steinhauser, 2008). Dry matter is inversely proportional to water content. Thus moisture also is an essential factor to be considered in biogas.

The C/N ratio depends on the water content. The ratio is calculated by the level of dry matter content measured in the number of water contained in the substrate. C/N ratio of units representing organic matter compounds in the carbon and nitrogen element comparisons. Microorganisms use both of these elements in their metabolic processes. Carbon is used as an energy source, while nitrogen is used in protein synthesis. If the nitrogen content is too much and the carbon content is small, then the gas production will decrease. As a result, there is much nitrogen in the mud. Conversely, if the nitrogen content is low and the carbon content is high, the nitrogen will quickly run out, and gas production stops. Dry matter content or moisture also participates in microorganism activity. Each type of microorganism has a water capacity (*aw*) for its activity and life. When it is optimal, the activity of microorganism is also optimal. The effect will also be better for gas production. Thus, biogas processes strongly influenced by the content of dry matter or moisture content. The optimum dry matter content ranged from 7-9% (Panjaitan and Pemayun, 1985) and 25% (Deublein and Steinhauser, 2008).

There are two several ways for making biogas, namely dry fermentation and wet fermentation. Dry fermentation is carried out on a substrate which has a water content less than 85%, whereas wet fermentation is carried out on a substrate with a water content of 85% or more (Deublein and Steinhauser, 2008). However,

the dry matter content of the substrate should not be less than 5% (Werner et al., 1989). Therefore, the water content of the substrate should not be more than 95%. Also, the total substrate material content should be more than 30% (Waltenberger and Krichmayr, 2013). Accordingly, the water content used in the study ranges from 60 to 90%. Under that matter, hence research aims to know the influence of water content of substrate on quantity and quality of sludge which result from making of biogas from a mixture of dairy cow faeces and rice straw.

MATERIALS AND METHODS

The material in this research was used dairy cow faeces and rice straw. Faeces of the dairy cow were obtained from Dairy Production Laboratory, Faculty of Animal Husbandry of Padjadjaran University, while rice straw was carried out from Cianjur, West Java. This experimental study was performed based on Complete Randomized Design (CRD) with three substrate water content treatments, there are P1 = 60%, P2 = 75%, and P3 = 90%. Each treatment has six replicates.

Before starting the experiment, the materials preparation should be done. Faeces dairy cows and rice straw were dried bypassed it with the wind until both materials have 60% water content. Then both of materials are taken samples for analyzed the content of organic carbon and nitrogen. Organic carbon was analyzed by Walkley and Black method, while nitrogen was analysed by Kjeldahl method. Based on the results of the analysis, the calculation of C/N ratio is presented in Table 1. By using C/N ratio 30 (Kurnani et al., 2012), the proportion of dairy cow faeces and rice straws for 1 kg of biogas substrate with 60% water content, there are 0.85 kg and 0.10 kg respectively.

Table 1. Organic carbon and nitrogen concentration of dairy cow faeces and rice straw

Materials	Carbon (%)	Nitrogen (%)	C/N Ratio
Dairy cow faeces	43.16	1.77	24.38
Rice Straw	45.92	1.06	43.32

The biogas substrate was collected then it was incubated in a plastic bag for seven days. After the water content reaches 30% on the substrate, it will be extracted using hot water (90^{o}C). The extraction uses 12 liters of water per 1 kilogram of the substrate. After that, the residue was diluted with water according to the treatment up to a total volume of 150 liters. Each substrate is fed into a 200-liter volume digester. Biogas process will finish in 30 days. The parameters are the quantity and quality of residual sludge of biogas manufacture. The quality of sludge consists of chemical parameters of N, P, K and Cation Exchange Capacity (CEC), heavy metals (As, Hg, Pb and Cd), microelements (Fe, Mn, and Zn) and biological parameters of *E. coli* and *Salmonella sp*.. Chemical parameters were analysed using Spectrophotometer, and the data were analysed statistically using SPSS 17, while the biological parameters were identified by plate count method and the result was described by the descriptive method.

RESULTS AND DISCUSSIONS

Effect of substrate water content on sludge quantity

The quantity of sludge from the biogas manufacture of dairy cow faeces and rice straw varies according to the water content of the substrate. The ANOVA results show that the water content of the substrate affects the sludge quantity produced. To know the differences between the treatments on the sludge quantity deeply, the result of the Tukey-test showed in Table 2.

Table 2. Effect of substrate water content on the quantity of the sludge

Treatment	Sludge (%)	Significance (α<0.05)
P3	0.10	a
P2	0.24	b
P1	0.35	c

The results of this study showed that the water content of P1 produced sludge of 0.35% which was significantly higher than other treatments, there are P2 (0.24%) and P3 (0.08%) respectively. It can be seen that P2 significantly has a higher sludge than P3. Based on that, it can easily to understand that the total sludge is

directly proportional to the substrate dry material.

Effect of substrate water content on the sludge nitrogen

The analysis results of nitrogen content in biogas sludge from dairy cow faeces and rice straw showed that the reactions gave varying results, and then a variety analysis test was conducted to determine the effect (ANOVA). The analysis shows the water content of the substrate influences sludge nitrogen. The effect of treatment was determined using Tukey-test, which showed in Table 3.

Table 3. Effect of substrate water content on the sludge nitrogen

Treatment	Mean	Significance (0.05)
P2	3.47	a
P1	3.25	ab
P3	3.05	b

Percentage of nitrogen in sludge is higher than other minerals. The result of percentage nitrogen in sludge biogas from dairy cow faeces and rice straw after implementation water content showed variance effect. The Tukey test in Table 3, showed that the substrate produces the different effect on the nitrogen sludge. It turns out that the water content in biogas manufacture P2 (75%) gives the highest nitrogen sludge (3.47%). This is because the most of the nitrogen (99%) is present in the sludge, while the residue became a gas, then flew into the air (Apandi, 1979). It is also suggested that about 17% of the total nitrogen are present in the water-soluble form, NH_3, and the remainder is organic nitrogen form. Thus, at 90% water content treatment, most of the nitrogen is released, and therefore the nitrogen in sludge is lower than other water content treatments.

Moreover, the loss of nitrogen occurs through two levels of decomposition, namely aerobic and anaerobic. The aerobic process occurs very quickly, resulting in high calorie and loss of nitrogen in the form of ammonium and nitrate. After the oxygen supply is reduced, the condition will change to anaerobic and facultative anaerobic. In anaerobic conditions, reduced nitrogen release in the compounds form (Ismawati, 2003). Although there are variations of nitrogen, the sludge biogas accomplishing the international standards with the value is greater than or equal to 2.3%.

Effect of substrate water content on the sludge phosphor

The content of phosphor sludge biogas varies from 1.04% to 1.34%. The result of ANOVA showed that the treatment of water content of biogas manufacture from dairy cowsfaeces and rice straw gave a real effect to phosphor sludge biogas content. This analysis was continued with Tukey-test, and the result is presented on Table 4.

Table 4. Effect of substrate water content on the sludge phosphorus

Treatment	Mean	Significance (0,05)
P2	1.34	a
P1	1.11	b
P3	1.04	b

The content of phosphorus in sludge biogas varies from 1.04% to 1.34%. The result of analytical statistic shows that sludge biogas from dairy cow faeces and rice straw gives an effect to the phosphorus content of sludge. P2 with water content 75% can produce 1.34% phosphor higher than 1.04% phosphor in P3. Water content 60% and 90% give similar phosphor content. The content of phosphor in sludge is related to the nitrogen content in the substrate resulted from biological degradation. The microorganisms will be involved with phosphor to build up their bodies, such as cell nuclei and protoplasm. The process involves an enzyme called phosphatase (Stofella and Kahn, 2001).

Effect of substrate water content on the sludge potassium

The analysis of the potassium content of the sludge also varied from 1.23% to 1.27%. To find out a variety of treatment effects analysis of variance (ANOVA) test was conducted. The result of the variance analysis showed that the water content treatment had a significant effect on the potassium content of the sludge. Accordingly, the test is continued with the Tukey-test whose results are presented in Table 5.

It can be seen in the Table 5, P2 with water content 75% results in 1.53% potassium, higher than P1 and P3, whileP1 and P3 have asimilar percentage at approximately 1.2%.

Table 5. Effect of substrate water content on the sludge potassium

Treatment	Mean	Significance (0,05)
P2	1.53	a
P1	1.27	a
P3	1.23	b

The analytical statistic result is significantly differentiated of all treatments. The high number of potassium in the P2 (75%) is caused by the condition of the acidity degree is more suitable for bacterial growth. Already composed at 75% water content, the acidogenic bacteria proliferate to produce volatile fatty acids. These acids can change the pH of the substrate being acid, and in this situation, potassium will release in inorganic potassium form, which is useful for microorganisms in the later fermentation stage. From these data can also be known potassium in sludge biogas is lower than that contained in liquid organic fertilizer (1.76%). It is easy to understand because the element of potassium in the substrate comes washed out during the extraction. Furthermore, the content is lower than that in liquid organic fertilizer, and potassium content is still by SNI 19-7030-2004 (0.20%).

Effect of substrate water content on calcium content of the sludge

ANOVA test was conducted to know the effect of treatment on calcium level. Apparently the treatment of water content in the manufacture of biogas from dairy cows faeces and rice straw affects the calcium sludge content. Therefore, the test is continued with a Tukey test whose results are presented in Table 6.

Table 6. Effect of substrate water content on the sludge calcium

Treatment	Calcium Content	Significance (0,05)
P2	1.26	a
P1	1.21	ab
P3	0.82	c

The Tukey-test results showed that the P2 with 75% water content also produced a calcium content (1.26%) higher than the P3 with 90% (0.82%) water content, but no different from the calcium content of the P1 with 60% (0.21%). Calcium content of 60% water content treatment is not different with the result of 90% water content treatment.

Calcium plays a role in cation exchange, so it has an important role in determining the quality of a fertilizer. In the substrate, most of the calcium comes from rice straw. In the early decomposition, the faeces of dairy cows and rice straw are degraded by microorganisms through enzymatic processes. Calcium in the substrate is utilized by microorganisms as micronutrients and is returned into inorganic calcium when degradation is complete. Inside of substrate, Ca act as a buffer to keep the acidity (Diaz and Savage, 2007). At 75% water content, the process of organic matter degradation is better because the growth of acidogenic bacteria is faster, then resulting more enzymes to degrade crude fibre.

Effect of substrate water content on the sludge magnesium

The average magnesium sludge content ranges from 1.04% to 1.41% in each 90% and 75% water content treatments. To find out the influence of treatment of water content on the sludge biogas made by dairy cows faeces and rice straw on variation magnesium content was conducted by ANOVA. The results of the variance analysis showed that the water content treatment affected the magnesium sludge content. To know the effect of treatments, Tukey-test results are presented in Table 7.

Table 7. Effect of substrate water content on the sludge magnesium

Treatment	Mean	Significance (0,05)
P2	1.41	a
P1	1.26	ab
P3	1.04	b

According to the data in Table 7, it is known that in the 75% water treatment, magnesium content is (1.41%) higher than the 90% (1.04%) water treatment, but no difference with the 60% water content. Like calcium, magnesium also

plays a role in cation exchange in the soil, which is an essential indicator in determining the quality of solid fertilizer. This element is mostly derived from rice straw. In the rice straw, magnesium binds to amylopectin and hemicellulose (Insam and Bertoldi, 2007). Therefore, the availability of magnesium is influenced by the biological straw degradation of rice through enzymatic processes. At 75% water content, microbial activity is higher than 60% and 90% water content in biogas. In general, the microbial activity is higher with water conditions >90%, but in the fermentation process, it can be changed, because other factors can influence the temperature and pH fermentation. In the biogas, fermentation takes place under thermophile anaerobic conditions and acidic pH, so that is only high temperature and acid resistant microorganisms who can survive at this stage. These microorganisms will break down the crude fibre into volatile fatty acids. In this condition, magnesium is released in an inorganic form that will be used by other microorganisms or plants that fertilized with this organic sludge from biogas production.

Effect of substrate water content on cation value of exchange capacity of the sludge

The results showed that biogas sludge water content treatment of dairy cows faeces and rice straw produced average CEC in varied from 31.56 cmol/kg up to 35.88 cmol/kg. To find out whether the variation is influenced by the water content of the substrate at the time of biogas manufacture, the Tukey test was performed the results, and there are presented in Table 8.

Table 8. Effect of substrate water content on the cation exchange capacity

Treatment	Mean	Significance (0.05)
P2	35.42	a
P1	34.88	a
P3	31.56	b

The Tukey-test results in Table 8 show that the 75% water content produced CEC value (35.42 cmol/kg) higher than the 90% (31.56 cmol/kg) water content, but it has a similar result with CEC in 60% water content treatment. Treatment of 75% water content is also not different with 60% moisture treatment. The CEC value is an indicator of nutrient availability that can be absorbed by plants. This value is related to the content of calcium and magnesium and other elements. Also, the value is also related to the degree of organic matter degradation. As the degradation progresses, the particle size of the substrate will decline, so the surface area will increase and can affect to increase the CEC value. Increased surface area of the substrate, usually occurs in the water content of 75%, because the growth of acidogenic bacteria will faster, followed by occurred in 60% and 90% water content.

Effect of substrate water content on heavy metal and other elements contents

The heavy metal content of As, Hg, Pb, and Cd is so small that it cannot be detected by an Atomic Absorption Spectrophotometer. This is due to the initial content of heavy metals in the substrate components (dairy cowsfaeces and rice straw) which is also too small. Similarly with the microelements Fe, Mn, and Zn. The microelements have a low concentration, for example, Fe, Mn and Zn with 90 ppm, 110 ppm, and 40 ppm respectively. The content of these elements is lower than the solid organic fertilizers required. According to the Minister of Agriculture No.70/Permentan/ SR.140/10/2011, namely the value maximum is 500 ppm, 5000 ppm, and 5000 ppm.

Effect of substrate water content on *Escherichia coli*

The number of *E. coli* bacteria on sludge biogas of dairy cow's faeces and rice straw is varied, according to substrate water content, such as consecutive 0.26 x 102 CFU/g for water content 60%, 0.10 x 102 CFU/g for water content 75%, and 0.35 x 102 CFU/g for water content 90%. To determine whether this variation is due to water content, ANOVA is performed. The analysis results showed that the water content treatment significantly affected the number of *E. coli*. Therefore, further testing is done by using Tukey-test which results are presented in Table 9.

Based on the Tukey test result, it was found that the treatment of 90% substrate water content gave *E. coli* (0.35 x 102 CFU/g) significantly higher than *E. coli* due to water content of 75% (0.10 x 102 CFU/g) and water content 60% (0.26 x 102 CFU/g). The lowest number of *E.coli* resulted in 75% water treatment.

Table 9. Effect of substrate water content on the number of *E. coli*

Treatment	Mean	Significance (0.05)
P3	0.35	a
P1	0.26	b
P2	0.10	c

This is due to a better fermentation process at water content. The acidogenic bacteria play a role in the acidogenesis stage grows and develop rapidly, and also produce more organic acids. As a result, pH sludge biogas decreases, so the atmosphere becomes more acidic and being the inhibits for *E. coli* growth. In addition, acid-producing bacteria metabolism increases in temperature 60-70^{o}C. This temperature is not suitable for *E. coli*. The stage of acidogenesis gradually reaches anaerobic conditions faster than other treatments, so *E. coli* bacteria which is gram negative and anaerobic facultative bacteria more quickly decreased.

Effect of substrate water content on *Salmonella sp*.

The number of *Salmonella sp.* have varies from an average of 0.09 x 102 CFU/g to 0.21 x 102 CFU/g. To know whether the water content of 60%, 75% and 90% effect on the number of bacteria *Salmonella sp.* was conducted by ANOVA. The analysis results revealed that the water content treatment affected the number of *Salmonella sp.* in sludge biogas from dairy cowsfaeces and rice straw. Accordingly, the test is continued by using the Tukey-test whose results are presented in Table 10.

Table 10. Effect of substrate water content on *Salmonela sp*.

Treatment	Mean	Significance (0.05)
P3	0.21	a
P1	0.14	ab
P2	0.09	b

Tukey-test results in Table 10 provides information that there are significant differences in the effect of treatment on the number of *Salmonella sp.* bacteria. In the Table it clearly stated that the 90% water treatment resulted in the number of *Salmonella sp.* (0.21x102 CFU/g) higher than 60% (0.14x102 CFU/g) and 75% (0.09x102 CFU/g) water contents. Conversely, *to E. coli* bacteria, *Salmonella sp.* is an anaerobic bacteria. Thus it is disturbed by reducing a substrate oxygen level. However, *Salmonella* sp. bacteria cannot stand at 72^{o}C in 15 seconds. Because the temperature of the biogas process can reach temperature >70^{o}C, regarding that in a long time total of *Salmonella sp.* bacteria will decrease rapidly.

CONCLUSIONS

It can be concluded that the water content of the substrate 75% produces medium the quantity, and quality of sludge biogas better than 90% ($P<0.05$), but produces sludge biogas quality which almost equal to 60% water content. This finding suggests that for obtaining a good sludge from the biogas manufacture made from dairy cows faeces and rice straw using batch type digester, it is recommended to use substrate with water content of 75%. Chemical composition of the resulted sludge meets the standard quality of solid organic fertilizer.

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RESEARCH ON QUANTITATIVE PARAMETERS IN THE MEAT PRODUCTION AT LAMBS OBTAINED FROM CROSSBREEDING ILE DE FRANCE BREED X PALAS MERINO BREED

Andrei PUIE, Ion RĂDUCUȚĂ, Nicoleta TEODORESCU (ROȘU), Laurenția DUMAN, Cornel FRUJINĂ, Vasile DREVE, Ion CĂLIN

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: raducion@yahoo.com

Abstract

The aim of this work is to bring new information on the possibilities of increasing production and improving the quality of meat, obtained from local Palas Merino breed by crossbreeding with Ile de France breed. The subject is topical, bearing in mind that the main production at European level for exploiting sheep is meat production and the demand for sheep meat has increased considerably, both in traditional consuming countries and outside in developed ones, but especially in countries with a developing economy, and the sheep meat price in certain countries is with 20-30% higher than the price of poultry or pork meat. The experiments were carried out in two successive years on three lots of lambs, respectively a control batch one and two experimental lots, during the suckling period of lambs until weaning them, respectively for a period of 60 days. During this period lambs have had permanent access to maternal milk but also at good quality hay and concentrated feeding stuffs the last one being administered to discretion in special places. Results regarding average daily gain and total growth weight of lambs during suckling period were performed by individual weighing of lambs at birth, at 30 days and 60 days, depending on the type of birth and sex of lambs. The results of the experiment highlighted the fact that the crossbred lambs from the third group have recorded the best quantitative performances in the meat production.

Key words*: crossbreeding, lambs, meat production, quantitative parameters.*

INTRODUCTION

The importance of sheep rearing is fed to both biological and economic value of the carried out productions, but also to the maximum value of sheep, transforming in meat, milk, wool, pelts and skins, grass on the pasture and agricultural by-products (Călin, 2003).

The aim of this work is to bring new information on the possibilities of increasing production and improving the quality of meat at lambs obtained from local Merino breed of Palas crossed with Ile de France breed.

The subject is topical, bearing in mind that the main production at European level for exploitting sheep is meat production and the demand for sheep meat has increased considerably, both in traditional consuming countries and outside in developed ones, but especially in countries with a developing economy, and the sheep meat price in certain countries is with 20-30% higher than the price of poultry or pork meat.

The fastest way to increase meat production of local breeds is by crossing them with specialized breeds of meat (Taftă et al., 1997; Răducuță, 2010; Călin et al., 2010).

MATERIALS AND METHODS

The experiments were carried out in two successive years on three lots of lambs. The period of suckling lambs has lasted for 60 days during which lambs have had permanent access to sheep's milk moms but also good quality hays and concentrated feeding stuffs administered to discretion in special places.

Results on body weight gain and growth during lactation has determined the body weight at birth, at the age of 30 days, and at the age of 60 days, depending on the type of birth and sex of lambs, as well as the average daily increase in 0-30 days, 31-60 days and 0-60 days. Experimental determinations were performed by weighing individual lambs.

For carrying out the proposed studies two experimental batches and a control batch have been created, as follows (Table 1):
- Batch no. 1 – Merino breed of Palas females that were crossed with Merino breed of Palas males (control batch);
- Batch no. 2 - Merino breed of Palas females that were crossed Ile de France breed males (experimental batch no. 1);
- Batch no. 3 - F_1 half breed sheep (obtained by the crossing between Merino breed of Palas females with Ile de France breed males), that were crossed with Ile de France breed males care (experimental batch no. 2).

Table 1. Studied biological material

Crt. no.	Experimental year	Biological material
1	Year I	Batch 1 ♂MP x ♀ MP
2	Year II	
3	Year I	Batch 2 ♂IF x ♀ MP
4	Year II	
5	Year I	Batch 3 ♂IF x ♀ (MP x IF)
6	Year II	

During the experiment period lambs supplementary feeding was performed, since a very early stage, starting with the age of 8-10 days, in specially designed pens where only lambs had access. Additional food consisted of high quality alfalfa hay and farm mixed fodder ad-libitum. Farm mixed fodder consisted of 45% maize, 50% barley and the difference was the sunflower meal 5% (Table 2).

Table 2. The chemical composition of mixed fodder

Farm mixed fodder composition	Unit of measure	Quantity
Energy	(MNC/kg)	1.09
Digestible crude protein	(g/kg)	156.64
Crude protein	(%)	15.72
Raw fat	(%)	2.52
Raw cellulose	(%)	6.84
Ash	(%)	6.22
Calcium	(%)	1.07
Total Phosphorus	(%)	0.43
Total chlorides	(%)	0.025
Vitamin A	(UI/kg)	9626.45
Vitamin D3	(UI/kg)	2188.70
Vitamin E	(mg/kg)	19.46

*MNC = meat nutrition units.

All lambs obtained from the two experimental batches, as well as the ones from the control batch were used in determining body weight during the experimental period, respectively during the 60 days of suckling period (birth – weaning).

RESULTS AND DISCUSSIONS

The highest birth weights in both year I and year II were obtained by males from Batch no. 3 (♂IF x ♀ (Mp x IF) coming from single parturitions achieving a weight of 4.869 ± 0.136 kg in year II, 3% more weight than the weight achieved with lambs from Batch no. 2 (♂IF x ♀ MP) in year II. The lower weights have been achieved, both in the year I, as well as in year II by the lambs from Batch no. 1 (♂MP x ♀ MP), with 9% lesser, indicating the positive performance of the Ile de France breed towards meat production and, especially, transmitting power of superior characteristics as regards meat production to descendants (Table 3).
As regards the weight at parturition, females recorded similar results. Thus, the best performances are made by females achieved by the Batch no.3 (♂IF x ♀ (Mp x IF) with single parturition: 4.467 ± 0.089 kg in year II, almost 17% more than females belonging Merino breed of Palas. Between Half breed batches are not registering significant differences, respectively Batch no. 3 registered a weight with 8% superior to that achieved by females belonging to Batch no. 2 (♂IF x ♀ MP).

Table 3. Lambs weight from experimental batches at birth

Lots	Year	Single parturition						Twin parturition					
		Male			Female			Male			Female		
		n	$\overline{X} \pm s_{\overline{X}}$	V (%)	n	$\overline{X} \pm s_{\overline{X}}$	V (%)	n	$\overline{X} \pm s_{\overline{X}}$	V (%)	n	$\overline{X} \pm s_{\overline{X}}$	V (%)
1	I	15	4.372±0.093	8.24	15	3.728±0.093	9.82	15	3.625±0.110	11.79	15	3.268±0.106	12.60
	II	15	4.442±0.097	8.52	15	3.824±0.077	7.88	15	3.713±0.105	10.98	15	3.470±0.090	10.04
2	I	14	4.512±0.134	11.10	14	3.951±0.119	11.29	14	3.831±0.132	12.92	14	3.483±0.125	13.46
	II	14	4.732±0.161	12.78	14	4.121±0.112	10.22	14	3.946±0.141	13.37	14	3.652±0.090	9.23
3	I	13	4.611±0.125	9.83	13	4.313±0.092	7.75	13	4.242±0.119	10.11	13	3.709±0.132	12.87
	II	13	4.869±0.136	10.13	13	4.467±0.089	7.22	13	4.392±0.162	13.31	13	3.929±0.120	11.04

At the age of 30 days the same trend between batches persists still the half breed batches groups are achieving the best results (Table 4).

Thus as regards males it can be observed that compared to the Batch no.1 (♂MP x ♀ MP) the difference in weight is increasing. Batch no. 3 (♂IF x ♀ (Mp x IF) achieving an average weight of approx. 13.0 kg in year II with more than 2.0 kg higher (over 19%). Also Batch no. 2 reaches a weight of 12.028 ± 0.415 kg by more than 1.13 kg superior (approx. 10%).

Concerning males obtained out of twin parturition they gain less weight at this age than those from single parturitions with approx. 6-7% (differences between similar groups), but it should be noted that the experimental batches recorded weights higher than those achieved by the control batch males obtained from single parturitions.

Females follow the same growth curve at the age of 30 days. The best results being obtained by animals from experimental batches with simple parturition, particularly Batch no. 3 in year II when females at the age of 30 days reached a weight of 12.088 ± 0.297 kg, superior to the weight achieved by the other batches (approx. 6% more compared to Batch no 2 and 13% compared to the control batch).

In the case of twin parturitions the weight of females achieved at this age are inferior with approx. 5% than the weight of females obtained from single parturitions that are similar to those identified by other authors (Teodorescu et al., 2013).

The increase in weight of the experimental batches gained both by females and especially males are due to higher feed conversion capacity and outstanding breed precocity registered by the paternal breed and the expression of these traits to their descendants.

Table 4. Lambs weight from experimental batches at the age of 30 days

Lots	Year	Single parturition						Twin parturition					
		Male			Female			Male			Female		
		n	$\overline{X} \pm s_{\overline{X}}$	V (%)	n	$\overline{X} \pm s_{\overline{X}}$	V (%)	n	$\overline{X} \pm s_{\overline{X}}$	V (%)	n	$\overline{X} \pm s_{\overline{X}}$	V (%)
1	I	15	10.608±0.306	11.17	15	10.181±0.280	10.67	15	10.018±0.336	13.01	15	9.634±0.279	11.20
	II	15	10.901±0.262	9.30	15	10.672±0.273	9.91	15	10.286±0.336	9.07	15	10.023±0.329	12.71
2	I	14	11.785±0.345	10.96	14	11.035±0.358	12.14	14	11.006±0.300	10.22	14	10.420±0.333	11.98
	II	14	12.028±0.415	12.92	14	11.360±0.265	8.72	14	11.339±0.351	11.59	14	10.707±0.274	9.57
3	I	13	12.534±0.396	11.38	13	11.814±0.03	10.00	13	11.534±0.446	13.96	13	11.098±0.321	10.44
	II	13	12.979±0.365	10.14	13	12.088±0.29	8.86	13	12.026±0.364	10.91	13	11.373±0.300	9.51

The best results as regards body weight at the age of 60 days were achieved by males from Batch no. 3 obtained from single parturition, which recording the highest weight at weaning both in year I and year II (20.107 ± 0497 kg or 20.657 ±0.434 kg), consecutive to a variation coefficient of 7.51% which indicating a very good homogeneity of the analyzed batch (Table 5).

Between experimental and control batches significant differences were registered, differences that were highlighted last weighing. Thus, between Batch no. 3 and the control batch (Batch no. 1) in year II, differences of 3.1 kg have been achieved (with more than 50% higher than the differences at age to 30 days) and between Batch no. 2 and the control batch differences with more than 7% were recorded at the end of the experimental period.

The accumulation of body weight at the end of the experimental period for lambs obtained from single parturition in study year II were as follows: 13.12 ± 0.383 kg control batch; 14.18 ± 0.440 kg Batch no. 2 and 15.79 ± 0.383 kg Batch no. 3.

Outstanding results were also recorded in the case of males from experimental groups obtained from twin parturitions, which achieved weights with approx. 6% lower than males obtained from single parturitions.

The results obtained in this experiment are similar to those found by other authors (Teodorescu et al., 2012; Teodorescu et al., 2013), but higher than those achieved by the control batch, particularly in the case of Batch no. 3 which gained at the end of the experimental period a higher weight (with over 11%).

At the end of the experimental period the batches achieved the following body weight accumulations: 12.63 ± 0312 kg control batch; 13.82 ± 0.314 kg Batch no. 2 and respectively 15.15 ± 0.424 kg Batch no. 3.

Females belonging to Batch no. 3 from single parturition have achieved the best growing performance which achieving an average weight of 18.892 ± 0.334 kg in study year II a total weight increase of approx. 14.424 ± 0.341 kg (approx. 12.6% more than the accumulation of body weight carried by the control batch and with 2.2% more than the increase gain achieved by Batch no. 2.

Females obtained from twin parturitions achieved modest results with a total increasing gain of 12.44 ± 0.286 kg in the case of the control group 13.75 ± 0.377 kg for Batch no. 2 and 14.26 ± 0.356 kg for Batch no. 3. Between the performances achieved by Batch no. 3 and Batch no. 1 significant differences were recorded.

Table 5. Lambs weight from experimental batches at the age of 60 days

Lots	Year	Single parturition						Twin parturition					
		Male			Female			Male			Female		
		n	$\overline{X} \pm s_{\overline{X}}$	V (%)	n	$\overline{X} \pm s_{\overline{X}}$	V (%)	n	$\overline{X} \pm s_{\overline{X}}$	V (%)	n	$\overline{X} \pm s_{\overline{X}}$	V (%)
1	I	15	17.351±0.344	7.69	15	16.366±0.266	6.30	15	16.018±0.296	7.16	15	15.426±0.253	6.36
	II	15	17.566±0.272	5.99	15	16.633±0.345	8.03	15	16.343±0.324	7.67	15	15.913±0.252	6.14
2	I	14	18.498±0.363	7.35	14	17.787±0.302	6.35	14	17.484±0.399	8.54	14	17.045±0.326	7.17
	II	14	18.910±0.411	8.14	14	18.228±0.396	8.12	14	17.764±0.366	7.70	14	17.403±0.259	5.57
3	I	13	20.107±0.497	8.92	13	18.503±0.370	7.21	13	18.807±0.618	11.8	13	17.615±0.458	9.38
	II	13	20.657±0.434	7.51	13	18.892±0.334	6.37	13	19.538±0.416	7.67	13	18.196±0.372	7.37

CONCLUSIONS

Crossing Merino breed of Palas females with Ile de France males has a direct effect on the main meat production related indicators.

Half breed lambs from Batch no.3 registered the highest weight at birth regardless of the type of birth and sex. Lambs belonging to the control batch achieved the lowest weights at birth both in year I and year II and Batch no. 2 lambs recorded intermediate weights at birth.

Half breed lambs from Batch no. 3 both males and females have achieved in both study years the highest average weights both at the age of 30 days as well as at the age of 60 days, while the lowest values were achieved by the Merino breed of Palas lambs. Lambs belonging to Batch no. 2 were on the second place according to the body weight achieved both at the age of 30 days and at the age of 60 days.

Body weight differences recorded between the experimental batches at the age of 60 days, both in the first study year and in the second study year, were very significant between lambs from Batch no. 3 and the maternal breed. Body weight differences recorded were significant among half breed females from Batch no. 2 and Merino breed of Palas females.

The maternal Merino breed of Palas has a particular combinatorial capacity with breeds specialized for meat production and especially with the Ile de France breed. The resulting F1 half breeds are showing high abilities towards birth weight growth gain, feed conversion capacity and weight achieved at weaning.

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EVOLUTION OF PIGS' LIVESTOCK AND PORK PRODUCTION IN THE WORLD

Ilie ROTARU

State Agrarian University of Moldova, MD-2049 58 Mirceşti Street, Kishinev, Republic of Moldova
Ministry of Agriculture, Regional Development and Environment, MD-2005, Constantin Tănase 9 Street, Kishinev, Republic of Moldova

Corresponding author email: i.rotaru@uasm.md

Abstract

This paper work covers the evolution of pigs` livestock, meat production, and annual meat consumption per person during the years 2005-2016. Based on the obtained data was established a continuous growth of world pigs` livestock and meat production. In 2016 compared to 2005, the livestock on pig increased by 9.2% and the meat production was increased by 14.18%, what proves that production processes have been using the intensive methods and the increase in meat production has been based on the increase of animal production. The world pork consumption has been increased by 20.68%. That means that the total of 6.5% in growth was based also on the meat import.

Key words: *evolution, pork and worldwide production.*

INTRODUCTION

In our days and in perspective the consumer is the main factor in the development of meat production. The pork is an important source of nutrition; this is why the safety of products is an undeniable requirement.
The meat has to be tasteful, with a high nutritional value and an affordable price.
As a result the competitive meat producers, which insure the internal and external market with quality meat will develop based on the gained profit from the efficient delivery of meat products.
It is important also to produce meat with superior technological qualities.
The animal breeder has to identify intensive methods of pork production in order to satisfy the consumer and the processor requirements (Rotaru, 2016; FAO).

MATERIALS AND METHODS

The results presented by FAO during the years 2005-2016 have been used in this research. It was studied the evolution of pigs `livestock, meat production and the total meat consumption per capita butalso in different countries.
The results were analysed based on the calculations for the differences assessment between various countries during the indicated period.

RESULTS AND DISCUSSIONS

Evolution of swine population. The swine population varied in the period of 2005-2016, but on the international level a considerable growth has been recorded and represents an increase of 9.2%.
In Brasil, Russian Federation, Spain and USA the population increase was between 14.2 and 60.2%.
In 2016 the total number of swine population in China has reached 456.773 thousands pig, in United States -74.500 thousands pig.
The results listed in the above table show that some countries like Belgium, Denmark, Poland, Mexico and France the total amount declined considerably, especially in Poland and France.
In the last two years (2014-2016) China had a small decrease of swine population as well. Regardless of that, China remains the world leader with 50% of the world population (Table 1).

Table 1. The evolution of swine population for the period of 2005-2016, thousands

Country	2005	2010	2013	2014	2015	2016	2016/2005,%
World	906,663	965,885	977,020	987,854	990,507	981,797	108.22
Belgium	6,318	6,430	4,243	6,350	6,364	6,176	97.75
Brasil	34,063	38,956	39,040	37,930	39,795	39,950	117.31
Canada	14,810	11,835	12,879	12,940	13,247	12,770	86.22
Denmark	13,534	13,173	12,075	12,331	12,537	12,383	91.36
France	14,951	14,531	13,487	13,335	13,162	12,709	85.00
Germany	26,857	*26,509*	27,790	28,338	27,652	27,376	101.93
Italy	8,9 71	9,157	8,662	8,676	8,674	8,477	105.68
Mexico	18,112	14,865	16,038	16,098	16,364	16,753	92.49
Poland	18,112	14,865	11,162	11,724	11,639	10,865	60 00
RepublicofMoldova	397	377	410	419	472	453	114.15
Romania	6,495	5,793	5,234	5,180	5,041	4,926	75.84
RussianFederation	13,412	17,231	18,816	19,081	19,546	21,506	160.24
Spain	24,884	25,342	25,495	26,567	28,367	29,231	159.91
Ukraine	6,466	7,576	7,577	7,764	7,350	7,079	109.48
U S A	60,975	64,887	64,775	67,776	68,389	71,500	117.26
Vietnam	27,434	27,373	26,261	26,761	27,750	29,075	105.98
China	428,505	476,237	482,248	480,095	471,550	456,773	106.59

Table 2**.** The evolution of meat production for the period of 2005-2016, thousands tons

Country	2005	2010	2013	2014	2015	2016	2016/2005
World	103,488	109,260	115,106	117,263	117,876	118,168	114.18
Belgium	1,915	1,925	1,130	1,118	1,124	1,060	104.43
Brasil	3,110	3,078	3,117	3,192	3,430	3,514	112.99
Canada	1,920	1,925	1,981	1,962	1,974	2,047	106.61
Denmark	1,793	1,668	1,589	1,593	1,600	1,579	88.06
France	2 ,018	2,,259	2,130	2,120	2,148	2,185	108.27
Germany	4,500	5,455	5,506	5,527	5,570	5,589	124.20
Italy	1,515	1,673	1,652	1,327	1,491	1,544	101.91
Mexico	1,103	1,174	1,283	1,290	1,322	1,376	124.75
Poland	1,956	1,894	1,775	1,864	1,976	2,008	102.05
Republic of Moldova	39	56	64	61	71	72	183.20
Romania	651	425	396	459	470	500	76.80
Russian Federation	1,520	2,307	2,81	2,973	3,098	3,368	220.90
Spain	3,168	3,368	3,431	3,555	3,8548	3,947	124.58
Ukraine	493	631	748	742	759	748	151.72
U S A	9,383	9,101	10,524	10,368	11,120	11,320	120.64
Vietnam	2,288	3,036	3,228	3,351	3,491	3,664	164.45
China	51,202	51,719	51,917	57,661	55,834	55,040	107.45

World Pork Production. In 2005, on the global level, 103.488 thousand tons of pork meat were produced vs 118.168 thousandtons in 2016, that represents 14.18% of increase. The global pork meat production leaders are China with 55.834 thousand tons, USA with 11.320 thousand tons and Germany with 5.589 thousand tons. The meat production in the described period, increased over 24% in Germany, Mexico and Spain. In Ukraine, Vietnam and Republic of Moldova, the pork meat production increased by over 50%, in Russian Federation - 120%.

At the same time, in some countries like Denmark and Romania the volumes decreased in the same period. As a result, the data reflected in the table shows a variable growth of meat production. This fact is explained by a different rate of intensive methods implementation, as well as usage performing genotypes in the production process (Cărătuș et al., 2016; Rotaru, 2013).

Table 3.Consumption of pork meat in the period of 2005-2013 (thousand tons)

Country	2005	2009	2010	2011	2012	2013	2013/2005
World	93263	103823	106282	106784	110568	112552	120.68
Belgium	364	364	394	386	447	429	131.59
Brazil	1902	2260	2408	2475	2513	2525	132.75
Canada	967	1027	990	1000	1006	961	99.37
Denmark	2391	107	126	102	333	291	121.75
France	2114	2194	2179	2128	2151	2125	102.52
Germany	4461	4437	4500	4433	4342	4286	96.07
Italy	2270	2390	2534	2436	2422	2457	108.23
Mexico	1411	1688	1700	1647	1772	1864	130.10
Poland	1982	2009	2033	2055	1831	1791	90.36
RepublicofMoldova	50	48	62	69	71	69	130.00
Romania	667	751	663	638	604	556	83.35
RussianFederation	2110	2848	3137	3258	3535	3671	173.98
Spain	2463	2218	2280	2247	2282	2296	93.21
Ukraine	545	685	739	801	929	930	171.27
USA	8734	8952	8692	8385	8552	8728	99.93
Vietnam	2281	3033	3027	3093	3151	3209	107.64
China	41644	48358	50009	50292	53248	54730	131.42

The Pork Consumption. The results presented in the Table 3 show that the pork meat consumption was equal to 112.552 thousand tons. This represents an increase of 20.68 % if compared with 2005 results. In Belgium,Brasil, Mexico, Republic of Moldova and China a 30-31% increase in the meat consumption was recorded, In Russian Federation – 73.98%. Even if the pork meat consumption decreased in Germany, France &USA, the total market demand is considerable in the period of 2009-2013. In these circumstances we can't neglect the population number of each of these countries, the larger the population the bigger is the consumption. In this case the better picture is offered by the consumption kg per person per year. This data is offered in the Table 4.

Table 4. Consumption of pork meat for the period 2005-2013 (kg/capita/year)

Country	2005	2009	2010	2011	2012	2013	2013/2005
World	15.00	15.42	15.61	15.5	15.89	16.02	106.8
Belgium	34.18	33.48	36.05	35.1	40.4	38.65	113.07
Brasil	11.85	11.68	12.33	12.57	12.65	12.6	106.32
Canada	25.22	25.82	24.45	24.43	24.28	*22.81*	*90.44*
Denmark	44.04	19.38	22.78	18.25	25.22	24.87	56.47
France	32.84	34.88	34.47	33.46	33.63	33.05	100.63
Germany	53.84	53.34	54.21	53.48	52.43	51.81	96.22
Italy	42.22	39.69	41.87	40.12	39.78	40.28	95.40
Mexico	13 53	14.50	14.42	13.80	14.66	15.23	112.56
Poland	51.20	48.73	50.23	51.24	47.03	46.19	90.21
RepublicofMoldova	13.22	13.26	17.25	19.46	20.34	19.83	140.54
Romania	30.11	33.53	29.58	28.4	27.38	25.31	84.05
RussianFederation	14.62	19.79	21.82	22.67	24.63	25.66	175.51
Spain	57.04	48.48	49.38	48.32	48.8	48.92	85.76
Ukraine	11.63	14.81	16.04	17.50	20.41	20.56	176.78
USA	29.37	29.07	27.99	26.81	27.21	27.64	94.10
Vietnam	26.82	34.39	33.99	34.39	34.7	35.00	130.49
China	34.91	34.91	35.89	35.82	37.73	38.6	109.78

The consumption of pork meat per capita on global level in 2013 was 16.3 kg, this represents an increase of 6.8% vs 2005. The largest numbers of consumption were recorded

in Germany - 51.81 kg, Spain - 48.92 kg, Poland - 46.18 kg, Italy - 40.28 kg, Belgium - 38.65 kg. The smallest numbers are for Brasil-12.6 kg and Mexico - 15.23 kg. The average consumption of pork meat in USA, Canada, Denmark, France, Romania, Republic of Moldova is over 20 kg. In Russian Federation, Ukraine an increase of 70% was recorded (Cărătuș et al., 2016; Găureanu et al., 2017).

Table 5.The evolution of meat productivity per animal in the period of 2013-2016 Hg/animal head

Country	2013	2014	2015	2016	2016/2005
World	792	798	803	799	100.88
Belgium	949	943	946	948	99.89
Brasil	859	860	874	883	102.79
Canada	947	961	970	976	103.06
Denmark	832	845	855	860	103.36
France	874	909	922	905	103.54
Germany	939	938	937	940	100.10
Italy	1262	1215	1319	1303	103.24
Mexico	763	785	808	819	107.33
Poland	876	861	909	909	103.76
RepublicofMoldova	817	720	775	774	94.73
Romania	826	811	816	837	102.44
RussianFederation	853	868	878	873	102.34
Spain	828	818	840	835	100.84
Ukraine	874	864	890	875	100.11
USA	938	969	963	957	102.02
Vietnam	702	710	685	717	102.13
China	770	774	777	769	99.87

The following productivity averages represented in Hg/head were recorded in the leading countries like Italy with 1303 Hg/head, Canada -976, USA - 957, Belgium - 948, Germany - 940. In other countries the productivity varies from 769 to 799 Hg/Head. In the described period (2013-2016) the productivity index didn't change in the following countries: Germany, Spain, Ukraine, China, Belgium. At the same time there was an increase in these countries: Canada, Denmark, France, Italy, Mexico, Romania, Russian Federation, USA, Vietnam (in average 2-7%). This demonstrates that the average weight of the animals delivered on the market was bigger.

CONCLUSIONS

Pigs `livestock during the period 2005-2016 globally has increased relatively continuously, in 2016 in comparison with 2005, the percentage of animalsincreased with 9.2%. The biggest amount of swine is in China which consists in 456.773 thousands of pigs, the total of 50% of a global amount. A big amount of swine we can find also in USA with a total number of 71500 thousands of pigs, in Brazil with 39 950 thousands of pigs and in Germany with 27 316 thousands of pigs.

During the period 2005-2016 the pork production has increased by 14.8% worldwide and in 2016 was consisted in 11816 tons of meat. The largest meat producers are China with 55834 tons, USA with 11320 tons and Germany with 5589 with an increase of 7-24%.

The global consumption consisted in 16.02kg per capita, which is a 6.8% increase compared to 2005. Germany consumes annually a 51.81kg per capita, Spain 48.92kg, Italy 40.28 kg and in Brazil and Mexico it is between 12-15kg per capita. In many countries such as: Canada, France, Romania, Denmark, Russia Ukraine the consumption is over 20kg per capita. China consumes around 38.6 kg of meat per capita.

The global productivity per animal Hg/animal head, in 2016 represented 799Hg/animal head. The following results were recorded in other countries like: Italy -1303Hg/animal head, Canada – 976Hg/animal head, USA-957 Hg/animal head, Belgium – 948Hg/animal head and Germany -940 Hg/animal head.

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RESEARCHES ABOUT INFLUENCE OF BREEDING TECHNOLOGY ON EJACULATE pH OF BROILER BREEDER ROOSTERS

Ioan CUSTURA[1], Minodora TUDORACHE[1], Ilie VAN[1], Monica MARIN[1], Georgeta DINIȚĂ[1], Paul ANTON[2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Animal Science / Faculty of Veterinary Medicine, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Aviagen Romania

Corresponding author email: johncustura2000@yahoo.com

Abstract

Researches are part of a large study to analyze semen material and breeding efficiency of broiler breeding hybrid parents. Study aimed to analyze influence of some microclimate factors (light intensity, bird's density and sex percentage) and litter material on semen quality (semen pH) in ROSS 308 broiler breeding males. Three experimental groups were formed (A – with analyze parameters sub-standard and litter made of chopped straws B – with analyze parameters above standard and litter made of rice hulls and C – with analyze parameters at the level recommended by the manufacturer of biological material and litter made of wood shavings) and in each trial 25 roosters and 250 laying hens from the ROSS 308 commercial hybrid were used. Researches were performed during a two years period with 3 control weeks (25, 35 and 45) during breeding period (19-64 weeks). Ejaculation pH has been between 7.30 ±0.08 in week 25 – trial A and 7.70 ± 0.10 in week 35 – trial B. Results about semen pH would seem to support usage of microclimate technological parameter values under standard recommendations and a litter made of chopped straws which would produce the lowest level of stress and so it would perturb the least reproduction of ROSS 308 broiler breeding males.

Key words: *litter, ejaculation pH, roosters, density, light intensity.*

INTRODUCTION

Bird's usual spermatogram is varying according to some factors among whom the most significant are: specie, race, age, management, feed, breeding usage regime (Vacaru Opriș, 2002; Bunaciu, 1977; Sexton, 1986, 1987, Jarinkovicova et al., 2012).

PH is concentration of hydrogen ions released by dissociation acids from sperm. Sperm pH in birds is close to blood pH and it is not significantly variable by specie (Peters et al., 2008, Hani et al., 2016). Sperm reaction is very important at membrane level where metabolically changes are taking place. Sperm reaction is different by level of genital apparatus segment. Adding of cloacae seminal plasma is slightly alkalinizing the sperm and so semen pH is varying from an ejaculation to another based on fluid quantity added.

PH, sperm concentration and metabolism are closely related (Orunmuyi et al., 2013, Almahdi et al., 2014). PH is having an influence on superficial tension, viscosity and absorption values. Viscosity increases direct proportional with temperature and sperm concentration and inverse proportional with pH (Dumitrescu, 1978; Fujihara, 1985). Hydrogen ions are influencing enzymatic reactions and they are able to accelerate or delay chemical processes (Bunaciu, 2009). During conservation perm reaction is evidently changing and becomes slightly alkaline.

MATERIALS AND METHODS

Broiler breeder farm are one of the most important link in poultry meat production chain. In this activity is imperative to understand basic reproductive physiology of both sexes to be able to apply management rules about feeding, maintenance, lighting programs and sanitary and veterinary management.

So, researches from this paper aimed to study efficiency of reproduction in ROSS 308 hybrid roosters concerning influence of some microclimate factors (light intensity, bird's density and sex percentage) and some other

characters which determine together rooster's semen quality influences hatchability and finally biologic and economic efficiency of reproduction.

So considering the goal aimed works were performed during two years in three houses, one for each trial: Avicola Călăraşi, S.C. Agrafood S.A. and Avicola Focşani, on 25 roosters and 250 partner hens in 3 control weeks (25, 35 and 45) during production period (19-64 weeks).

In trial A aiming influence of some microclimate factors at sub-standard values on characters determining semen quality following microclimate parameters are considered:

- litter: chopped straws;
- sub-standard light intensity: 30 lux;
- sub-standard bird density: 3 males/m^2;
- sex proportion under standard: 25 weeks - 8 birds, 35 weeks – 7.5 birds, 45 de weeks – 6.5 birds.

Trial B analyzed effect of microclimate parameters above limits on characters which determine semen quality and microclimate parameters considered were:

- litter: rice hulls;
- light intensity above standard: 70 lux;
- bird density over standard: 5 males /m^2;
- sex proportion above standard: 25 weeks - 9 birds, 35 weeks – 8.5 birds, 45 weeks – 7.5 birds.

Trial C analyzed influence of keeping microclimate parameters at standard value on semen quality and microclimate parameters were:

- litter: wood shavings;
- light intensity standard: 40 lux;
- bird density standard: 4 males/m^2;
- sex proportion standard: 25 weeks – 8.5 birds, 35 weeks - 8 birds, 45 weeks -7 birds.

Poultry were kept in uniform conditions in the three houses (corresponding to the three experimental groups), on permanent litter (large captivity), in upgraded houses, with feed and water delivered according to the technical book of the hybrid.

During production period was analyzed *semen quality* (ejaculation pH) found with a pH-meter. Phonotypical characterization of groups was performed by classical statistical methods (Sandu, 1995) and study of parameters variation which has a normal repartition was performed using *Student* test to compare average homogeneities of two samples (Sandu, 1995; Dragomirescu, 1999).

RESULTS AND DISCUSSIONS

Semen quality is influenced by many factors (genetical type, season, age, feeding, maintenance system, etc.) but only litter type and microclimate factors were taken into account to evaluate and compare results about semen quality (ejaculation pH).

Semen pH appreciation is particularly important especially if artificial insemination is practiced or when biologic material (gene) banks are founded when conserving roosters sperm by freezing is needed.

There is a relationship between semen quality and its pH. As good a sperm is in aspect and mobility of spermatozoa as acidic its pH is.

Obtained values for pH of ejaculation from individuals in trial A are inside normal limits for species concerned and a low variability is noticed during all three control weeks (Table 1 and Figure 1). During first two weeks these tended to migrate to neutral zone probable in correlation with the other sperm qualitative parameters.

Table 1. Average values of ejaculate pH for first experience series

Week	n	$\bar{X} \pm s_{\bar{X}}$	s	c.v.%
25	25	7.40 ± 0.08	0.42	5.68
35	25	7.30 ± 0.08	0.42	5.79
45	25	7.50 ± 0.05	0.27	3.59

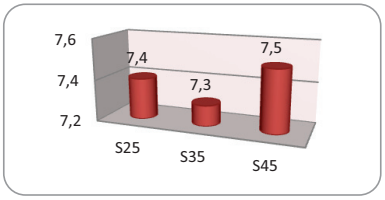

Figure 1. Average values of ejaculate pH for first experience series

Differences observed between average values of pH during the three control weeks were statistically tested (Student test, Table 2) and are not significant being caused by chance or individual variation.

Table 2. Testing the significance of differences observed between the three weeks in terms of pH of ejaculat, first series

Specification	S25	S35	S45
S25	-	0.42^{NS}	1.05^{NS}
S35		-	1.52^{NS}

Values for ejaculation pH from individuals inside trial B (Table 3, Figure 2) are revealing pH values of species with a low heterogenity of observations during the three observation weeks as in trial A.

Table 3. Average values of ejaculate pH for second experience series

Week	n	$\bar{X} \pm s_{\bar{X}}$	s	c.v.%
25	25	7.70 ± 0.10	0.48	6.24
35	25	7.50 ± 0.12	0.58	7.78
45	25	7.60 ± 0.09	0.45	5.98

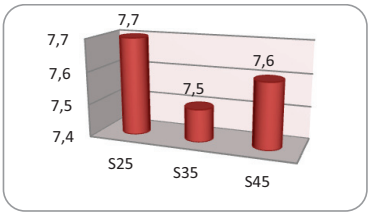

Figure 2. Average values of ejaculate pH for second experience series

Student Test (Table 4) is revealing statistically significant differences of pH value between weeks 25 and 35 other differences being caused by chance or individual variation with no statistical significance.

Table 4. Testing the significance of differences observed between the three weeks in terms of ejaculat pH, second series

Specification	S25	S35	S45
S25	-	1.71^{*}	0.93^{NS}
S35		-	1.32^{NS}

PH values in trial B were higher aiming to basic zone so we could expect to a lower semen quality. Considering that individuals received the same feeding condition and it was the same genetic type this time results seem to emphasize an unfavorable influence of technological parameters values and litter type (rice hulls) on sperm quality.

PH values in trial C (Table 5, figure 3) are found inside normal limits with lower variability among all trials most probable due to environmental conditions at standard values and usage of a classical wood shavings litter.

Table 5. Average values of ejaculate pH for third experience series

Week	n	$\bar{X} \pm s_{\bar{X}}$	s	c.v.%
25	25	7.60 ± 0.03	0.17	2.27
35	25	7.50 ± 0.05	0.26	3.52
45	25	7.60 ± 0.05	0.24	3.19

Noticed differences between average values of semen pH during the three control weeks of adult period were tested for statistical significance (Table 6) and they were found not significant statistical.

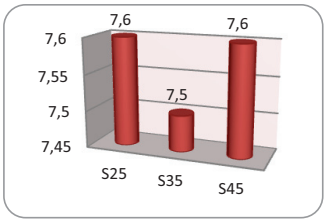

Figure 3. Average values of ejaculate pH for third experience series

Table 6. Testing the significance of differences observed between the three weeks in terms of ejaculate pH, third series

Specification	S25	S35	S45
S25	-	0.57^{NS}	1.05^{NS}
S35		-	0.76^{NS}

Differences observed between averages registered in the three trials are revealing some important aspects of rooster's semen quality (Figure 4). On one side lowest pH values were obtained inside trial A where apparently usage of some technological parameters under standard values and a chopped straws litter would have a favourable influence on roosters reproductive capacity by diminishing pH value which is correlated as known with a better value of the other quality indexes. On the other side highest pH values were obtained in week 25 of life when physiological processes on which spermatogenesis is based probable are not at the peak. Thirdly using some microclimate parameters above standard technological values has a negative influence on semen pH and very possible on the other quality indexes because of stress. It is well

known that stress affects firstly functions linked to adaptation process including reproduction. Although we noticed that in all three trials pH values are inside normal limits so biological and economical efficiency of houses should not be a problem.

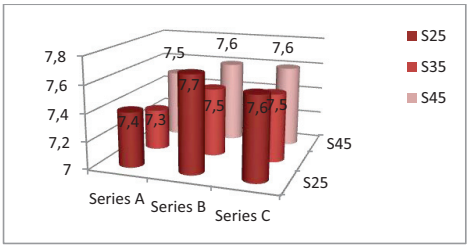

Figure 4. Comparative between the three experimental series on ejaculate pH

Calculated values of Student test shown in tables 7-9 are revealing differences with differed degree of statistical significance between pH averages registered in trial A and those registered in the other two trials excepting week 45 of life. Differences between trials B and C are not significant or zero for weeks 35 and 45.

Table 7. Testing of significance for differences between experimental series, 25th week, for ejaculate pH

Specification	t test value
A-B	4.37^{***}
A-C	2.86^{**}
B-C	1.46^{NS}
$t_{49;0.05} = 1.68$; $t_{49;0.01} = 2,40$; $t_{49;0.001} = 3,50$	

Table 8. Testing of significance for differences between experimental series, 35th week, for ejaculate pH

Specification	t test value
A-B	2.02^{*}
A-C	2.77^{**}
B-C	0
$t_{49;0.05} = 1.68$; $t_{49;0.01} = 2,40$; $t_{49;0.001} = 3,50$	

Table 9. Testing of significance for differences between experimental series, 45th week, for ejaculate pH

Specification	t test value
A-B	1.58^{NS}
A-C	1.61^{NS}
B-C	0
$t_{49;0.05} = 1.68$; $t_{49;0.01} = 2,40$; $t_{49;0.001} = 3,50$	

So considering the semen pH results seem to plead for usage of values under the technological standard of macroclimate parameters de and a litter of chopped straws condition which would produce lower amount of stress and so would perturb the least ROSS 308 male's reproduction. We emphasize however that this conclusion is only partial and more investigations are necessary to draw correct conclusions.

CONCLUSIONS

1. In trial A are noticed differences with no statistical differences between average values of ejaculation pH in the three control weeks.
2. In trial B calculated values of Student test are revealing significant differences in pH values between weeks 25 and 35 the others being caused by chance or individual variation with no statistical significance. Registered pH values are higher aiming to basic zone of pH so we could expect to a lower semen quality.
3. In trial C observed differences are found not significant statistical.
4. Considering the semen pH it could be recommended usage of values under the technological standard of macroclimate parameters de and a litter of chopped straws.

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DIVERSITY OF THE SOIL MITE POPULATIONS FROM AN ANTHROPIC ECOSYSTEM FROM HUNEDOARA COUNTY-ROMANIA

Minodora MANU, Viorica HONCIUC, Marilena ONETE

Romanian Academy, Institute of Biology Bucharest, Department of Ecology, Taxonomy and Nature Conservation, 296 Splaiul Independenţei Street, Bucharest, Romania

Corresponding author email: minodora_stanescu@yahoo.com

Abstract

In 2015, the diversity of the soil mite populations from an anthropic ecosystem, as a tailing pond, was investigated. The study area was located on Mealului Valley, Certeju de Sus locality from Hunedoara County. In total, 210 soil samples were analyzed, from five transects. The vegetation was classified in two groups: tall plant species and herbaceous ones. The altitude from the studied transects variated between 325 and 363 meters. In total 1138 mites were extracted, belonging to three orders: Trombidiformes (87 ± 15.22 individuals), Sarcoptiformes (992 ± 147.6) and Mesostigmata (59 ± 7.01 individuals). Dominant were decomposer mites, followed by the predators' ones. Analysing the Shannon diversity and dominance index, we observed that the highest values was obtained in transects characterized by an herbaceous layer, in comparison with those with tall vegetation. Using correspondence analysis, we revealed that the mite fauna grouped in two classes: one the preferred the small herbaceous layer (especially invertebrates from Mesostigmata order and mites from six families belonging to Trombidiformes order) and one that is correlated with tall vegetation (as Oribatida).

Key words: *anthropic, coverage, mite, soil, vegetation.*

INTRODUCTION

Mites represent one of the most abundant fauna group from soil. Due to their small dimensions (microscopic ones) they are many times neglected, although they are very important in direct or indirect decomposing of organic matter. Edaphic communities are important for soil formation, they contribute to the improvement of some characteristics of soils and they play key roles in many processes that enhance the success of ecological restoration (Cole et al., 2005; Lützow et al., 2006; Saitoh et al., 2011; Menta et al., 2014).

Ecological studied revealed that diversity and structure of the mite population vary depending on the type of investigated ecosystems and on the environmental factors (Nielsen et al., 2012; Birkhofer et al., 2012; Hasegawa et al., 2013; Manu et al., 2013; Dirilgen et al., 2016). Due to these characteristics, mostly often soil mite populations are considered bioindicators, they being used in assessment of successional stage of different anthropic ecosystems (as coal dump areas, deforested surfaces or other industrial areas). Mining causes significant damage to the environment: the removal of top layers of soil causes loss of structure and functionality, with a subsequent reduction in biodiversity. Unfortunately, soil mite fauna are poorly monitored even though they represent a good tool for assessing soil quality (Menta et al., 2014). The most studied mite orders were Sarcoptifomes (Oribatida) and Mesostigmata (Beaulieu and Weeks, 2007; Havlicek E., 2012).

In Europe, the majority of studies on the soil mite populations from anthropic ecosystems were made in mine area or other industrial disturbed soils from Italy, Austria, Germany, Svalbard and Poland (Wanner and Dunger, 2002; Caruso and Migliorini, 2007; Menta et al., 2014; Madej and Kozub, 2014; Coulson et al., 2015).

In Romania, studies on soil mite communities from anthropic ecosystems are few and fragmented. If we take into consideration the industrial areas from Romania, the most recent acarological investigations were made in spoilt dumps from Retezat Mountains – Hunedoara county, heavy metal polluted grasslands from Zlatna –Alba county or polluted forest with cement dust from Câmpulung Muşcel - Argeş county (Manu, 2010; Călugăr, 2013; Manu et

al., 2016). A detailed research presents the taxonomical and numerical structure of the gamasid populations from four spoilt areas from Retezat and Tarcu-Petreanu mountains. Eighteen species were identified, belonging to 14 genera and 9 families. Species *Cheroseius borealis* and *Hypospis aculeifer* were dominant and present in all studied surfaces. The mite populations had structural and dynamical differences, caused by the various environmental conditions (like vegetation, soil humidity and pH). To evidence these modifications were analyzed the following statistical parameters: number of species, numerical density, relative abundance, dominance, constancy. Comparative with predator mite populations from the natural ecosystems, the values of these parameters obtained for gamasids from the spoilt areas were very low. It shows that in anthropized ecosystems the influence of the specifically biotic and abiotic factors on the mite populations was significant.

In this context, the objective of the present study was to assess the diversity aspects of all identified edaphically mites in correlation with vegetation type, specific for an anthropic area as a tailing pond (Manu, 2010).

MATERIAL AND METHODS

The present study was developed in Mealului Valley, Certeju de Sus locality from Hunedoara County (Figure 1).

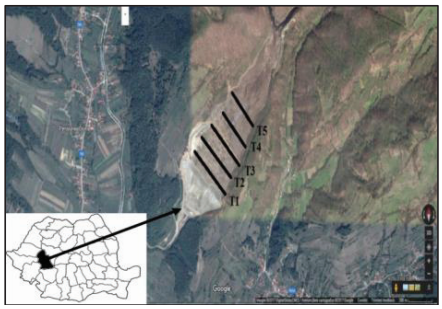

Figure 1. The geographic description of investigated ecosystem from Mealului Valley

Due to the gold and silver exploitation from the communism period (1565-1990), the landscape was modified from a natural meadows and forests to an anthropic ecosystem (a tailing pond).

This tailing pod was situated at $45^{0}57'38.97"$ N and $22^{0}58'55.39"$ E.

Altitude has varied between 325 and 370 meters. The soil samples were collected in September 2015.

The dominant high plant species (1-5 meters) from the tailing pond were: *Calamagrostis arundinacea* (L.) Roth*, Phragmites australis* (Cav.) Trin. Ex Steud, *Robinia pseudoacacia* L., 1753 (in T1, T2, T3 and T4) and *Betula pendula* Roth*, Salix purpurea* L., *Phragmites australis* (Cav.) Trin. Ex Steud (in T5). The coverage percentage was: 72% in T1 and T3; 42% in T2; 98.8% in T4 and 88.75% in T5 (Figure 2A).

If we take into discussion the herbaceous vegetation with a height of 0-1 meter from the soil, the dominant species were: *Conyza canadensis* (L.) Cronquist*, Medicago sp*. (in T1,T2), *Agrostris sp.* (in T2, T3), bryophytes (T1, T2, T3, T4), *Equisetum palustre* L. (in T3), *Tussilago farfara* L. (in T4) and *Epilobium palustre* L. (T5).

The coverage percentage was: 24% in T1, 37% in T2, 21% in T3, 12% in T4 and 11.5% in T5 (Figure 2B). In order to investigate the heterogeneity of the tailing pond, five transects were established in this ecosystems.

The distance between transects is about 25 meters. In each transect, 14 sampling points were established.

From each sampling point, three soil samples were collected. In total, 210 samples were analyzed (42 samples/transect X 5 transects), with a MacFadyen corer, on a depth of 10 cm, by 5 cm diameter.

The mites were extracted with a modified Berlese-Tullgren funnels, in ethyl alcohol, clarified in lactic acid and identified to family level, using actual published identification keys (Balogh, 1972; Balogh and Mahunca, 1983; Ghiliarov and Bregetova, 1977; Karg, 1993; Gwiazdowicz, 2007; Krantz and Walter, 2009).

In order to assess the diversity aspects of edaphically mites, a comparative analyze was made between the five transects from the anthropogenic ecosystem. Mite diversity (Shannon index), dominance (D index) and equitability (J index) and the correspondence analysis (CA) were calculated using the statistical soft PAST (Hammer et al., 2001).

A. Tall vegetation

B. Herbaceous layer

Figure 2. The vegetation from investigated ecosystem from Mealului Vallety (A- tall vegetation; B- herbaceous layer)

RESULTS AND DISCUSSIONS

In total, 1138 mites (including immatures) were extracted from the 210 soil cores (87±15.22 individuals from Trombidiformes order, 992 ± 147.6 from Sarcoptiformes order and 59 ± 7.01 individuals from Mesostigmata).

From Trombidiformes order, six families were identified, the dominant ones being Trombidiidae and Cunaxidae (Table 1).

Table 1. Numerical abundance of the investigated mites' taxa from Mealului Valley

	Taxa	T1	T2	T3	T4	T5	Total
Order	Trombidiformes						
Family	Tydeidae		6	4	1	1	12
	Trombidiidae		3		13	5	21
	Cunaxidae		5	7	1	5	18
	Tetranychidae			1			1
	Cheyletidae			3			3
	Scutacaridae			1		31	32
	Total		14	16	15	42	87
Order	Sarcoptiformes						
Suborder Oribatida							
Family	Ceratozetidae	2	12	4		5	33
	Astegistidae		90	68	6	16	180
	Opiidae	5	106	78	102	344	635
	Eulohmanniidae	1					1
	Galumnidae				6	15	21
	Liacaridae				1		1
	Belbidae	1					1
	Mycobatidae				1	4	5
	Phenopelopidae		1	1	2		4
	Protoribatidae					1	1
	Suctobelbidae	1	1	2			4
	Tectocepheidae	1	55	17		1	74
Suborder Astigmata							
Family	Acaridae		18	12		5	35
	Glycyphagidae			4		3	7
	Total	11	283	186	118	394	992
Order	Mesostigmata						
Family	Ascidae		6	7		16	29
	Amblyseidae		1			1	2
	Lealapidae		8	6	4	2	20
	Rhodacaridae		1				1
	Uropodidae	5		1		1	7
	Total	5	16	14	4	20	59

Analyzing the dominance and Shannon diversity index, we observed that the highest values were obtained in T3, followed by the T2 and T1.

The equitability index revealed that in transects with high values of mite diversity, the taxa were represented by a similar number of individuals (Table 2).

Table 2. Population parameters of the investigated mites from Mealului Valley

Transect	T1	T2	T3	T4	T5
No.of taxa	7	14	16	10	17
No. ind.	16 (±1.41)	313 (± 28.19)	216 (± 19.86)	137 (± 20.32)	456 (± 68.26)
Dominance_D	0.23	0.23	0.24	0.57	0.58
Shannon_H	1.68	1.75	1.84	1.03	1.11
Equitability_J	0.86	0.66	0.66	0.45	0.39

Using correspondence analysis (CA), in order to evaluate the relationship between mite taxa abundance and habitat, we determined the preferences of the all soil invertebrates for the investigated transects from tailing pond.

The eigenvalue (the dispersion of the sites/species distribution along the ordination axis) was significant for axis 1 (k = 0.37) and axis 2 (k = 0.34) (Figure 3).

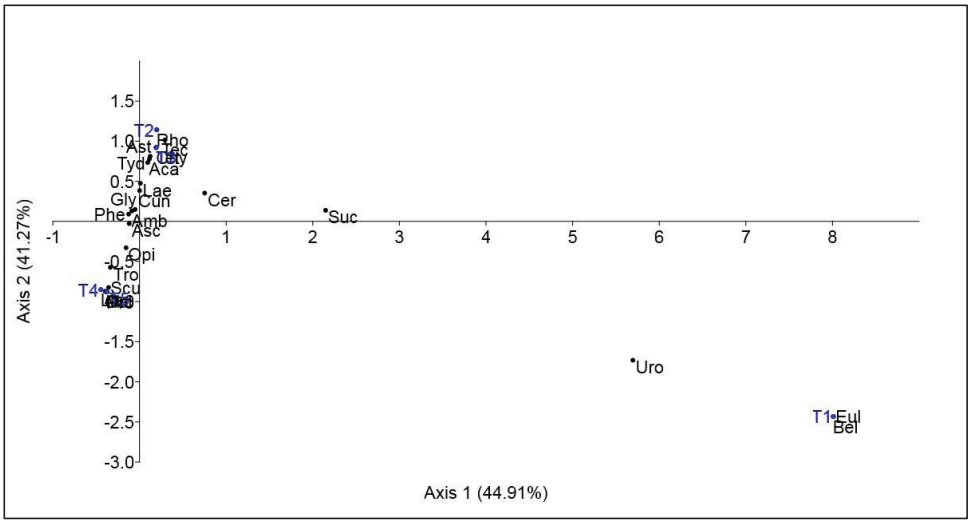

Figure 3. Correspondence analysis (CA) of the taxa abundance and investigated transects (Eul=Eulohmanniidae; Phe=Phenopelopidae; Aca=Acaridae; Amb=Amblyseidae; Asc=Ascidae; Ast=Astegistidae; Bel=Belbidae; Cer=Ceratozetidae; Chy=Chyletidae; Cun=Cunaxidae; Gal=Galumnidae; Gly=Glycyphagidae; Lae=Laelapidae; Lia=Liacaridae; Mic=Micobatidae; Opi= Opiidae; Pro=Protoribatidae; Rho= Rhodacaridae; Scu= Scutacaridae; Suc=Suctobelbidae; Tec=Tectocepheidae; Tet=Tetranychidae; Tro=Trombidiidae; Tyd=Tydeidea; Uro=Uropodidae)

Axis 1 divided the mite fauna in two groups, differentiated by their preferences to certain transect: T2-T3-T1 (with the highest coverage percent of herbaceous layer) and T4-T5 (with a lowest coverage percent of herbaceous layer, but highest at tall plant species).

Mites associated with the first group were: species from Tydeidae, Cunaxidae and Cheyletidae families (Trombidiformes); Uropodidae and Laelapidae (Mesostigmata) and Ceratozetidae, Astegistidae, Suctobelbidae, Tectocepheidae and Acaridae (Sarcoptifomes).

The second group is formed from Trombidiidae, Scutacaridae (Trombidiformes), Mycobatidae, Opiidae and Galumnidae (Sarcoptiformes).

Mites from Cunaxidae family are present in various type of ecosystems (forest, grasslands, agricultural fields), but also in anthropogenically disturbed areas. They are opportunistic

predators, feeding with nematodes, collembolans, phytophagous mites or thrips (Skvarla et al., 2014). Tydeidae are among the smallest mites, many being fungus feeders, but others plant feeders, predators and scavengers (Da Silva et al., 2016).
Invertebrates from Cheyletidae family are phytophilous predators, searching prey on the leaf and steam surface of plants (Walter and Proctor, 2003).
Their preferences for the transects with abundant herbaceous vegetation, could be possible due to the specific more compact habitat, that created favorable environmental condition for these predators mites.
On the other hand, in transect T1, T2 and T3, a developed moss layer was identified, this representing a favorable habitat, for predator mites as Laeleapidae. The moss layer is rich in organic matter and other soil invertebrates, which are the trophic source for these mites (Salmane and Brumelis, 2008; Glime, 2013).
We also observed that in T4 and T5, was recorded the highest numerical abundance of oribatids. The results are confirmed by the studies of other specialists, which revealed that the densities of Oribatida were positively correlated with tall vegetation (as trees from broad-leaved forests) and the species richness of Mesostigmata was positively correlated with species richness of forest floor plants (Hasegawa et al., 2013; Schatz, 2015).
The second axis differentiates mites into two groups: T1-T4-T5 and T3-T2, the first group being correlated with transects were *Phragmites australis* was identified, in comparison with the last one, which preferred the habitat with *Agrostris sp.*(Figure 3).

CONCLUSIONS

Each investigated transects from tailing pond were characterized by specific vegetation composition and coverage. Considering the mite fauna, the most abundant group was represented by Sarcoptiformes order, mainly represented by oribatids, followed by Trombidiformes and Mesostigmata orders. Quantification of Shannon diversity index indicated that transects with herbaceous vegetation represented the most favorable habitats for the soil mites. The equitability index revealed that in transects with high values of mite diversity, the taxa were represented by a closed number of individuals.
Using multivariate analysis, the influence of vegetation on soil mite populations was investigated.
The results revealed that the mite fauna grouped in two classes: one the preferred the small herbaceous layer (especially Mesostigmata and some Trombidiformes) and one that is correlated with tall vegetation (as Oribatida).
In order to investigate more complex ecological process between soil mite and characteristic environment of an anthropic ecosystem, as tailing pond, further investigation are requested.

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CORRELATIONS BETWEEN SOIL MICROBIAL FLORA, PLANTS AND FARM ANIMALS HEALTH IN ORGANIC FARMING

Alexandru POPESCU

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: popescu.alexandru@ansvsa.ro

Abstract

This study regards organic and biodynamic agriculture as forms of nature expression without interference with synthetic substances. The work is about the soil microbiology, feed plants health and the quality parameters of animal products. The author had made a parallel between the mechanisms of antibiotic resistance in humans and animals and those that generate: soil microbial disorders, plant vulnerability to pest attack and the receptivity of farm animals to infectious diseases. The factors involved in these phenomena were herbicides, pesticides and synthetic fertilizers. Using the principles of homeopathic treatments, associated with the biodynamic farming doctrines for the rehabilitation of denatured soils, on new scientific basis, the study demonstrated the possibility of recovering degraded land from human actions.

Key words*: animal health, biodynamic agriculture, soil microorganisms.*

INTRODUCTION

Despite the fact that world agricultural production is steadily increasing, as a consequence of the increase in the world's population, more and more signals announce a collapse of the productive capacity of the soil with dramatic consequences for the future. Faced with these challenges, many companies have tried in recent decades to find solutions for avoiding a food catastrophe.

The variables are the following: soil health, fodder plant diseases, animal diseases and ultimately public health. Methods of control used up to now: pesticides, herbicides, antiparasitics, antibiotics, pH and GMOs reduce their efficiency from year to year. As a result, scientists are trying to find solutions to develop healthy and renewable farming methods, while pursuing the recovery of land compromised by conventional practices used in the past.

Starting from the concept of biodynamic agriculture (Steiner, 2012) enunciated by Rudolf Steiner in 1924 and subsequently applied in practice both in Europe and the USA, more and more farmers approached agricultural sciences at an unconventional angle, considered for decades to be a pseudoscience. In recent years, due to the advances made in the study of human microbiome, rhizomicrobiome and nanotechnology, these practices have begun to be reconsidered (Teruo and Parr, 1994). This study has demonstrated that biodiversity is the essence of balance in nature (Chhabra, 2017). Interaction between microbial flora populations often determines the biodiversity of plants.

Any human action designed to eliminate certain species considered harmful, both microscopically and macroscopically, inevitably leads to a global dysfunction of the biotope with adverse consequences already known in agriculture (Sohag et al., 2010). Recent data show that up to 10 billion bacteria and 10 million fungi (de Vrieze, 2015) can be found in the soil around each rhizosphere. Microbes have multiple functions in the soil: they can provide plants with nutrients and minerals from the soil, produce growth stimulating hormones, stimulate the immune system of plants, and trigger or mitigate stress responses.

MATERIALS AND METHODS

Four farms from different pedoclimatic areas were selected to track the soil, plant, animal or human route. The first location was in the Burnaz Plain on the Teleorman River bank, the second in Bărăgan Lehliu – Dor Mărunt area, the third on the bank of the Bârsa brook, on a

plot belonging to Vulcan village, and the fourth in the region of the Precarpathian hills in the Breaza area. Corn fodder and wheat have been grown in the meadow or in the plain areas. In the hilly region the basic crop was that of vegetables. Each ranch, with the exception of the vegetable one, owns a beef and/or a dairy farm. Soil samples were collected from each farm after protocols agreed by statisticians. Twenty dominant bacterial species and five mushroom species were identified. Laboratory examinations were done in the pedology laboratory in Pécs. In addition to qualitative tests, two simple and inexpensive methods were used to measure microbial activity in the soil: the respirometry method and the cotton strip test method (image analysis and tensometer) (Gunasekhar et al., 2007).

In November 2015 samples of compost soil with cow dung were collected and were placed in a cow horn. The horn was buried in the ground with the bottom down to about 20 cm deep. The central area of the plot was chosen as the place of choice for soil sampling and horn burial. In April each horn was dug out and the content was placed in a container of five hundred liters of water. The liquid was mixed continuously for an hour, using the Hanemann method, in order to obtain a dynamization dilution (Bellavite, 2005). With the help of sprinklers, the solution was sprinkled on three hectares of cereal crop for each cattle farm (six containers per hectare) and on 1000 m^2 of vegetable farm. A second identification of the microbial flora was performed one year after the first harvest. At the same time, the productive characteristics of the parcels were determined, following the quality of the vegetal material, the degree of parasitic attack, the health status of the animals fed with these fodders and the quality of the products obtained from them (Miller-Ensminger, 2018).

RESULTS AND DISCUSSIONS

The bacterial species identified in the previous year were also found in the following year, with the difference in population proliferation that changed sensitively. The species found in the soil were: *Acidobacterium capsulatum, Azotobacter agilis, Azotobacter salinestris, Azotobacter chroococcum, Arthrobacter aurescens, Bacillus thuringiensis, Bacillus coagulans, Bacillus subtilis, Thiobacillus thiooxidans, Thiobacillus denitrificans, Chromatium okenii, Frankia asymbiotica, Methanobrevibacter smithii adhaesiva, Rhizobium aggregatum, Methylobacterium organophilum, Nitrobacter vulgaris, Rhodopseudomonas palustris, Rhodobacter sphaeroides, Xanthomonas perforans* (Table 1, Table 2 and Table 3).

The identified mushroom families were: *Aspergillus, Rhizopus, Trichoderma, Penicillium, Fusarium*.

Table 1. Bacterial species ratio in farm 1

Bacterial species	2016 (%)	2017 (%)
1. Acidobacterium capsulatum	5.5	9.5
2. Azotobacter agilis	0.1	0.3
3. Azotobacter salinestris	10.1	5.4
4. Azotobacter chroococcum	1.7	2
5. Arthrobacter aurescens	1	0.5
6. Bacillus thuringiensis	14.7	5.8
7. Bacillus coagulans	5.1	8.1
8. Bacillus subtilis	2.8	1.8
9. Thiobacillus thiooxidans	5.3	9.4
10. Thiobacillus denitrificans	3.6	8.3
11. Chromatium okenii	2.8	1.3
12. Frankia asymbiotica	0.9	0.4
13. Methanobrevibacter smithii adhaesiva	4.8	3.1
14. Rhizobium aggregatum	1.5	2.1
15. Methylobacterium organophilum	2.3	3.4
16. Nitrobacter vulgaris	6.1	10.8
17. Rhodopseudomonas palustris	8.8	14.3
18. Rhodobacter sphaeroides	4.7	3.5
19. Xanthomonas perforans	6.1	3.8
20. Holophaga foetida	12.1	6.2

Table 2. Bacterial species ratio in farm 2

Bacterial species	2016 (%)	2017 (%)
1. Acidobacterium capsulatum	6.1	7.2
2. Azotobacter agilis	0.3	0.5
3. Azotobacter salinestris	8.7	7.2
4. Azotobacter chroococcum	2.2	1.3
5. Arthrobacter aurescens	1	1.5
6. Bacillus thuringiensis	15	11
7. Bacillus coagulans	7.8	5.8
8. Bacillus subtilis	4.3	4.1
9. Thiobacillus thiooxidans	3.1	2.8
10. Thiobacillus denitrificans	2.4	8.6
11. Chromatium okenii	4	3.3
12. Frankia asymbiotica	0.1	0.2
13. Methanobrevibacter smithii adhaesiva	6.2	7.9
14. Rhizobium aggregatum	3.9	3.6
15. Methylobacterium organophilum	1.8	1.1
16. Nitrobacter vulgaris	8.4	12.4
17. Rhodopseudomonas palustris	5.2	7.2
18. Rhodobacter sphaeroides	3	9.3
19. Xanthomonas perforans	2.5	2.6
20. Holophaga foetida	14	2.4

Table 3 – Bacterial species ratio in farm 3

Bacterial species	2016	2017
1. Acidobacterium capsulatum	7.5	2.9
2. Azotobacter agilis	0.5	4.1
3. Azotobacter salinestris	8	6.3
4. Azotobacter chroococcum	3.9	2.2
5. Arthrobacter aurescens	1.2	1.9
6. Bacillus thuringiensis	12.5	6.4
7. Bacillus coagulans	9.1	11.7
8. Bacillus subtilis	5.6	6.1
9. Thiobacillus thiooxidans	0.7	5.4
10. Thiobacillus denitrificans	1.2	8.9
11. Chromatium okenii	3	4
12. Frankia asymbiotica	1	0.5
13. Methanobrevibacter smithii adhaesiva	11	7
14. Rhizobium aggregatum	7.8	6.7
15. Methylobacterium organophilum	0.6	1.1
16. Nitrobacter vulgaris	0.8	0.4
17. Rhodopseudomonas palustris	18	9
18. Rhodobacter sphaeroides	2.8	5.8
19. Xanthomonas perforans	1.2	5.2
20. Holophaga foetida	3.6	4.4

By the respirometry method, microbial biomass was determined in the soil on the twelve day of incubation. On soils treated with dynamized solution, microbial biomass increased significantly between 200% and 250%. Testing the resistance of cotton tape on buried fibers for 35 days with the tensometer, as well as image analysis by the color intensity measurement technique, confirmed an increased enzyme activity in experimental plots.

The primary production obtained on the tested land parcels as compared to the witness one showed superior quality of the plant material used for silos with better consistency and an increase in the amount of gluten from the seeds. The palatability of the silo improved, the animals showing a higher appetite than those in the witness group. As far as the secondary production is concerned, the quality of the carcasses in Angus cows was higher by 5 - 8% compared to those fed with the feed on the witness group (Figure 2). The morbidity of juveniles was 0 whereas in the witness group there were 5 cases of illness (group of 25 heads). The amount of milk harvested from the experimental plots was 8 -10% higher than the witness one (Figure 1), the microbial load and the number of somatic cells, lower.

In terms of soil fertility, three large groups of microorganisms have been identified, that live in different proportions. The first is that of positive microorganisms involved in soil regeneration. The second is that of negative organisms that contribute to soil degeneration.

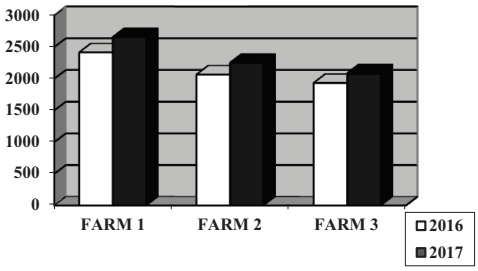

Figure 1. Evolution of milk production
2016 – FARM 1: 105 dairy cows *23 l/day
FARM 2: 86 dairy cows *24 l/day
FARM 3: 69 dairy cows *28 l/day
2017 – FARM 1: amount of milk higher 10%
FARM 2: amount of milk higher 9%
FARM 3: amount of milk higher 8%

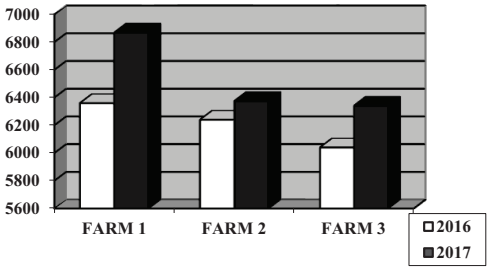

Figure 2. Evolution of the carcasses quality
2016 – FARM 1: 20 Angus cows *318 kg/carcass
FARM 2: 20 Angus cows *312 kg/carcass
FARM 3: 20 Angus cows *302 kg/carcass
2017 – FARM 1: quality of carcasses higher 8%
FARM 2: quality of carcasses higher 6%
FARM 3: quality of carcasses higher 5%

The third one has a neutral but also opportunistic manifestation. It join the first group or the second group according to small changes in the environment. By multiplying these microorganisms we can potentiate the effect of the first or of the second. Conventional agriculture can destroy soil rhizobioma (microbial ecosystem) by using foreign substances, such as fertilizers and pesticides, without compensating for these effects.

Although the studies are still in a preliminary stage, many variables needing to be controlled, we can say that by the "potentialization" of the matter by dynamization, the soil microorganisms populations transfer positive properties to the inorganic substrate, affecting the structure and fertility of the soil. During plant domestication, they were selected for crop-related attributes, but not for plant-friendly associations with a beneficial microbiome.

Agriculture can destroy soil rhizobioma (microbial ecosystem) by using soil modifications, such as fertilizers and pesticides, without compensating for their effects. On the contrary, healthy soil can increase fertility in a number of ways, including supplying nutrients such as nitrogen and also protecting against pests and viral, bacterial or fungal diseases.

CONCLUSIONS

The composition of rhizobiome can change rapidly in response to changes in the environment. By Hanemannian, dinamization of the compost solution, opportunist organisms join the positive action helping to recover compromised soils.

Even minor changes in the amount of certain bacteria can have a major effect on plant defense and physiology.

On the contrary, healthy soil can increase fertility in several ways: providing nutrients such as nitrogen and protecting against pest and viral, bacterial or fungal diseases.

A more diversified soil microbe stimulates plant biodiversity and results in increased yields and reduced animal disease.

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TECHNOLOGIES OF THE AGRO FOOD PRODUCTS PROCESSING

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INVESTIGATION OF THE IMPACT OF BIOCHAR ON THE NECTARIFEROUS QUALITIES OF THE ZUCCHINI (*Cucurbita pepo* var. *giraumontia* Filov)

Tsvetelina NIKOLOVA, Vera PETROVA, Milena YORDANOVA, Asia PETROVA

University of Forestry, Faculty of Agronomy, 10KlimentOhridski Blvd, 1797, Sofia, Bulgaria,

Corresponding author email: c.alipieva@abv.bg

Abstract

The aim of the study was to determine the influence of biochar, incorporated as a soilmeliorant in the cultivation of zucchini, on the nectariferous qualities of their flowers. The experiment was carried out in 2017 on the experimental field of the University of Forestry – Sofia. In the spring cultivation of the soil were incorporated biocar and manure, and were developed different variants: 1) control - no biochar and manure; 2) only with manure – 4 t/ha^{-1}; 3) biochar - 500 kg/ha^{-1}; 4) manure + reduced amount of biochar (250 kg/ha^{-1}); 5) manure + optimal amount of bio char; 6) manure + increased amount of biochar (750 kg/ha^{-1}).Forthepurpose of thestudyit wasfollowed: the development of the plants, the flowering duration, the number of flowers per plant and the amount of separate nectar and pollen. It was recorded the visit of bees on flowers of the studied plants. This determines the nectariferous potential of zucchini. The increase of nectar productivity by the flowers was observed in the variants with combination of biochar and manure.

Key words*: bio char, Cucurbita pepo var. giraumontia Filov, honeybee, nectar productivity, zucchini.*

INTRODUCTION

Some of the vegetable plants are of no importance to bees, and some of the more valuable honey plants. Grown throughout the country, asseparate area in mixed plantings which is why they are of interest to bees. These crops provide food for people and some of them are juicy fodder for domestic animals (Bizhev, 2003).

Cucurbita pepo is an one yearl dicotyledonous plant of the family Cucurbitaceae and constitute a separate variety group there to *ser. var. Giromontia*. They come from Asia Minor. The valuable biological and economic qualities of courgettes have given rise, they are rapidly spreading to southern Europe and the Mediterranean. In our country they are grown in almost the whole country.The species from family Cucurbitaceae depend on the pollination of honeybees for fruit production. Representatives of this family have large yellow-colored colors, which emit a large amount of nectar and pollen and are visited very well by bees. Female flowers produce large amounts of nectar, and males - mostly pollen and less nectar. They bloom for a long time and provide good grazing for bees.

Biochar not only enriches the soil with nutrients but also reduces acidity. It also creates more favorable conditions for the development of useful microflora and leads to increased yields. Inaccessible to the microorganisms compounds are embers of wood, which are carbonated materials, a product of incomplete combustion of different organic materials. The studies of Glaser et al. (2002) prove that charcoal is suitable for maintaining a high level of soil organic matter.

Biochar resulting from pyrolysis of plant materials are called bio-coal and are subject to research in some countries as a means of improving the structure and fertility of soils. This direction of their application dates back to at least 2500 years ago, as is the case for areas close to the Amazon river (Woods et al., 1999; Maris, 2006; Glaser, 2007).

Manure is characterized by its high content of macroelements, vitamins, growth regulators and useful microorganisms. Its use leads to an increase in yield and production quality. (Sreenivasaetal., 2010; Natarjan, 2007).

The main objective is to study the impact of imported carbonated plant residues as a soil improver, on the nectariferous quality of the species *Cucurbita pepo var. giraumontia*. Determine the amount of individual pollen nectar from each variation.

MATERIALS AND METHODS

Studies were conducted in the learning experience of base "Hostile"of the University of Forestry - Sofia, in 2017.On alluvial meadow soil, an area of 150 m^2. The experiment was set by the standard method and circuit 100 x 60 x 50. After pretreatment of the soil are sized test Partselki. Biochar and manure were imported in March on the following scheme:

variant I - Control - cleansoil
variant II - Fertilizer 4 t/da = 140 kg
variant III - Biochar 500 kg/da = 23.5 kg
variant IV - Fertilizer 140 kg + Biochar 250 kg/da – 12 kg
variant V - Fertilizer 140 kg + Biochar 500 kg/da - 23.5 kg
variant VI - Fertilizer 140 kg + 2750 kg/da – 35 kg
variant VII – Fertilizer 4 t/da = 140 kg

Fenological and biometric measurements were performed on five plants of each variant in the phases - the beginning of flowering, the mass flowering and the end of flowering when the signs. We tested indicators by the method of Simidchiev 80: Start and duration of flowering; Flowering duration of a separate color; Number of colors on one plant; Amount nectar of 1 color for 12 h in mg; Percentage sugar content in the nectar; Quantity of pollen in mg;

RESULTS AND DISCUSSIONS

Despite the differences between experiments and control plants, it has to be noted that in the beginning of mass flowering the differences between the different variants are very small.At the beginning of blossom blossomed only female color, a few days later began flowering and onmalecolor.

Table1. Phonological indicators for zucchini related to the determination of the honey productivity

Variant	I	II	III	IV	V	VI	VII
Initial flowering (days)	08 June	08 June	08 June	08 June	08 June	08 June	08 June
Mass tsaf. (Days)	28 June	28 June	28 June	28 June	28 June	28 June	28 June
End of flowering (days)	10 July	10 July	10 July	10 July	10 July	10 July	10 July

Table 2. Flowering duration for *Cucurbita pepo var. giromontia*

Variant	I	II	III	IV	V	VI	VII
Flowering time. per plant (days)	33 days	33 days	33 days	33 days	33 days	33 days	33 days
Initial flowering (days)	2 ♀	2 ♀	2 ♀	2 ♀	2 ♀	2 ♀	2 ♀
Mass tsaf. (days)	7 ♀, 6 ♂	7 ♀, 6 ♂	7 ♀, 6 ♂	7 ♀, 6 ♂	7 ♀, 6 ♂	7 ♀, 6 ♂	7 ♀, 6 ♂
Duration of tsaf. one color (days)	4 days	4 days	4 days	4 days	4 days	4 days	4 days

♀ - female color, ♂-malecolor

Table 3. Quantity of nectar and sugar content in colors of *Cucurbita pepo var. giromontia*

Variant	Amount of nectar of 1 color in (ml)		Sugars in the nectar, (%)		Amount of pollen,(mg)
	♀-color	♂-color	♀-color	♂-color	♂-color
I	75.5	70.5	29.9	31.5	0.15
II	76.9	72.6	30	32.9	0.2
III	78.6	73.1	31.6	32.7	0.14
IV	89.5	72.8	30.5	32.6	0.2
V	89.9	73.2	37.2	35.1	0.22
VI	89.4	73	30.9	31.8	0.23
VII	76.9	72.5	30	32.9	0.2

From female flowers, a greater amount of nectar was released in all assay variants from 75.5 mg to 89.9 mg.Because of the species specificity of *Cucurbita pepo var. giraumontia*, we recorded lower nectar values in male flowers from 70.5 to 73.2 mg. Sugar content in nectar is relatively high from 29.9% to 37.2% with a maximum of 40% sugar content. Highest values are reported for V variant. Our results are close to those ofMassimo 1993, 2001.

The obtained results show that the biochar introduced into the soil has a positive effect on

the color of the zucchini (Table 1, Table 2). For variants VI, V and VI the colors produce higher amounts of nectar and pollen (Table 3).

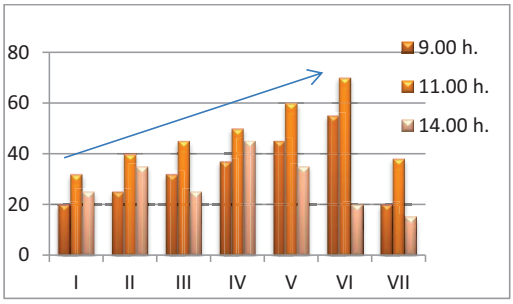

Figure 1. Visiting the bees by the colors of *Cucurbita pepo var. giromontia.*

Figure 1 shows that bees began to visit flowers in 9:00 hours. Mass visit was reported with increasing temperature and solar radiation for 11 hours and then decline before closing color. The highest number of bees was recorded for option VI. The inputs of biochar and manure in the soil in this variant have a favorable effect on the quantities of nectar and pollen which have been shown, as evidenced by the higher values shown in Figure 1.

CONCLUSIONS

The results of this study show that the colors of *Cucurbita pepo var. giraumontia* produce an abundance of nectar and pollen that are available in quantities sufficient to maintain bee families in large plantations.Through proper farming practices create the most favorable conditions for plant growth, and indirectly to improveseparation of the nectar.

The increase in nectar production by the colors is observed in the bio-manure and manure variants.

In conclusion, it can be concluded that the introduction of these soil improvers is reflected positive on all tested signs.

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STUDY OF THE HONEY PRODUCTIVITY OF SOME TREE SPECIES

Tsvetelina NIKOLOVA, Asia PETROVA, AleksandraYORDANOVA

University of Forestry, Faculty of Agronomy, 10 Kliment Ohridski Blvd,
1797, Sofia, Bulgaria,

Corresponding author email: c.alipieva@abv.bg

Abstract

For the proper development of the bee families and the best and varied grazing, the beekeeper needs to know theavailableplantsbearing honey that bees will use as a source of food. And all the factors that affected the flowering period of individual honey plants and conditions ensuring best separation of nectar.To clarify the underlying factors, we started a series of studies.Thepurpose of our study was to establish the phenology of flowering, nectar-productivity, sucrose productivity and honey productivity of tree species in urban conditions. This determines the nectariferous potentialof the species of the family Fabaceae and genus Tilia. Briefly flowering period these species emit a significant amount of nectar with high sugar content.

Key words*: honey qualities, tree honey species, honey productivity, blossoming phenology.*

INTRODUCTION

The life of bees is closely related to the bearing-honey plants as a source of food. From these plants, bees receive essential nutrients-nectar and pollen (Bizhev, 2003). Family *Fabaceae* includes over 19,400 species in about 730 genera, which is the third largest family among the angiosperms. In Bulgaria there are present 291 species, 49 of which are tree plants. Black locust is one of the most honey- bearing wood in our country and for many regions it appears to be the firstmajor grazing. In the lower and richest soils regions of Bulgaria, especially in northern Bulgaria along the Danube and Dobrudja, it produces much more nectar in comparison to those in the semi-mountainous and mountainous regions. Acacia's grazing it is very unreliable because it blooms during a period of the most variable weather, with frequent winds and rainfalls and bees very rarely use it fully (Bizhev, 2003).

Blossoms are white and emit very strong and pleasant aroma, which attract the bees. *Styphnolobium japonicum* (*Sophora japonica*) produces a lot of nectar and bees visit it intensely, including the fallen blossoms on the ground. Honey locust is very valuable honey bearing tree species. It flowers immediately after the acacia and provides the preparation of very aromatic honey. The Lindens (*Tilia*) is a genus of trees which includes about 40 species. In Bulgaria are mainly distributed three species of linden- large-leaf, small-leaf and silver-leaf. These three species in our country are appreciated as the most honey-bearing plants, releasing nectar in large quantities (Bizhev, 2003).

They are not flowering at the same time and that is how they provide longer period of honey collecting. The phenology of flowering of honey bearing plants is directly dependent on the climatic conditions of the geographical area (Fukusima-Hein et al., 1986; Moreti, 1992).

Bee hives and bee families can also successfully survive in urban conditions. This type of beekeeping has gained great popularity over the last few years. Growing conditions are more appropriate for these purposes, the temperatures in the cities are higher, and there is a variety of vegetation in the parks and the green areas. In these city areas the pesticides are not use and genetically modified organisms (GMO) are not grown.

The dirty air and exhaust gases from the cars are not obstacles, as the bees have the unique ability to filter and purify the nectar they collect (Ilieva, 2017).

MATERIALS AND METHODS

The Sofia valley is located between the mountainous parts of Stara Planina mountain in the northeast and the mountains Lyulin,

Vitosha and Lozenska in the southwest. It is the largest of all off-Balkan valleys in Bulgaria at altitude 560 m.

The study was carried out in 2017. For the purpose of the experiment are used single trees in the parks in the central city part of Sofia.

In order to establish the period and the duration of the flowering, are performed phenological observations in the area. The quantities of the collected nectar, its sugar content and honey productivity of the plants are determined by the method of Simidchiev (1980).

We determined the quantities of nectar by the capillary method and the sugar concentration of the nectar by the refractometer.

We determined the honey productivity by multiplying the total quantity by a coefficient 1.25 (Radoev and Ilieva, 1955), as the water content in the honey is 20%.

For the purpose of the experiment we chose branches of different heights and from different locations of the crown for study. We isolated the flowering branches with tulle for 24 hours. Depending on the flowering period, we studied the blossoms of each tree.

RESULTS AND DISCUSSIONS

Phenological observations of honey-bearing plants have been done by following: the flowering period by months (Table 1).

Table 1. Flowering period of tree species of family Fabaceae and genus Tilia

Latin name	March	April	May	June	July	August	September
Robinia pseudoacacia	-	-	⬢	-	-	-	-
Laburnum anagyroides	-	-	⬢	-	-	-	-
Cercis siliquastrum	⬢	⬢	-	-	-	-	-
Gleditschia triacanthos	-	-	⬢	⬢	-	-	-
Amorpha fruticosa	-	-	-	⬢	⬢	⬢	-
Albizzia julibrissin	-	-	-	⬢	⬢	⬢	⬢
Tiliagrandifolia	-	-	-	⬢	-	-	-
Tiliaparvifolia	-	-	-	⬢	-	-	-
Tiliaargentea	-	-	-	⬢	⬢	-	-
Sophora japonica	-	-	-	-	⬢	⬢	-

The results of Table 1 show that the *Albizzia julibrissin* has the longest period of flowering from June to September. It is pleasure for the bees to visit it for collecting nectar and pollen. The shortest period of flowering we observed in *Robinia pseudoacacia*, *Laburnum anagyroides*, *Tilia grandifolia* and *Tilia parvifolia* for an average of about 10 days.

The short flowering period of these species is due to species and climatic conditions during the period. Their flowering is very intensive in spring and stimulates the development of bee families.

False indigo-bush (*Amorpha fruticosa*) blooms late in May-early in June. It has long flowering period 20-25 days. It has a high capacity of honey, and if there are more plantings, it can strengthen the main bee grazing.

In our country common species of Linden bloom a little after the blossomed of *Acacia* and *Honey locust,* which coincides with the beginning of the summer. Earliest starts to flower *Tilia parvifolia*. After that*Tilia grandifolia* starts to flower *Tilia argentea* which is the most common in Bulgaria flowers at the latest.

Honey locust (*Gleditsia triacanhos*) is very good honey-bearing plant. Starts its flowering after the blossomed of Black locust. If there are these two species, the major grazing is extended by about two weeks.

Blossoms of the Honey locust produce less quantity of nectar than the blossoms of the Black locus (Table 2), but, if there are greater number of plants, her honey productivity is also high.

Table 2. Honey productivityof honey-bearing tree species

Latin name	Flowering duration (days)	Quantitynectar of 1 blossom in period of mass flowering (mg)	Sugar content (%)
Robinia pseudoacacia	10	3.5	43.2
Laburnum anagyroides	7	0.5	35.3
Cercis siliquastrum	13	0.95	41.5
Gleditschia triacanthos	15	0.42	37.8
Amorfa fruticosa	23	0.97	43.2
Albizzia julibrissin	34	0.16	32.6

During the flowering of the Honey locust we have established better weather conditions than those in the flowering of the Acacia. Under these conditions the major gazing from Honey locust is more permanent and more sure.
Flowering of *Styphnolobium japonicum* (*Sophora japonica*) we noted in July-August when the other flowering honey-bearing vegetation is too scarce. For that reason the *Styphnolobium japonicum* is very suitable for planting near the apiary to improve bee grazing
The flowering period of trees in the region of Sofia averages between 7 and 34 days for different species. It is significantly affected by climatic factors such as temperature and rainfall.
On the Table 2 is shown the amount of nectar of one blossom and sugar content in the nectar. The trees of the Fabaceae family have a high sugar content of 32.6 % for the Persian silk tree and up to 43.2 % for the Acacia.
In addition to nectar, bees also collect pollen from the flowers.
All tree species from the Fabaceae family, in table. 2 are good honey-bearing plants. They can provide the necessary quantities of nectar and pollen for bee colonies grown in large cities.
All three types of Linden are widespread in the area of the study. They are excellent hony-bearing plants and are well attended by bees for nectar and pollen throughout the day (Table 3).

Table 3. Honey productivity of genus *Tilia*

Latin name	Quantity nectar of 1 blossom in period of mass flowering	Sugar content (%)	Honey productivity (kg/dka)
Tilia grandifolia	9.5	48	15.2
Tilia parvifolia	3.9	50.3	14.5
Tilia argentea	4.7	28	4.8

The flowers are small, tender, gathered in the inflorescence of the midnight, stained in pale yellow. In one inflorescence there are from 5 to 7, with some limes being able to reach up to 15 colors. Depending on the type, it can be with or without hairs.

Of the three types of Linden, which are the subject of the study, the highest sugar content was recorded at *Tilia parvifolia*– 50.3%.
At Tilia argentea were recorded the lowest level of sugar content – 28% and relatively low honey productivity – 4.8 kg/dka.
The highest amount of nectar was recorded in *Tilia grandifolia* 9.5 mg, and the lowest for *Tilia parvifolia* 3.9 mg, but the sugar content is at the highest percent of 50.3. The median productivity is the lowest in *Tilia argentea* 4.8 kg/dka and approximately the same in the other two species (14.5p-15 kg/dka).
With the highest honey productivity – 15.2 kg/dka was *Tilia grandifolia*, and the reported sugar content (48%) is approximately as in the *Tilia parvifolia*.

CONCLUSIONS

The studied tree species successively bloom and create conditions for long and varied bee grazing. Bees are transferred fromone to theotherspeciesaccording to their flowering.
For the different species are reported the difference between the quantities of nectar and the sugar content of the flowers is due to the species specificity. The largest contribution as a source of nectar has the Black locust and the three species of Linden, which are very good pastures for bees and obtaining high yields of commodity honey for a honey.
With the made evaluation of honey-bearing tree species the possibility of developing apiculture in urban conditions has been explored.

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COW MILKMARKETED AT DISPENSERS - CHEMICAL COMPOSITION AND TOTAL NUMBER OF GERMS

Mircea-Valentin MUNTEAN[1*], Ovidiu-Marius MARIAN[1], Aurelia COROIAN[2], Zamfir Ioan MARCHIS[2], Bogdan ONICA[1], Sorin TERHES[3]

[1]Faculty of Agriculture, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Manastur Street, 400372, Cluj-Napoca, Romania.
[2]Faculty of Animal Science and Biotechnologies, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Manastur Street, 400372, Cluj-Napoca, Romania.
[3]S.C. Farm Ecolact SRL, 111 Bujorilor Street, Zalau,Romania

[*]Corresponding author e-mail: ovidiu.marian@usamvcluj.ro

Abstract

The processing of raw milk for human consumption and its sale on the market requires compliance with certain quality standards. According to criteria of quality and food safety the Regulation of European Parliament and the council no. 853/2004, milk quality is influenced by many factors: lactation, fat, protein, lactose,total number of germs. Analysing some of these factors on samples collectedin October 2017 from four milk dispenser shows large variations for certain parameters that are outside the quality criteria. These values denotes that it iscomplies with hygiene conditions and raw milk is delivered to collection points uncontaminated which means lack of hygiene conditions and milk contamination. Following the analysis of the 40 samples the results obtained from the four dispensers studied, milk can be consumed sure from dispenser D2 and also with certain restrictions from the D3 dispenser. It is not recommended to consume milk from the D1 and D4 dispenser. The aim of this study is to determine whether milk marketed by dispensers under the conditions specific to this period is affected in terms of qualitative parameter analysis. The biological material study was represented analyzed by 40 samples of milk from fourcow milk dispensers functional located in this period in Cluj-Napoca city. These samples were collected at the same time period during 9- 20 October2017.

Key words: *fat, lactose, milk, protein, total number of germs.*

INTRODUCTION

The national program on the quality of raw cow's milk and the timetable for the measures taken to comply with the EU legislation of the European Union is finalized (Raducu et al., 2016). According to this, farmers will have to comply with European hygiene quality standards in order to sell raw milk.In order to process the raw milk and to comply with the Quality and Food Safety Criteria, the Regulations (CR) of the European Parliament and Council no. Regulation (EC) No 853/2004 laying down specific hygiene rules forFoods of animal origin, milk quality is still one of the most important problems in cow's farms in Romania, especially hygienic parameters.

Starting from the premise that the qualitative parameters of the fresh milking milk are in line with these requirements, the study follows the qualitative changes that may occur in milk marketed by dispensers. Number of people who consume raw milk Is growing because of its taste, beneficial effects for human health, non-destructive valuable nutrients By thermal treatments (Oliver et al., 2009).

Direct sales to the consumer through milk dispensers are given by the following standards: A.N.S.V.S.A.111/2008; A.N.S.V.S.A. no. 55/2010): total number of germs (maximum 100,000 / ml and somatic cells (400,000 / ml) without antibiotic healthy animals that do not suffer from diseases which can be transmitted to people through milk (Ștețca et al., 2014).

Milk sold through dispensers is sold at a lower price than packed in packs and the milk price can also be influenced by the following factors: total number of germs (TNG), number of somatic cells, and percentage of fat, protein, lactose, casein.

The total number of germs is one of the main indicators of the hygienic quality of milk (Velea et al., 2012; Barreto et al., 2013). The total number of germs increases in the

possibility of bacterial infections or tissue damage (Nagahata et al., 1987) and negatively influences the quality of the cheese by proteolytic enzymes present in the mass milk, decreases milk production and affects its physic-chemical composition, its taste and its nutritional value (Nagahata et al., 1987; Norman et al., 2011; Souza et al., 2012).
The aim of this study is to determine whether milk marketed by dispensers under the conditions specific to this period is affected in terms of qualitative parameter analysis. These samples were collected at the same time period during.

MATERIALS AND METHODS

During 9- 20 October 2017, qualified personnel collected 40 samples from four cow milk dispensers functional in this period in Cluj-Napoca city. The samples were collected in special containers purchased from the milk quality foundation in Cluj-Napoca. The samples were collected from each dispenser at the same time (12.00-13.00, each day), ambient temperature at the time of collection varied between 7 degrees to 21 degrees Celsius which were kept atrefrigeration temperature for about one hour until the moment of analyse. All dispensers are equipped with milk refrigeration systems.Analyzes were made at Foundation for Milk Quality Control, Str. Avram Iancu, 407280, Floreşti, Romania.
Analysis of milk chemical composition and total numbers of germs (TNG) (Ştețca et al., 2014): To determine the chemical composition of raw milk, the following parameters were determined: fat content, protein, lactose, pH and total numbers of germs (TNG).
Based on international standard methods (ISO 9622:2013) milk components were determined using CombiFoss™ FT+, a combination apparatus between MilkoScan™ FT+ and Fossomatic™ FC (FOSS, Denmark). MilkoScan returns milk chemical component values by using infrared light measurement (Fourier Transform Infra-Red Spectrophotometry principle). Analysis duration was about 30 seconds. The electronic instrument, Fossomatic™ FC, used for the detection of somatic cell count (SCC) in milk, works based on the principle of coloring the somatic cells, than continues by counting them (flow cytometry). Milk sample was mixed with a fluorescent dye which colors the somatic cell's DNA molecules. A very thin string of milk was put under the counting unit, which permitted only one somatic cell to pass at a time. In the counting unit sample was exposed to blue light, which cause them to emit red light. Thus, the instrument can count the cells. All samples were analyzed in triplicate and the final results are listed below.
The European rules, represented by European Regulations 853/2004 and 854/2004, stipulate that the total number of germs (TNG) for healthy milk is below 200,000/ml of milk. Comparative determination of the number of total number of germs (TNG) from the samples taken from four dispensers considered reveals that TNG increases with conditions of poor hygiene and non-treatment of diseases in the mammary gland.The fat quality indicator states that the values obtained must be between 3.5 - 4.5%.The protein quality indicator states that the values obtained should be between 3.0 - 3.5%. Percentage, the lactose quality indicator indicates that the values obtained must be between 4.0 - 4.5%.The values presented in the table 1 are analyzed statistically by the program R-Project, ANOVA and LSD test (Vidican et al., 2013).

RESULTS AND DISCUSSIONS

From the indices presented in Table 1, the total number of germs (TNG), fat, protein, lactose and pH content were considered to be relevant for this study relative to the temperature.
For processing the data presented in Table 1, each sample received a codeand the following notations were made: D1 for milk collected from first automatic milk dispenser machine (ex D1-08 - sample 8), D2 for milk collected from second automatic milk dispenser machine (ex D2-06 - sample 6). Similarly, the sample codes for the dispensers 3 and 4 were noted D3 and D4.

Table 1. Raw milk from milk dispenser's indices analysed

Identification sample	Day	Temp [^{0}C]	Fat (g/100g)	Protein (g/100g)	Lactose (g/100g)	pH	TNG/ml x1000
D1 - 01	9-Oct-17	7	3.76	3.38	4.7	6.58	715
D2 - 01	9-Oct-17	7	3.9	3.42	4.6	6.49	140
D3 - 01	9-Oct-17	7	3.35	3.36	4.66	6.42	215
D4 - 01	9-Oct-17	7	3.8	3.44	4.53	6.44	305
D1 - 02	10-Oct-17	8	3.74	3.39	4.75	6.56	716
D2 - 02	10-Oct-17	8	3.85	3.45	4.68	6.48	162
D3 - 02	10-Oct-17	8	3.35	3.36	4.66	6.42	198
D4 - 02	10-Oct-17	8	3.7	3.44	4.53	6.41	220
D1 - 03	11-Oct-17	12	3.74	3.46	4.78	6.56	620
D2 - 03	11-Oct-17	12	3.84	3.38	4.67	6.48	144
D3 - 03	11-Oct-17	12	3.33	3.36	4.76	6.42	240
D4 - 03	11-Oct-17	12	3.7	3.43	4.61	6.41	325
D1 - 04	12-Oct-17	16	3.92	3.31	4.77	6.66	516
D2 -04	12-Oct-17	16	3.98	3.45	4.76	6.68	230
D3 - 04	12-Oct-17	16	3.35	3.36	4.72	6.47	314
D4 - 04	12-Oct-17	16	3.91	3.39	4.69	6.56	420
D1 - 05	13-Oct-17	14	3.72	3.36	4.79	6.62	680
D2 - 05	13-Oct-17	14	3.58	3.49	4.57	6.48	169
D3 -05	13-Oct-17	14	3.42	3.37	4.63	6.51	226
D4 - 05	13-Oct-17	14	3.76	3.44	4.71	6.47	287
D1 - 06	16-Oct-17	18	3.72	3.32	4.72	6.51	560
D2 - 06	16-Oct-17	18	4.04	3.48	4.68	6.56	206
D3 - 06	16-Oct-17	18	3.51	3.33	4.73	6.62	280
D4 - 06	16-Oct-17	18	3.78	3.42	4.75	6.54	240
D1 - 07	17-Oct-17	19	3.34	3.39	4.77	6.56	420
D2 - 07	17-Oct-17	19	3.78	3.53	4.73	6.48	176
D3 - 07	17-Oct-17	19	3.46	3.44	4.57	6.55	209
D4 - 07	17-Oct-17	19	3.82	3.53	4.79	6.63	206
D1 - 08	18-Oct-17	21	3.72	3.36	4.68	6.58	380
D2 - 08	18-Oct-17	21	3.92	3.45	4.75	6.61	216
D3 - 08	18-Oct-17	21	3.52	3.41	4.64	6.64	270
D4 - 08	18-Oct-17	21	3.83	3.39	4.79	6.49	320
D1 - 09	19-Oct-17	18	4.02	3.32	4.77	6.57	446
D2 - 09	19-Oct-17	18	3.76	3.48	4.72	6.55	199
D3 - 09	19-Oct-17	18	3.5	3.44	4.68	6.49	189
D4 - 09	19-Oct-17	18	3.67	3.39	4.73	6.62	214
D1 - 10	20-Oct-17	17	3.88	3.34	4.67	6.63	360
D2 - 10	20-Oct-17	17	3.88	3.41	4.57	6.68	210
D3 - 10	20-Oct-17	17	3.47	3.42	4.63	6.59	180
D4 - 10	20-Oct-17	17	3.78	3.38	4.66	6.56	215

Analysing the obtained charts, the following are observed:

- The variation of protein must be between the permissible values of 3.0 ... 3.5 %; the results of the four dispensers in the study highlights the fact that all samples were within the normal range which shows that feed rations were carefully monitored and that there was concern for improving milk obtained (Figure 1);
- The fat variation must be between the permissible values of 3.5 ... 4.5%. Lower values than the admissible limit of 3.5% (3.35) of fat content were recorded only at dispenser D3 (Figure 2). Samples with fat percentages below this limit were either in the case of sampling techniques errors or by incorrect methods of determination; in the case of the other 3 dispensers (D1, D2 , D4), their values show an average of 3.8% and the maximum values exceed by 3.9% which denotes a low fat content but in the admissible limit values;
- The lactose variation must be between the permissible values of 4 ... 4.5 %.The diagram analysis in figure 3 indicates that the maximum value is exceeded by up to 6% by dispenserD1 and by 5.5% by dispenser D 4. These variations may occur due to the structure of the food ration, which shows concentrations different carbohydrates;

- The total number of germs (TNG)must be less than 200,000/ml. Variation indicates (Figure 4) in the case of the first dispenser D1average values to 2.75, which indicate a major problem of hygiene conditions regarding milking and / or contamination of milk. In the case of the fourth dispenser D4, average values reach 1.375 which puts the same problem as with the D1 dispenser; values closer to the upper limit are for the D3 dispenser of 1,075;

- In the case of the four metered dispensers, the only one whose average values fall within the permissible range is the D2 metering device, which indicates that the hygienic conditions of the milks are complied with and that the raw milk is delivered to the uncontaminated collection points.

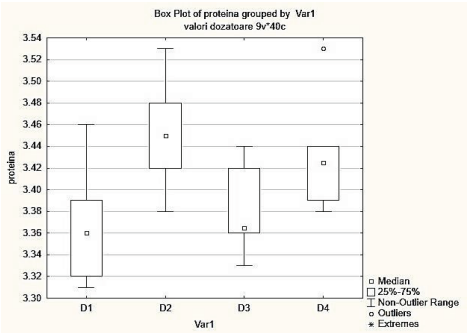

Figure 1. Protein variation

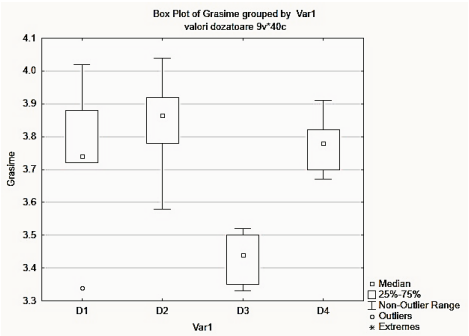

Figure 2. Fat variation

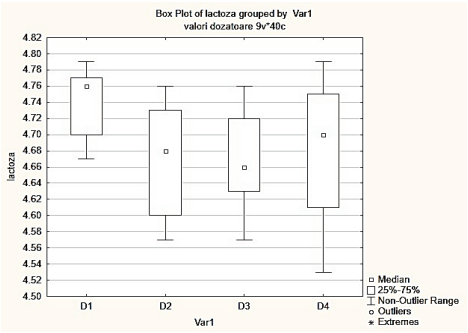

Figure 3. Lactose variation

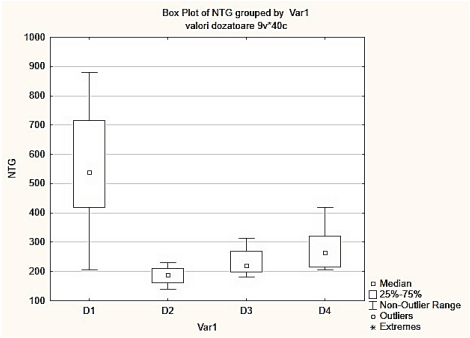

Figure 4. Total numbers of germs (TNG) variation

CONCLUSIONS

Total number of germs (TNG) is dependent on many factorsand in the case of analysed samplescan draw a conclusion on how they performed milking hygiene and animal health which have a direct impact on milk quality.

Analysing the total number of germs (TNG) in the period 9-20 October 2017 it is noted that in case of samples collected from first automatic milk dispenser D1 they exceed until 2.75 times the maximum admissible values. In case of D4 dispenser, the maximum admissible value is exceeded by 1,375 times and for D3 the excess is reduced by 1,075 times.

The samples collected from second automatic milk dispenser D2 are up to the maximum allowable values which show thatmilking hygiene and animal healthare at the European standards required.

Consumption of milk from this dispenser can be done without endangering the health.

The fat content analysis shows that only the dispenser D3 has a fat content lower than the minimum admissible value and the other three dispensers are closer to the minimum value but are within the range provided by the standards.

For protein, the normal protein content should be between 3-3.5%.Protein varies according to many factors including diet, season, stabbing, grazing, lactation cycle.Analyses of the four dispensers show that the values analysed are in the parameters required by the standards, indicates that animals are included in a selection and improvement program.

The values obtained for lactose are higher than 4.5%, which is the upper limitthese variations canit is due to the composition of the food ration, which has different carbohydrate concentrations

Following the analysis of the 40 samples the results obtained from the four dispensers studied, milk can be consumed sure from dispenser D2 and also with certain restrictions from the D3 dispenser.

Number of people who consume raw milk Is growing because of its taste, beneficial effects for human health, non-destructive valuable nutrients by thermal treatments (Oliver et al., 2009).

It is not recommended to consume milk from the D1 and D4 dispenser.

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STUDY ON THE ASSESSMENT OF SOME BEVERAGES AND FOODS BY THE iTQi AT WORLD LEVEL

Cecilia POP, Gabriela FRUNZĂ

University of Agricultural Sciences and Veterinary Medicine Iasi, Romania

Corresponding author email: frunza.gabriela27@gmail.com

Abstract

The paper aims carrying out an analysis of the results of the evaluation of beverages and food products by the International Institute for Taste and Quality (iTQi) worldwide. Similar to the `Michelin Red Guide` authority, iTQi does not organize a competition, but rewards each product for its psychosensory characteristics. Methodological, were analyzed, hierarchized and synthesized information collected from the iTQi database. The data processed in the study covers the period 2015-2017 and refers to the distribution of the distinctions obtained by the evaluated products, by category of prizes (the Diamond Taste Award - DTA, the Crystal Taste Award - CTA and the Superior Taste Award - STA) and geographical areas. The conclusions of the study reveals the increasing trend in the number of distinctions granted by iTQi, from 1243 in 2015 (1194 STA, 41 CTA and 8 DTA) to 1553 in 2017 (1513 STA, 30 CTA and 11 DTA). Romania occupies 14th place in Europe with 36 STA obtained during the analyzed period, a situation that should be improved through measures of stimulation of a more active participation and targeting the higher categories of distinctions, DTA and CTA.

Key words: *quality, taste, awards, food products.*

INTRODUCTION

Food products are characterized through an ensemble of specific and variable psycho-sensorial properties as number and intensity. These properties are very important to consumers, being one of the important criteria for accepting or rejecting a product and implicitly for adopting the purchasing decision.

In the literature it is appreciated that these properties participate with a weight of about 60% in the whole characteristics who compete to achieving the overall quality of a food product (Necula V., 2010).

The assessment of these properties is accomplished through sensory organs, which transmit central nervous system information; the latter compares those with the information already stored in its database, which implies a high degree of subjectivity.

For this reason, research done in the last decades have targeted the elaboration of some methods of sensory analysis with a very high degree of objectivity of the results.

With the improvement of sensory analysis methods and specialized institutions for the evaluation of beverages and food products have appeared, one of these being the International Taste and Quality Institute (iTQi).

Headquartered in Brussels, Belgium, iTQi has been active since 2005 and is evaluating any beverage or food product available in retail (Rodrigues Do Carmo 2013, Zanca, 2016). Like the "Michelin Red Guide" authority, iTQi does not organize a competition but rewards each product for its psycho-sensory characteristics after a "blind" assessment. These awards have been awarded since 2005 and represent a special international recognition (Lee et all 2015, Montse Vázquez, et all 2013, Rodrigues Do Carmo 2013, Zanca, 2016) centered on the unbiased judgment of 140 sommeliers and Chefs (specialists of different nationalities) trained in the application of the sensory analysis methodology of food. They have practiced their profession in Europe, but also in North American, Central and South American countries as well as in Japan, China, Thailand, Australia.

The iTQi jury is unique in the world because it is selected through its exclusive partnership with 15 prestigious associations of professional sommelier and chefs from Europe.

Members of the jury are recognized by authorities such as Michelin Red Guide, Gault Millau Guide being selected for exceptional expertise in objective appreciation of the psychosensory properties of beverages and

food products as well as for the ability to suggest potential improvements as a result of the sensory analysis performed.
The iTQi is an independent organization dedicated to certifying the taste of food and beverages around the world; the validity of the distinctions granted is authorized for a period of three years.
To ensure that the products tested have the same quality as well as those that reach consumers, iTQi has the right to evaluate in the accredited laboratories the physico-chemical characteristics of the samples received. The products are presented to the jury prepared, heated or cooked in conformity with the instructions of producer.
Based on the sensory analysis methodology and depending on the intensity of satisfaction felt at tasting, the jury gives each product a maximum of 100 points, targeting the following criteria: first impression, appearance, smell, texture, taste and aftertaste (retro-olfaction).
DTA is the highest distinction granted by iTQi (the product analyzed obtaining over 90 points, seven times in the last 10 years), followed by CTA (product achieving over 90 points, three years in a row). All products with a minimum score of 70 points are valued as STA and awarded as follows: a gold star for products with "Notable taste" (70-79 points), two gold stars for "Remarkable" products (80-90 points) and three gold stars for "Exceptional" products (over 90 points) [7, 8, 9, 10].

MATERIALS AND METHODS

There have been processed, analyzed and synthesized the information obtained after studying the database managed by iTQi. The iTQi database includes drinks and food products proposed for evaluation by their manufacturers. The data introduced and processed in the study targeted the entire period for which there are records (2015-2017).
The hierarchy, processing and synthesizing data has been achieved according to geographical regions of which the products originate (Asia, North America, Central America and South America, Africa, Oceania and Europe) and according to the distinctions obtained by beverages and food products assessed by the iTQi jury: DTA (over 90 points, seven times in the last 10 years), CTA (over 90 points, three years in a row) and STA ("Notable taste", 70-79 points, "Remarkable" products, 80-90 points, and "Exceptional" products, over 90 points).
The expression of results was made in absolute and relative values to be able to be compared, analyzed and interpreted more easily. The interpretation of data has been completed with an analysis of the results of iTQi's evaluation of beverages and food products worldwide. This analysis led to formulation some conclusions and recommendations to improve the level of participation of countries to these types of assessments, accessing to a larger extent and the higher categories of distinctions, PDG and PCG.

RESULTS AND DISCUSSIONS

Sensory analysis applied correctly and scientifically is part of the assembly of modern analytical methods which allows the realization of a real and complete assessment of food products quality, which could not be achieved using only physicochemical and microbiological methods. Through its work, iTQi highlights the sensory value of the analyzed products, decisive value in the purchasing behaviour of consumers. Thereby, during the study period, iTQi has granted worldwide **4174** distinctions for beverages and food products evaluated: 1242 (29.8% of the total period) in 2015, 1383 (33.1%) in 2016 and 1549 (37.1%) in 2017. Of these, only 0.7% (28) were DTA and 2.9% (119) had CTA; the majority share was represented by STA, respectively 86.4% (4027).
Depending on the geographic regions of the world, Europe holds 58.03% from awards (with 36 participating countries), followed by Asia, 30.47% (with 25 participating countries), by North, Central and South America, 10.21% of awards (with 19 participating countries), by Africa, 0.67% (with nine participating countries) and Oceania, 0.60% (with three participating countries). In European countries, iTQi has granted 2429 awards (58.03% of total), respectively 797 awards in 2015, 799 awards in 2016 and 833 in 2017 (Table 1 and table 2). First place, of 91 participating countries, there was **Japan**, she amassing 778 awards (214 in 2015, 257 in 2016 and 307 in

2017), holding a share of 18.60% of the total. From these 778 products awarded, 50.77% obtained between 80 and 90 points, 29.82% obtained over 90 points and 13.75% obtained between 70 and 80 points. Only 4.11% obtained CTA and 1.54% obtained DTA.

Table 1. Category of prizes awarded by iTQi over the period 2015-2017 worldwide

Regions	COUNTRY	DTA				CTA				STA				TOTAL AWARDS			
	Year	2015	2016	2017	Total	2015	2106	2017	Total	2015	2016	2017	Total	No.	%DTA	%CTA	%STA
Asia	Japan	2	5	5	12	7	14	11	32	205	238	291	734	778	1.5	4.1	94.4
	China	-	-	-	-	1	-	2	3	29	58	104	191	194	-	1.5	98.5
	Thailand	-	-	-	-	-	-	-	-	10	31	44	85	85	-	-	100
	Korea	-	-	-	-	-	-	-	-	5	24	34	63	63	-	-	100
	India	-	-	-	-	-	-	-	-	13	4	9	26	26	-	-	100
	Myanmar	-	-	-	-	-	-	-	-	2	6	13	21	21	-	-	100
	Singapore	-	-	-	-	-	-	-	-	4	9	3	16	16	-	-	100
	Malaysia	-	-	-	-	-	-	-	-	1	5	5	11	11	-	-	100
	Vietnam	-	-	-	-	-	-	-	-	2	5	3	10	10	-	-	100
	Other [1]	-	-	-	-	1	1	-	2	16	31	12	59	61	-	3.2	96.8
Total region		**2**	**5**	**5**	**12**	**9**	**15**	**13**	**37**	**287**	**411**	**518**	**1216**	**1265**	**-**	**-**	**-**
America	Canada	-	-	-	-	-	-	-	-	16	31	8	55	55	-	-	100
	U.S.A.	-	-		-	1	-	-	1	15	10	13	38	39	-	2.6	97.4
	Mexico	-	-	-	-	-	-	1	1	8	4	9	21	22	-	4.6	95.4
	Colombia	-	-	1	1	2	-	-	**2**	28	27	46	101	104	0.97	1.9	97.1
	Ecuador	-	-	-	-	-	1	-	1	16	14	24	54	55	-	1.8	98.2
	Peru	-	-	-	-	1	-	-	1	11	18	20	49	50	-	2	98.0
	Brazil	-	-	-	-	-	-	-	-	11	14	16	41	41	-	-	100
	Panama	-	-	-	-	-	-	-	-	5	6	4	15	15	-	-	100
	Other [2]	-	-	-	-	-	1	1	2	16	10	17	43	45	-	4.5	95.5
Total region		**-**	**-**	1	**1**	4	**2**	**2**	**8**	**126**	**134**	**157**	**417**	**427**	**-**	**-**	**-**
Africa[3]		**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**7**	**10**	**11**	**28**	**28**	-	-	100
Europa	Germany	2	1	2	5	10	10	1	**21**	105	120	111	336	362	1.39	5.81	92.8
	Greece	-	-	-	-	1	3	2	**6**	118	106	119	343	349	-	1.7	98.3
	Spain	1	3	2	6	1	4	3	**8**	72	91	121	284	298	2.0	2.7	95.3
	Belgium	-	-	-	-	2	3	2	**7**	100	82	100	282	289	-	2.4	96.6
	Turkey	-	-	-	-	1	1	-	**2**	42	71	93	206	208	-	0.97	99.0
	Italy	2	-	-	2	4	1	4	**9**	53	50	68	171	182	1.1	5	93.9
	Netherlands	1	-	-	1	2	-	-	**2**	39	34	43	116	119	0.8	1.7	97.5
	Croatia	-	-	-	-	-	2	-	**2**	27	34	23	84	86	-	2.3	97.7
	Un. Kingdom	-	-	-	-	-	-	-	**-**	26	22	8	56	56	-	-	100
	Portugal	-	-	1	1	-	1	-	**1**	20	16	16	52	54	1.9	1.9	96.2
	Lithuania	-	-	-	-	1	3	-	**4**	25	14	6	45	49	-	8.1	91.8
	Poland	-	-	-	-	1	-	1	**2**	17	19	9	45	47	-	4.3	95.7
	Belarus	-	-	-	-	1	1	-	**2**	15	15	12	42	44	-	4.6	95.4
	Romania	**-**	**-**	**-**	**-**	-	-	-	**-**	7	12	17	36	36	-	-	100
	Russia	-	-	-	-	-	-	-	**-**	18	0	5	23	23	-	-	100
	Austria	-	-	-	-	1	-	-	**1**	4	10	8	22	23	-	4.4	95.6
	France	-	-	-	-	-	-	1	**1**	10	8	2	20	21	-	4.8	95.2
	Norway	-	-	-	-	-	-	-	**-**	9	3	9	21	21	-	-	100
	Other [4]	-	-	-	-	2	1	1	4	57	58	43	158	162	1.2	2.5	96.3
Total region		**6**	**4**	**5**	**15**	27	**30**	**15**	**72**	**764**	**765**	**813**	**2342**	**2429**	-	-	-
Oceania	Australia	-	-	-	**-**	**-**	**-**	**-**	-	5	2	4	11	11	-	-	100
	Fr. Polynesia	-	-	-	**-**	1	-	**-**	1	-	1	-	1	2	-	50	50
	Fiji	-	-	-	**-**	**-**	**-**	**-**	-	3	4	5	12	12	-	-	100
Total region		-	-	-	**-**	**1**	**-**	**-**	**1**	**8**	**7**	**9**	**24**	**25**	-	-	-
WORLD TOTAL		**8**	**9**	**11**	**28**	**42**	**47**	**30**	**119**	**1192**	**1327**	**1508**	**4027**	**4174**	**0.7**	**2. 9**	**96.4**

[1] Countries with less than 10 products awarded during the analyzed period (Sri Lanka, Philippines, Pakistan, United Arab. Emirates, Iran, Azerbaijan, Kazakhstan, Afganistan, Saudi Arabia, Oman, Palestina, Kuwait, Hong Kong, Nepal)

[2] Countries with less than 10 products awarded during the analyzed period (Bolivia, Uruguay, Argentina, Chile, Guatemala, Venezuela, Paraguay, Suriname, Cuba, Saint Lucia, Costa Rica)

[3] Countries with less than 10 products awarded during the analyzed period (S. Africa, Cape Verde, Tunisia , Algeria, Togo, Congo, Morocco, Namibia, Reunion)

[4] Countries with less than 10 products awarded during the analyzed period (Slovenia, Luxembourg, Hungary, Sweden, Switzerland,Finland, Latvia, Serbia,Ukraine, Cyprus, Bosnia Herz., Ireland, Czech , Bulgaria , Liechtenstein, Iceland, Slovakia)

Second place in the world (first place at European level) is occupied by **Germany**, with a share of 8.63%.

Table 2. The total number and % total of prizes awarded by iTQi over the period 2015-2017 worldwide

Regions	COUNTRY	2015		2016		2017		Total 2015-2017		
		No.	% region	No	% region	No.	% region	No.	% total region	%total worldwide
Asia	Japan	214	71,8	257	59.8	307	57.2	778	61.5	18.6
	China	30	10.1	58	13.5	106	19.7	194	15.3	4.6
	Thailand	10	3.3	31	7.2	44	8.2	85	6.7	2.0
	Korea	5	1.7	24	5.6	34	6.3	63	5.0	1.5
	India	13	4.4	4	0.9	9	1.7	26	2.1	0.6
	Myanmar	2	0.7	6	1.4	13	2.4	21	1.7	0.5
	Singapore	4	1.3	9	2.1	3	0.6	16	1.3	0.4
	Malaysia	1	0.3	5	1.1	5	0.9	11	0.8	0.3
	Vietnam	2	0.7	5	1.2	3	0.6	10	0.8	0.3
	Other [1)]	17	5.7	31	7.2	13	2.4	61	4.8	1.5
Total region		**298**	**100**	**430**	**100**	**537**	**100**	**1265**	**100**	**30.3**
America	Canada	16	12.2	31	22.6	8	5.0	55	12.9	1.3
	U.S.A.	16	12.1	10	7.3	13	8.2	39	9.1	0.9
	Mexico	8	6.1	4	2.9	10	6.3	22	5.1	0.5
	Colombia	30	22.9	27	19.7	47	29.6	104	24.4	2.5
	Ecuador	16	12.3	15	10.9	24	15.1	55	12.9	1.3
	Peru	12	9.2	18	13.2	20	12.6	50	11.7	1.2
	Brazil	11	8.4	14	10.2	16	10.0	41	9.6	1.0
	Panama	5	3.8	6	4.4	4	2.5	15	3.5	0.4
	Other [2)]	16	13.0	11	8.8	18	10.7	46	10.8	1.1
Total region		**131**	**100**	**137**	**100**	**159**	**100**	**427**	**100**	**10.2**
[3)]**Africa**		**7**	**100**	**10**	**100**	**11**	**100**	**28**	**0.67**	**0.67**
Europa	Germany	117	14.7	131	16.4	114	13.7	362	14.9	8.7
	Greece	119	14.9	109	13.6	121	14.5	349	14.4	8.4
	Spain	74	9.3	98	12.3	126	15.2	298	12.3	7.1
	Belgium	102	12.8	85	10.6	102	12.3	289	11.9	6.9
	Turkey	43	5.3	72	9.0	93	11.2	208	8.6	5.0
	Italy	59	7.4	51	6.4	72	8.7	182	7.5	4.4
	Netherlands	42	5.3	34	4.3	43	5.3	119	4.9	2.9
	Croatia	27	3.4	36	4.5	23	2.3	86	3.5	2.1
	Un. Kingdom	26	3.3	22	2.8	8	1.0	56	2.3	1.3
	Portugal	20	2.5	17	2.1	17	2.0	54	2.2	1.3
	Lithuania	26	3.3	17	2.1	6	0.7	49	2.0	1.2
	Poland	18	2.3	19	2.4	10	1.2	47	1.9	1.1
	Belarus	16	2.0	16	2.0	12	1.5	44	1.8	1.0
	Romania	7	0.9	12	1.5	17	2.0	36	1.5	0.9
	Russia	18	2.3	0	0.0	5	0.6	23	0.9	0.6
	Austria	5	0.6	10	1.2	8	1.0	23	1.0	0.5
	France	10	1.3	8	1.0	3	0.4	21	0.9	0.5
	Norway	9	1.1	3	0.4	9	1.1	21	0.8	0.5
	Other [4)]	59	7.3	59	7.4	44	5.3	162	6.7	3.9
Total region		**797**	**100**	**799**	**100**	**833**	**100**	**2429**	**100**	**58.3**
Oceania	Australia	5	55.6	2	28.6	4	44.4	11	44	0.2
	Fr. Polynesia	1	11.1	1	14.3	0	0.0	2	8	0.1
	Fiji	3	33.3	4	57.1	5	55.6	12	48	0.3
Total region		**9**	**100**	**7**	**100**	**9**	**100**	**25**	**100**	0.6
Total annual worldwide		**1242**	-	**1383**	-	**1549**	-	-	-	-
Total period worldwide		**4174**								**100**

[1)] Countries with less than 10 products awarded during the analyzed period (Sri Lanka, Philippines, Pakistan, United Arab. Emirates, Iran, Azerbaijan, Kazakhstan, Afganistan, Saudi Arabia, Oman, Palestina, Kuwait, Hong Kong, Nepal)

[2)] Countries with less than 10 products awarded during the analyzed period (Bolivia, Uruguay, Argentina, Chile, Guatemala, Venezuela, Paraguay, Suriname, Cuba, Saint Lucia, Costa Rica)

[3)] Countries with less than 10 products awarded during the analyzed period (S. Africa, Cape Verde, Tunisia , Algeria, Togo, Congo, Morocco, Namibia, Reunion)

[4)] Countries with less than 10 products awarded during the analyzed period (Slovenia, Luxembourg, Hungary, Sweden, Switzerland,Finland, Latvia, Serbia,Ukraine, Cyprus, Bosnia Herz., Ireland, Czech , Bulgaria , Liechtenstein, Iceland, Slovakia)

It had fewer award-winning products (362) compared to Japan, but has a higher share of the taste quality of the evaluated products, 34.53% being considered exceptional, obtaining between 90 and 100 points (about 5% more than Japan). Also, the awards for the last category of prizes (70-80 points) are fewer for Germany (7.18% versus 13.75%). For the DTA and CTA categories, the two countries have accumulated close values 1.38% versus 1.54 and 5.80% versus 4.11%.

On 3rd place in the world it is situated **Greece** with a share of 8.34% of the total, very close to the second place, with 349 products awarded. It is noticed that a share of over 40% of iTQi awards were received by only six European countries: Germany 8.63%, Greece 8.34%, Spain 7.12%, Belgium 6.91%, Turkey 4.97% and Italy 4.35% of the total of 91 participating countries at tasting sessions.

Romania occupies 23rd place in the world (14th place in Europe) receiving a total of 36 awards: seven awards in 2015 (19.4% of the total), 12 awards in 2016 (33.3% of the total) and 17 awards in 2017 (47.3% of the total); most of them were awarded for "remarkable" products (22 products, 61.1%), ten products (27.8%) being appreciated as "exceptional" and four products (11.1%) being considered with "notable taste".

Following the evaluation of the products by the iTQi jury, manufacturers receive:

• a free sensory analysis chart summarizing the opinion of the jury;

• a sensory analysis report that provides information on five main sensory criteria (first impression, vision, smell, texture, taste, aroma) with a total of 12 to 25 sensory subcriteria and includes suggestions for product improvement, if is the case;

• an association report of food with drinks detailing more combination of tastes, customized for tasting products.

The information provided by ITQI helps manufacturers to improve the quality of their products, being used by those involved in the activity of research, development and quality control.

Prizes awarded iTQi stands for promotion tools in marketing campaigns but also for negotiating the sales price of the respective products.

Regional data analysis highlights the first three places: Europe with a share of 58.3% of the total prizes awarded (2429 awards), Asia with 30.3% of the total (1265) and America with 10.2% of the total (427).

This situation reflects the reality of the efforts made by these regions in the direction of quality improvement. "New European Vision for Quality" cultivates the concept of "**MADE in EUROPE**" with the mission to become the modern emblem of the products and services offered by Europeans to all international trade, a philosophy of quality and respect for the consumer (Pop Cecilia et all, 2013).

At the same time, Europe is also recognized for the most demanding food safety legislation in the world. As for Asia, we mention that Japan obtained 61.5% of the total awards to this region and 18.6% of the world total. Moreover, Japan occupies and the world's first place, earning 778 awards followed by Germany with 362 awards; both countries are recognized worldwide for product quality realized.

CONCLUSIONS

The results obtained for the analyzed period (4174 distinctions granted) indicates a worldwide increase of about 25% of the interest shown for participation in such assessments (from 1242 prizes in 2015 to 1549 prizes in 2017).Of these, only 0.7% (28) were DTA and 2.9% (119) CTA; the majority share was represented by STA, respectively 86.4% (4027). These results, reveals in the equal measure, both the rigorousness of the assessment made by the iTQi jury as well as the need to intensify research to improve the sensory quality of beverages and food products.

On first place, of 90 participating countries, was placed Japan, she earning 778 prizes, followed by Germany with 362 awards, Greece with 349 awards, Spain with 298 awards, Belgium with 289 awards and Turkey with 208 awards. China occupies the seventh place worldwide (194 awards), USA 22nd place (39 awards), and Romania ranks 23rd in the world and 14th place in Europe (with 36 STAs in the analyzed period).

Considering the fact that consumer attitude in the buying process is based and the cognitive component (which targets elements such as: informing relating to the characteristics of the product, the extent to which the product it will give him satisfaction, the degree of trust that can be attributed to it) we consider that participation as broad as possible of manufacturers at iTQI ratings should be more supported and encouraged.

This support could be achieved by promoting the benefits of iTQi assessment at the level of Professional Associations of manufacturers and processors. The final results obtained by the iTQi evaluators are confidentially communicated to the participating manufacturers together with an analysis report, comments and additional suggestions about their products, which can help them greatly in the process of improving the sensory quality of foods. At the same time, the iTQi emblem, which can be applied on the packaging/label, constitutes a "trust bulletin" of the product for consumers, which helps to increase the sales and at the same time the visibility of the products on the national and international market.

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COMPARASION OF CARCASS YIELD AND WHOLESALE MEAT CHARACTERISTICS OF HOLSTEIN AND BROWN SWISS CATTLE

Yalçın BOZKURT*, Cihan DOGAN

Suleyman Demirel University, Faculty of Agriculture, Department of Animal Science, Isparta, Turkey

*Corresponding author e-mail: yalcinbozkurt@sdu.edu.tr

Abstract

This study aimed to compare carcass and wholesale beef cut yields of Holstein and Brown Swiss male cattle. The data from Holstein (17) and Brown Swiss (17) cold carcasses were obtained from 34 animals in total slaughtered in a commercial slaughterhouse in Isparta province located in the west Mediterranean region of Turkey. For this purpose, carcasses were fabricated into four primal wholesale carcass bone-in cuts as combined with sub-primal retail cuts. Therefore, cold carcass weights (CCW), killing-out percentages (KO%), forequarter, hindquarter, chuck (combined with brisket), rib (combined with plate), loin (combined with sirloin and flank) and round were recorded in kilograms and as percentages of carcass weight (CW%). There were no significant differences (P>0.05) in CCWs and KO% between breeds. Average CCWs and KO% of each breed were 242.1 and 250 kg and 51.02% and 50.4% for Brown Swiss and Holstein, respectively. There were also no significant differences (P>0.05) in forequarter and hindquarter of breeds. Average weights and CW% of forequarters were 128.4 and 131.9 kg and (53.1% and 52.8%) for Brown Swiss and Holstein, respectively. Similarly hindquarters were 113.7 and 118.1 kg and (46.9% and 47.2%) for Brown Swiss and Holstein, respectively. While there were no significant differences (P>0.05) in other cuts only rib values obtained for Holstein cattle were significantly greater (P<0.05) than those of Brown Swiss cattle. It was observed that there was a tendency for all carcass characteristics of Holstein cattle to be higher than Brown Swiss cattle. However, both cattle could be recommended for beef producers in the region.

Key words: *Holstein, Brown Swiss, Carcass Cuts, Wholesale, Meat Traits.*

INTRODUCTION

Marketable different beef products has become a priority for beef producers in the last decade. Factors affecting ruminant carcass and meat quality are directly related to the animal and its environment (Dannenberger et al., 2006), and probably the most important are breeds and feeding strategies.

Differences in retail market value are available in different parts of the carcass (Morris et al., 1999). Farmers must be rewarded for the production of a larger amount of these high market value beef cuts.

The value of carcass cuts should recognise both the demand and reliability of the consumer as well as the marketing standards that emphasize meat quality, uniformity and consistency. For this reason, an assessment procedure to estimate the weight and yield of carcasses and beef retail cuts is of great importance to the beef industry (Cross and Belk, 1994).

Many published reports on carcass performance comparisons of different breeds have been well-documented and compared carcass characteristics of different breeds slaughtered at the same slaughter weight and fed under different feeding conditions prior to slaughter. It appears that the results of such comparisons including different breeds grown in different climate regions and environmental and marketable conditions are, however, limited in the literature. For the meat production purpose, production potentials of Brown Swiss and Holstein male cattle are usually measured in terms of growth performance, carcass yield and meat quality since the males of both breeds are kept for beef production purposes. Very limited studies are available to recommend and comprehensively compare the carcass and meat qualities of the breeds mentioned in this study.

Therefore, it was aimed to compare carcass and wholesale beef cut yields of Holstein and Brown Swiss cattle in this study.

MATERIALS AND METHODS

Animal

The animals used in this study were comprised of Brown Swiss and Holstein male cattle previously grown in a 12-month feedlot beef system.

Dried alfalfa and hay roughages, as well as ground barley and cattle fattening dietary coarse feed as a concentrate, were provided to achieve 1 kg daily live weight gain and rations were re-adjusted to live weight changes of animals. The study began in December 2011 and was experimented on animals in the university research farm and lasted for 12 months.

The data from Holstein (17) and Brown Swiss (17) cold carcasses were obtained from 34 animals in total slaughtered in a commercial slaughterhouse in Isparta province located in the west Mediterranean region of Turkey. Slaughter data collection and slaughtering process continued from December 2012 to March 2013 for 3 months.

Slaughtering procedure

After slaughter hot carcasses the animals were weighed and then chilled at a temperature of 4°C for 24 h and the cold carcass weights were recorded and used to calculate the killing-out percentage.

Each cold carcass was split into left and right sides by longitudinal sawing along the middle of the vertebral column and then the left side was divided between 12^{th} and 13^{th} thoracic vertebra into forequarter and hindquarter and weighed. Then killing-out percentage was calculated.

Carcass fabrication

Carcasses were divided into major cut groups based on their quality and were fabricated into four primal wholesale carcass bone-in cuts as combined with sub-primal retail cuts (Weniger et al., 1963). Therefore, CCW, KO%, forequarter, hindquarter, chuck (combined with brisket), rib (combined with plate), loin (combined with sirloin and flank) and round were recorded in kilograms and as percentages of carcass weight (CW%).

The retail cuts were weighed using a mobile weighing scale. All weights were taken by the same individuals throughout the experimental period.

Statistical Analysis

Student's *t* test was used to analyse the data and the differences in retail cuts in carcasses of each breed were determined by using statistical software program. (Minitab v.16)

RESULTS AND DISCUSSIONS

Carcass (bone in weight) performance means of breeds are shown in Table 1. Wholesale (bone in weight) beef cuts means of breeds are shown in Table 2.

There were no significant differences (P>0.05) between in all parameters studied.

Table 1. Carcass (bone in weight) performance means

N (34)	BS (N:17)	BS (s.e.)	H N:17)	H (s.e.)	Coefficient of variation	
					BS	H
HCW(fat)	254.4	6.27	261.6	2.69	10.16	4.24
HCW(trimmed)	245.6	6.15	252.8	2.52	10.32	4.11
Chilling loss(kg)	12.25	0.60	11.62	0.55	20.22	19.58
Chilling loss(%)	4.82	0.21	4.43	0.19	17.52	17.77
CCW(kg)	242.1	6.02	250	2.48	10.26	4.08
KO(%)	51.1	0.27	50.4	0.35	2.31	2.82

HCW: Hot carcass weight, CCW: Cold carcass weight, KO: Killing-out percentages, BS: Brown Swiss, H: Holstein

The average HCW (fat) and HCW (trimmed) was 254.4, 261.6 and 245.6, 252.8 kg for Brown Swiss and Holstein groups respectively. There were no significant differences (P >0.05) in HCW (fat) and HCW (trimmed) between breeds.

The average chilling loss as kg and chilling loss as % were 12.25, 11.62 kg and 4.82%, 4.43% for Brown Swiss and Holstein, respectively. There were no significant differences (P>0.05) in chilling loss kg and chilling loss% between breeds.

The average CCWs and KO% of each breed were 242.1, 250 kg and 51.1%, 50.4% for Brown Swiss and Holstein, respectively. There were no significant differences (P>0.05) in CCWs and KO% between breeds (Table 1).

The killing-out percentages found in this study for Holstein (50.4%) groups and for Brown Swiss (51.1%) were higher than the results of reports (Alpan, 1972; Tüzemen et al., 1990; Çatıkkaş and Koç, 2017), but lower than the results of some previous reports (Akbulut and Tüzemen, 1994; Koç and Akman, 2003; Önenç, 2003; Sağöz et al., 2005; Aslan and Zülkadir, 2009; Garip et al., 2010).

The presented values in Table 1 for carcass performances of breeds in this study were found to be higher than the reported carcass yields for Brown Swiss and local breeds and Holstein breed crosses (Akcan and Alpan, 1984; Tüzemen et al., 1990; Karakaş, 2002; Özdoğan, 2007).

Table 2. Means of wholesale beef cuts (Bone in weight)

N (34)	BS (N:17)	BS (s.e.)	H (N:17)	H (s.e.)	Coefficient of variation	
					BS	H
Forequarter (kg)	128.4	2.97	131.9	1.44	9.55	4.51
Forequarter (%)	53.1	0.39	52.8	0.19	3.02	1.51
Hindquarter (kg)	113.7	3.28	118.1	1.21	11.89	4.22
Hindquarter (%)	46.9	0.39	47.2	0.19	3.41	1.69
Chuck (kg)	79.5	2.04	80.1	0.96	10.61	4.95
Chuck (%)	32.84	0.32	32.06	0.35	4.01	4.46
Rib (kg)	48.9	1.06	51.8	1.07	8.91	8.50
Rib (%)	20.25	0.23	20.69	0.28	4.60	5.66
Loin (kg)	32.2	1.10	33.3	0.44	14.15	5.46
Loin (%)	13.26	0.23	13.31	0.17	6.99	4.82
Round (kg)	81.6	2.28	84.8	1.08	11.51	5.23
Round (%)	33.65	0.28	33.94	0.25	3.46	3.03

BS: Brown Swiss, H: Holstein

Average weights and CW% of forequarters were 128.4 and 131.9 kg and (53.1% and 52.8%) for Brown Swiss and Holstein, respectively. There were also no significant differences (P>0.05) in forequarter and hindquarter of breeds.

Similarly hindquarters were 113.7 and 118.1 kg and (46.9% and 47.2%) for Brown Swiss and Holstein, respectively. While there were no significant differences (P>0.05) in other cuts only rib values obtained for Holstein cattle were significantly greater (P<0.05) than those of Brown Swiss cattle. These results found for forequarter and hindquarter in this study were in line with the findings of the studies conducted by Dikeman et al., (1977) and Pabiou et al., (2014).

The average weight chuck, rib, loin and round were 79.5, 48.9, 32.2, and 81.6 kg for Brown Swiss and 80.1, 51.8, 33.3 and 84.8 kg Holstein groups respectively.

There were no significant differences (P>0.05) in chuck, rib, loin and round between breeds. The average percentage of retail cuts chuck, rib, loin and round were 32.84%, 20.25%, 13.26%, and 33.65% for Brown Swiss and 32.06%, 20.69%, 13.31% and 33.94% Holstein groups respectively.

There were also no significant differences (P >0.05) in chuck, rib, loin and round between breeds (Table 2).

Plascencia et al. (1999) reported wholesale cuts yield some groups of feedlot steers, although, the breeds used were unknown, as a percentage were 32.81%, 8.8%, 5.85% and 17.98% for round, sirloin, short loin and chuck with bone, 8.75% and 12.11% for flank and brisket with trim, 9.06% and 11.15% for rib and plate, respectively. Some of the beef cuts mentioned in their study are almost similar with the results found in this study.

CONCLUSIONS

It can be concluded that as Purchas et al. (1999) emphasized that the importance of improvements in producing the high value marketable carcasses and carcass cuts. It is

believed that these improvements would allow the incentives and premiums paid to beef producers on carcasses of the best quality to be obtained in a country.

It was observed that there was a tendency for all carcass characteristics of Holstein cattle to be higher than Brown Swiss cattle. However, the results in this study indicated that both cattle could be recommended for beef producers in the region.

However, since there is a meat-based payment system exist in the region and also carcass grade is based on only killing out percentages can have a stronger market signal to the producer rather than recommendation of breeds because the viability of the beef industry in a value-based marketing system depends on the production of high quality, consistent carcasses with good confirmation and carcasses with high killing out percentages.

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CONTENT OF PESTICIDE RESIDUES IN THE FLOWERS OF THE ACACIA AND LINDEN TREES FROM THE MOLDAVIAN CODRI AREA

Valentina CEBOTARI, I. BUZU, Olesea GLIGA, Olga POSTOLACHI, N. GRANCIUC

Institute of Zoology of Academy of Sciences of Moldova (ASM)
1, Academiei Street, MD 2028, Chișinău, Republic of Moldova

Corresponding author email: valentinaceb@yahoo.com

Abstract

The purpose of this research was to assess the conformity of the forest melliferous flora with organic apiculture, regarding concentrations of systemic pesticides, to predict the production of bio apicultural products. Flowers of the forest trees of white acacia (Robinia pseudoacacia) and large-leaf linden (Tilia platyphillos), which are the main sources of nectar and pollen for honey bees, were studied for the presence of 17 more commonly spread pesticides: pyrethroid insecticides (Cypermethrin, Deltamethrin, Pyrethrin, Tau-fluvalinate), neonicotinoid insecticides (Imidacloprid, Clothianidin, Thiamethoxam), organophosphorus insecticides (Fenamifos), triazole fungicides (Difenoconazole, Fenhexamid, Mepanipyrim, Cyprodinil), acaricides (Fipronil) and herbicides (Amidosulfuron, Amitrol, Glyphosate, Sulfosulfurol). Of the 17 investigated pesticides, in the white acacia flowers, 14 pesticide residues were found: Cypermethrin, Deltamethrin, Pyrethrin, Clothianidin, Fenamifos, Difenoconazole, Fenhexamid, Mepanipyrim, Cyprodinil, Fipronil, Amidosulfuron, Amitrol, Glyphosate and Sulfosulfurol. Average concentrations of pesticides residues in acacia flowers ranged from 0.003 mg/kg for Mepanipyrim and Fipronil, to 8.75 mg/kg for Difenoconazole and 0.25 mg/kg to Glyphosate. In the large-leaf linden flowers, out of the 17 investigated pesticides, the residues of 13 pesticides were registered, including 3 pyrethroid insecticides, 1 neonicotinoid insecticide, 1 organophosphorus insecticide, 3 triazole fungicides, 1 acaricide and 4 herbicides. Average concentrations of pesticide residues in the linden flowers ranged from 0.001 mg/kg for Fipronil up to 16.0 mg/kg for Difenoconazole and 1.25 mg/kg for Glyphosate. The obtained data attest that in the flowers of forest trees are detected low concentrations of residues of pyrethroid insecticides (Cypermethrin, Deltamethrin, Pyrethrin) from 0,003 up to 0,100 mg/kg, of neonicotinoid insecticide (Clothianidin) from 0,400 up to 0,425 mg/kg, of organophosphorus insecticide (Fenamifos) in an amount of 0.042 mg/ kg, triazole fungicides (Fenhexamid, Mepanipyrim, Cyprodinil) from 0,017 to 0,092 mg/kg, triazole fungicide Difenoconazole from 8,75 to 16,0 mg/kg, acaricide Fipronil from 0,001 up to 0,003 mg/kg, herbicides (Amidosulfuron, Amitrol, Sulfosulfurol) from 0,005 up to 0,013 mg/kg and herbicide Glyphosate from 0,25 up to 1,25 mg/kg. The detected concentrations of pesticide residues in the tree flowers (white acacia and large-leaf linden) was from 1.3 to 33.3 times lower than the maximum admisibil limits, according to national and EU standards. Therefore, the recorded pesticide residues in the flowers of forestry trees, are not harmful for the health of bees and fauna of studied ecosystem. In conclusion we can say that the flora of studied by us forest sector, are not polluted with pesticides, so organic beekeeping can be practiced here, with the production of organic apiculture products.

Key words*: pesticides, flowers, acacia, linden, Apis mellifera, organic apiculture.*

INTRODUCTION

Forest tree flowers has a particular interest as a source of harvesting for *Apis mellifera* bees. Usually, in apiculture of the Republic of Moldova, the first harvesting occurs in acacia or linden trees. The honey obtained from these harvests has a superior quality. The beekeeping branch has an essential importance for the national economy due to the value and quality of its products, the creation of jobs among vulnerable populations of rural areas, and conservation through pollination of homeostasis and of biodiversity of natural ecosystems. There are about 120 thousand families of bees in the country, from which about 2.0 to 2.2 thousand tons of honey is obtained annually, of which 600 tons are exported to different countries. Other bee products, quite important, such as wax, pollen, propolis, royal jelly, venom, are used in various fields of national economy (food, medicine, pharmaceuticals, cosmetics, plastic art, etc.). Analysis of the situation in recent years demonstrates that traditional technology of breeding and exploitation of bee families does not provide everywhere the production of organic and safety bee products of competitive quality, which could be sold at reasonable prices (Siceanu, 2012). EU certification and control

bodies (EC Regulation 834/2009, EU OJ L241, 2009, EC Regulation 271/2010, EU OJ L84, 2010) often detect residues of polluting substances in apiculture production, exported from the Republic of Moldova, which leads to its embargo and compromising the competitiveness of the branch and the national image internationally (Antonescu et al., 2001; Bogdanov et al., 1999; Buruian, 2011). Both farmers and beekeepers, until now haven't realized, that the uncontrolled using of pesticides for crops treating and of honey bees, has an unbalanced impact on the homeostasis of natural ecosystems, wich will finally diminish the health of bees and human.
In this context, the research of environmental pollutants and the identification of their impact on honey bees and apiculture products, the study of the conformity of organic apiculture in the conditions of different autochthonous anthropogenic ecosystems, the organic nutrition of bees during critical periods of harvest in nature, are current problems, and solving them it would allow the development of measures to adjust conventional apiculture to organic beekeeping, acoording to EU standards. The importance of organic beekeeping at current stage was demonstrated by some authors from EU (Antonescu et al., 2001; Siceanu, 2012). Bee products, as well as agricultural, bio-certified products from officially accredited units are sold on the markets at more advantageous prices. These have a higher demand on the market because its ensures the product's safety, its are of superior quality and have a more distinctive origin. In the EU, organic apiculture is governed by a number of European Commission (EC) regulations, the most important of them are: EC Regulation no. 834/28.06.2007 on organic production and labeling of organic products, EC Regulation no. 834/2009, EC Regulation no. 271/2010 and EC Regulation no. 392/2013, which provides the accreditation and certification by the European international bodies of apiaries and bee products of the conventional category into the bio (organic) category, according to unique criteria and standards.
The adjustment (transformation) of beekeeping of the Republic of Moldova to the organic category, requires a thorough compliance study of its main components such as: bee families, the environment, bee breeding and care infrastructure, methods of feeding and nutrition of bee families during of poor harvest in nature, the logistic of beekeeping collection (tools, equipment, machinery), primary processing and storage of apiculture products, the quality and safety of apiculture products.
The ecological status of melliferous base from ecosystems of productive activity of honey bee is a particular problem. Under the melliferous base are meant melliferous flowers that serve as harvesting source of nectar and pollen.
In this context, the investigation of pollution degree of of melliferous flowers, from the production area of bees, with different pesticide residues and elaboration on this base of proposals for enhancing the ecological and food safety are extremely important and current problems, both on nationally and internationally levels.
The residues, which accumulate in the inflorescence of melliferous plants, soil and water, are, in fact, the main pollutants of the environment where the honeybee carries out their vital activity. Among these are heavy metals, pesticides, antibiotics, radionuclides, nitrites and nitrates.
From the environment, pollutants reach in the bees 'food, having an impact both on bees' health and vitality, and on the quality and safety of bee products, affecting the balance of environmental factors, needed for organic apiculture.
Recently, finds particular concern in Europe and throughout the world the use of systemic pesticides in agriculture for seed treatment and spraying of crops to combat insect pests, fungi and weeds.
According to "Beyond Pesticides" – (formerly National Coalition Against the Misuse of Pesticides, is a nonprofit organization from USA) (http://www.beyondpesticides.org/ programs/bee-protective-pollinators-and-pesticides/ chemicals-implicated, 2016), neonicotinoid pesticides have adverse effects on reproduction, are neurotoxic and mutagenic for insects, birds, fish, freshwater snails, earthworms, dragonflies, mosquitoes and vertebrates, mentioning that "*neonicotinoids could represent the new contemporary ecological disaster, being a threat to nature*".
The World Health Organization (WHO) and the

International Agency for Research on Cancer (IARC) announced in March 2015 that the herbicide Glyphosate is carcinogenic and has negative effects on the endocrine system. Residues of these herbicides can be found in the urine and blood of animals as well as humans (http//www.maslina.slobodnadalmacija.hr/novosti/ID/19528/Glifosat, 2017). Harmful effects of systemic pesticides are confirmed by a large number of researchers (Alaux et al., 2010; http://www.greenpeace.org., 2014; Garry et al., 2016; Grill et al., 2012; Henry et al., 2012; Oliveira et al., 2014; Pettis et al., 2012; Tanner G., 2011; Schneider et al., 2012; Tome et al., 2012; Whitehorn et al., 2012).

In this context, researching pollutants and identifying their impact on honey bee and apiculture products, studying the conformity of organic beekeeping under the conditions of various native anthropogenic ecosystems, are important and current tasks.

The purpose of this research is to assess the conformity of the forest melliferous flora with organic apiculture, regarding concentrations of systemic pesticides, to predict the production of bio apicultural products.

MATERIALS AND METHODS

Residues of 17 more commonly spread systemic pesticides have been investigated, such as: pyrethroid insecticides *(Cypermethrin, Deltamethrin, Pyrethrin, Taufluvalinate),* neonicotinoid insecticides *(Imidacloprid, Clothianidin, Thiamethoxam),* organophosphorus insecticides *(Fenamifos),* triazole fungicides *(Difenoconazole, Fenhexamid, Mepanipyrim, Cyprodinil),* acaricides *(Fipronil)* and herbicides *(Amidosulfuron, Amitrol, Glyphosate, Sulfosulfurol)*. The investigations were carried out on the main component of the bee activity area – flowers of the entomophilous forest trees, which provide the first basic honey harvesting. Pesticide residues were determined in the flowers of white acacia (*Robinia pseudoacacia*) and large-leaf linden (*Tilia platyphillos*) trees. During the blooming period, 4 samples with mass 100-130 g each of flowers with petiole were collected from trees at different distances from beehives. Samples were collected from Ghidighici Forestry, Canton no. 9, Forest Sector no. 21, of S.E. for Silviculture Chişinău. The Ghidighici forestry is a part of Moldavian Codri area.

The sampling of the flowers has been carried out according to the sanitary-veterinary norms regarding methodology of the sampling, processing, packaging and transport of the samples for the laboratory examinations (Normă sanitar-veterinară - Sanitary-veterinary standard, 2010). The samples were packed in plastic bags and transported urgently (1-2 hours) to the laboratory for analysis.

Researches of pesticide residues were performed on the same day in the accredited Laboratory for the determination of pesticide residues of S.E. "Center of Quarantine, Identification, Arbitration on Expertise and Disinfection of Production" by Gas-Liquid Chromatography GC-MS and LC-MS Methods, described in the Collection of Standard Methods (Lazări et al., 2000).

The obtained results were compared with the maximum residue level (MRL) values according to the Sanitary Regulation on the Maximum Allowable Residue Levels of Phytosanitary Products in or on Food or Feed of Plant and Animal Origin for Animals, approved by the Decision of Government of Republic of Moldova no. 1191 of 23.12.2010, adjusted to EU requirements.

The obtained in experience data were statistically processed using computer software "STATISTICA - 6" and evaluated their certainty, according to variation biometric statistics, by methods of Plohinskiy (1989).

RESULTS AND DISCUSSIONS

Our research has shown that pyrethroid, neonicotinoid and organophosphorus insecticides, triazole fungicides, acaricides and herbicides are the most widespread pesticides in the environment (melliferous plants) where honey bees works. Among these groups and classes of pesticides, we have investigated 17 pesticides in the flowers of forest trees and we have recorded different detectable concentrations of 3 pyrethroid insecticides, 1 neonicotinoid insecticide, 1 organophosphorus insecticide, 4 triazole fungicides, 1 acaricide and 4 herbicides. In the white acacia flowers (*Robinia pseudoacacia*), from the 17 investigated pesticides, residues of 14

pesticides were found: *Cypermethrin, Mepanipyrim, Cyprodinil, Fipronil, Deltamethrin, Pyrethrin, Clothianidin, Amidosulfuron, Amitrol, Glyphosate and Fenamifos, Difenoconazole, Fenhexamid, Sulfosulfurol* (Table 1).

Table 1. The content of pesticide residues in acacia flowers from forest, mg/kg

No.	The name, class and group of pesticide	MRL*	Pesticide content (N=4), M ± m	Difference from MRL (3-4)	
				±d	times
1	2	3	4	6	7
	PYRETHROID INSECTICIDES				
1.	Cypermethrin	0.1	0.005±0.003	-0.095	-20.0
2.	Deltamethrin	0.05	0.008±0.005	-0.042	-6.3
3.	Pyrethrin	0.5	0.075±0.047	-0.425	-6.7
4.	Tau-fluvalinate (*L*)	0.01	n.d.	-	-
	NEONICOTINOID INSECTICIDES				
5.	Imidacloprid	0.05	n.d.	-	-
6.	Clothianidin	0.05	0.008±0.005	-0.042	-6.3
7.	Thiamethoxam	0.1	n.d.	-	-
	ORGANOPHOSPHORUS INSECTICIDES				
8.	Fenamifos	0.05	0.008±0.005	-0.042	-6.3
	TRIAZOLE FUNGICIDES				
9.	Difenoconazole	20.0	8.75±1.65	-11.25	-2.3
10.	Fenhexamid	0.1	0.005±0.003	-0.095	-20.0
11.	Mepanipyrim	0.02	0.003±0.002	-0.017	-6.7
12.	Cyprodinil (*L*+*R*)	0.05	0.014±0.004	-0.036	-3.6
	ACARICIDES				
13.	Fipronil	0.005	0.003±0.002	-0.002	-1.7
	HERBICIDES				
14.	Amidosulfuron	0.05	0.005±0.002	-0.045	-10.0
15.	Amitrol	0.02	0.005±0.002	-0.015	-4.0
16.	Glyphosate	2.0	0.250±0.240	-1.75	-8.0
17.	Sulfosulfurol (*SS*)	0.05	0.005±0.004	-0.045	-10.0

Notification: * - MRL (maximum residue level), according to the "Sanitary Regulation on the Maximum Allowable Residue Levels of Phytosanitary Products in or on Food or Feed of Plant and Animal Origin for Animals", approved by the Decision of Government of Republic of Moldova no. 1191 of 23.12.2010, www//lex.justice.md, vizited on 06.12.2017.

From 17 studied pesticides in the acacia flowers, no residues of 3 insecticides (pyrethroid *Tau-fluvalinate* and neonicotinoids *Imidacloprid* and *Thiamethoxam*) were detected.

The lowest concentrations of residues were registrated at acaricide *Fipronil* - on average 0.003±0.002 mg/kg, triazole fungicides *Mepanipyrim* - 0.003±0.002 mg/kg and *Fenhexamid* - 0.005 ± 0.003 mg/kg, as well as the pyrethroid insecticide *Cypermethrin* - 0.005±0,003 mg/kg. The highest residual concentrations of the studied pesticides in acacia flowers were recorded at triazole fungicide *Cyprodinil* - 0.014±0.004 mg/kg, pyrethroid insecticide *Pyrethrin* - 0.075±0.047 mg/kg, herbicide *Glyphosate* - 0.25±0.24 mg/kg and triazole fungicide *Difenoconazole* - 8.75±1.65 mg/kg.

It should be noted that these relatively "high" concentrations of pesticide residues in the acacia flowers from forest, actually, are very low, compared to the maximum admissible limits according to national and EU standards, of 3.6 times for *Cyprodinil*, 6.7 times for *Pyrethrin*, 8.0 times for *Glyphosate* and 2.3 times for *Difenoconazole*.

Thus, very low concentrations of residues of pyrethroid, neonicotinoid and organophosphorus insecticides, as well as triazole fungicides, acaricides and herbicides in acacia flowers from forest, have been detected, values that are well below the maximum admissible level according to national and the EU standards.

In this context, we can conclude that white acacia flowers, from the forest studied by us, are not polluted with pesticides. Therefore, these (flowers) are safety for practicing organic beekeeping and obtaining of bio apiculture products.

Such results were obtained by other authors (Ilavarasan and Vadivelu, 2017). The researchers have shown that *Acacia nilotica* and *Acacia leucophloea* flowers in the Thanjavur region, Tamilnadu, India, do not contain detectable concentrations of organochlorine and organophosphorus pesticide residues, which indicates their absence in the flowers of forest trees.

In the large-leaf linden flowers (*Tilia platyphillos*), out of the 17 investigated pesticides, the residues of 13 pesticides were registered, including 3 pyrethroid insecticides, 1 neonicotinoid in secticide, 1 organophosphorus insecticide, 3 triazole fungicides, 1 acaricide and 4 herbicides. The lowest residue concentration was registered at the acaricide *Fipronil* - 0.001 mg/kg and at the pyrethroid insecticide *Cypermethrin* - 0.003 mg/kg (Table 2).

Table 2. The content of pesticide residues in large-leaf linden flowers from the forest, mg/kg

Nr.	The name, class and group of pesticide	MRL*	Pesticide content, (N=4), M ± m	Difference from MRL (3-4)	
				±d	times
1	2	3	5	6	7
	PYRETHROIDE INSECTICIDES				
1.	Cypermethrin	0.1	0.003±0.002	-0.097	-33.3
2.	Deltametrine	0.05	0.020±0.004	-0.030	-2.5
3.	Pyrethrine	0.5	0.100±0.041	-0.400	-5.0
4.	Tau-fluvalinate (*L*)	0.01	n.d.	-	-
	NEONICOTINOID INSECTICIDES				
5.	Imidacloprid	0.05	n.d.	-	-
6.	Clothianidin	0.05	0.008±0.005	-0.042	-6.2
7.	Thiamethoxam	0.1	n.d.	-	-
	ORGANOPHOSPHORUS INSECTICIDES				
8.	Fenamifos	0.05	0.008±0.005	-0.042	-6.2
	TRIAZOLE FUNGICIDES				
9.	Difenoconazole	20.0	16.00±1.22	-4.00	-1.3 ori
10.	Fenhexamid	0.1	0.008±0.005	-0.092	-12.5 ori
11.	Mepanipyrim	0.02	n.d.	-	-
12.	Cyprodinil (*L+R*)	0.05	0.019±0.006	-0.031	-2.6
	ACARICIDES				
13.	Fipronil	0.005	0.001±0.0007	-0.004	-5.0
	HERBICIDES				
14.	Amidosulfuron	0.05	0.012±0.005	-0.038	-4.2
15.	Amitrol	0.02	0.009±0.003	-0.011	-2.2
16.	Glyphosate	2.0	1.250±0.478	-0.75	-1.6
17.	Sulfosulfurol (*SS*)	0.05	0.013±0.006	-0.037	-3.8

Notification: ***** - MRL (maximum residue level), according to the "Sanitary Regulation on the Maximum Allowable Residue Levels of Phytosanitary Products in or on Food or Feed of Plant and Animal Origin for Animals", approved by the Decision of Government of Republic of Moldova no. 1191 of 23.12.2010, www//lex.justice.md, vizited on 06.12.2017.

Some of the investigated insecticides in large-leaf linden flowers, in general, have not been detected. Thus, no detectable concentrations of residues of pyrethroid insecticide *Tau-fluvalinate*, neonicotinoid insecticides (*Imidacloprid, Thiamethoxam*) and triazole fungicide *Mepanipyrim* were registered.

The highest concentrations of residues in the linden flowers had been registered at triazole fungicide *Difenoconazole* - 16.00±1.22 mg/kg and controversial herbicide *Glyphosate* 1.250±0.478 mg/kg. It was observed that the linden flowers have a slight tendency to accumulate higher concentrations, compared with acacia flowers, of residues of some pyrethroid insecticides (*Cyprodinil, Deltamethrin, Pyrethrine*), triazole fungicides (*Difenoconazole, Fenhexamid*) and herbicides (*Amidosulfuron, Amitrol, Glyphosate, Sulfosulfurol*). At the same time, the detected concentrations of pesticide residues in forestry linden flowers are well below the maximum admissible level, according to the current standards, for the triazolic fugicide *Difenoconazole* on 1.3 times, herbicide *Glyphosate* on 1.6 times, up to 12.5 times for fungicide *Fenhexamid* and 33.3 times for pyrethroide insecticide *Cypermethrin*.

Also such results have been obtained in the researches of other authors (Łozowicka et al., 2014). In the North-East of Poland, the presence of 163 pesticides residues were evaluated (6 acaricides, 62 fungicides, 18 herbicides and 77 insecticides) in various herbs, including 3 samples of linden flowers. As a result pp′-DDD was found in the linden sample 0.02 mg/kg below MRL (MRL = 0.05 mg/kg). It is necessary to point out that pp′-DDD was found in one sample, and that this pesticide belongs to the chlorinated pesticide group and is a product of the breakdown of DDT, a pesticide banned for agricultural use worldwide. These results are important because lime flowers are part of medicinal plants collected from the forest. Good quality control and determination of the presence of toxic pesticides in herbs is essential to avoid their overconsumption and cumulative toxicities in long-term use.

If the presence of insecticide and fungicide residues in the flowers forest trees can be explained by their use in spray treatments against harmful insects and fungi, then, it is curious to note the fact of herbicides presence in the forest trees flowers. Where did these come from? We suppose that these pesticides could reach the flower samples by spraying them through aerosols in the nearby agricultural fields, through the vapors from the rainwater, from the groundwater and surface waters through infiltration or other less known by us pathways.

Our hypotheses find their confirmation in communications of researchers from Romania (Mihail et al., 2010). Studying the content of organochlorine insecticide residues in the forest soil (272 samples), they had found an average concentration of HCH equal to 0.03 mg/kg, ranging from 0.001 to 0.434 mg/kg, and an average of 0.032 mg/kg DDT, ranging from 0.001 to 0.264 mg/kg, compared to 0.01 mg/kg CMA according to EU standards. Thus, average concentrations of some pesticides in the soil, detected by these researchers have exceeded about 3.0 times CMA, according to EU standards.

In our research, if we compare indirectly the concentrations of pesticide residues researched in the flowers of the forest trees (acacia and linden) with the maximum residue levels (MRL), we find that these (concentrations) do not exceed the established national and EU standards.

Therefore, the recorded pesticide residues in the flowers of forestry trees, are not harmful for the health of bees and fauna of studied ecosystems. In conclusion we can say that the flora of studied by us forest sector, are not polluted with pesticides, so organic beekeeping can be practiced here, with the production of organic apiculture products.

CONCLUSIONS

In the flowers forest trees, such as acacia white (*Robinia pseudoacacia*) and large-leaf linden (*Tilia platyphillos*) low concentrations of pesticide residues were detected, as follows: pyrethroid insecticides (*Cypermethrin, Deltamethrin, Pyrethrine*) from 0.003 to 0.100 mg/kg, neonicotinoid insecticide (*Clothianidin*) from 0.400 to 0.425 mg/kg, organophosphorus insecticide (*Fenamifos*) in the amount of 0.042 mg/kg, triazole fungicides (*Fenhexamid, Mepanipyrim, Cyprodinil*) from 0.017 to 0.092 mg/kg, triazole fungicide *Difenoconazole* from

8.75 to 16.0 mg/kg, Fipronil acaricide from 0.001 to 0.003 mg/kg, herbicides (*Amidosulfuron, Amitrol, Sulfosulfurol*) from 0.005 to 0.013 mg/kg and herbicide *Glyphosate* from 0.25 to 1.25 mg/kg. Pesticide residues concentrations in the above-mentioned forest trees flowers (acacia and linden) are 1.3 to 33.3 times lower than the maximum admissible levels under national and EU standards.

Therefore, the recorded pesticide residues in the flowers of forestry trees, are not harmful for the health of bees and fauna of studied ecosystems. In conclusion we can say that the flora of studied by us forest sector, are not polluted with pesticides, so organic beekeeping can be practiced here, with the production of organic apiculture products.

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ASSESSMENT OF AVIFAUNA IN CORRELATION WITH ALTERATION-HYDROLOGY OF MANGLA WETLAND, AZAD KASHMIR, PAKISTAN

Muhammad UMAR[1], Muhammad ARSHAD[2], Mubashar HUSSAIN[1], Muhammad Faheem MALIK[1], Moazama BATOOL[3], Fatima ZAFAR[1]

[1]University of Gujrat, Faculty of Science, Department of Zoology, 50700, Hafiz Hayat, Gujrat, Punjab, Pakistan
[2]University of Sargodha, Faculty of Science, Department of Zoology, 40100, Sargodha, Punjab, Pakistan
[3]Government College Women University, Sialkot, 51310, Sialkot, Punjab, Pakistan

Corresponding author's email: dr.mubashar@uog.edu.pk

Abstract

Avifauna population estimation and hydrological analysis of surface water of Mangla dam Wetland was carried out for two consecutive years by conducting bird census in the months of January. The birds are counted by Point Count Method from a fixed point and for the fix time period in such a way that the Sun is always at the back. A total of 37,082 birds belong to 37 species visited the wetland in 2010 whereas 28 with population of 21,302 birds in 2011. Twenty one bird species were common at the study site for both years. The most abundant bird species were Black Headed Gull and Great Cormorant for both years 2010 and 2011. The census index (139.93 / km²) was higher in 2010 rather than (80.38 / km²) in 2011. The level of Co+2, Cu+2, Fe+2, Mg+2, Mn+2 and Zn+2 were found to be within the permissible limits in surface water analysis. However, the levels of Cr+2, Pb+2 and Cd+2 were high from safe limits. The study emphasized on extensive monitoring of the lake and catchment area in order to address the threats posed by heavy metals.

Key words: *avifauna, birds, Mangla dam, Pakistan.*

INTRODUCTION

Wetlands are the dynamic ecosystem consists of 6% of earth habitats (Maltby and Turner, 1983). These are considered as biodiversity hotspots due to presence of wide range of flora and fauna (Mitsch and Gosselink, 1986). The habitat of wetlands is important for most of the birds for feeding, nesting and roosting behavior (Stewart, 2001). Geographically, Pakistan is present in north western part of southern Asia and consists of wetlands of an area of 9.7% ranging from coastal mangroves to glacial lakes of Himalaya. Out of 48 wetlands of Pakistan, 19 are included as Ramsar sites (Scott, 1989). Pakistan wetlands are especially important for their distinctive and diverse habitat (Garstang et al., 2003). Mangla dam is 12th largest dam of the world and it is located in Mirpur, Azad Kashmir, with subtropical climate of average rainfall of 150mm, between latitude and longitude of 32 to 36 and 73 to 75 respectively.
The water body was constructed in 1967 and consists in an area of 26599 ha which receives influx from Jhelum River. Mangla wetland have vegetated area which provides habitat for more than 30,000 water birds such as grebes, gulls, cormorants, coots, waders and geese (Ali, 2005). A total of 347 birds species were identified which included as 176 non passerine while 171 passerine (Roberts, 1991, 1992). Another study on the avian fauna of wetland indicated that 336 species were present in 2001 which included as 115 winter visitors, 153 resident, 39 passage migrants, 15 summer visitors and 14 were traced species (Grimmett et al., 2001). The biodiversity, in general, and avian fauna, in specific, have severe threats due to anthropogenic activities (Ali, 2005). The avifauna of Pakistan consists of 668 species belonging to 85 families with large number of winter visitors (Roberts, 1991, 1992). It has been reported that 85% of the migratory fauna of Pakistan consists of ducks and waders which is higher than resident and summer breeder bird population (Alfred et al., 2001).
The ratio of long distance migrant birds is higher than regular winter visitors while 43%

birds come in Pakistan for breeding only (http://www.wwfpak.org/species_birds.php).

Water is known as medium of life. It is used for different purposes which include agriculture, recreational, domestic and industries (Dara, 1993). Fresh water is a source for the development of civilizations but due to pollution there is severe threat to natural fresh water reservoir (Benjamin et al., 1996). In developing countries, the increase in production in industrial and agricultural sectors due to population growth has resulted in the contamination of water reservoirs (Canpolat and Calta, 2001). Due to anthropogenic activities seasonal variations occurs in the water bodies which disturb the biodiversity (Odum, 1971). These variations such as temperature, transparency, pH, electrical conductivity, total hardness, nitrates and phosphates determine the quantity of planktonic biomass in the freshwater bodies (Mahboob and Sheri, 2001).

Water pollution is severe threat for biodiversity globally. The major cause of water pollution is industrial effluents, sewage waste and acid rain which deposits heavy metals into water bodies (Obasohan et al., 2008). Big cities of Pakistan produce average sewage of 11650 million gallons per day which is used to irrigate 32,000 hectares. However, this sewage contains high pH, temperature, chemical oxygen demand, nitrates and nitrites, and cations like calcium, sodium, potassium, and magnesium (Ghandour et al., 1985). Besides of these, a large amount of heavy metals i.e. zinc, nickel, iron, lead, chromium, arsenic, copper, cadmium and cobalt are also present in sewage which may accumulate in soil and transferred to vegetables and crops indirectly. The other sources for the contamination of wetlands are the substances released from industrial effluents, pesticides and nitrogenous fertilizers (Ali et al., 1996).

A few heavy metals are important micronutrient in low concentration like manganese, cobalt, zinc, nickel, lead and copper but their higher level poses risk for biodiversity (Nürnberg, 1982). These metals are released in industrial effluents in a higher concentration level that contaminate the water reservoir and ultimately toxic for aquatic life (Ibok et al., 1989). As these trace metals viz. nickel, copper, lead and zinc have great affinity for bio-magnification, ultimately entering into the food chain and can cause the severe health problems by effecting the liver, kidney, muscles and tissues (Dallinger et al., 1987). Metals like arsenic, cadmium, lead and chromium possess severe threat to biodiversity (Din et al., 1997, 2008). The current research work was undertaken to assess the physico-chemical parameters and concentration levels of various heavy metals in surface water of Mangla Wetland. Avifauna census was carried out to take into account the number of species and their population at the lake. The impact of anthropogenic activities and climate change (weathering process) was assessed by exploring diversity, abundance and species richness and evenness of avifauna visiting this water body. The study emphasized on working out major factors contributing in declining trend of bird species at this Ramsar site.

MATERIALS AND METHODS

The study was executed at Mangla wetland, situated in Mirpur, Azad Jammu & Kashmir (33.12 N, 73.39 E) with an elevation of 630 m and comprise of an area about 26500 ha. It is the 12th largest dam of the world which was constructed in 1967 at the southeast corner 100 km away from the Islamabad. This water body is of special concern because it provides wintering habitat for water birds.

Bird census was carried out for two consecutive years 2010 and 2011 in the months of January and February because a large number of migratory birds visit the lake. The area of the reservoir is divided into five observation points. The birds are counted by point count method from a fixed point or for the fix time period in such a way that Sun is always at the back. Relative abundance of each bird species is estimated by this method (Sutherland, 1996). The assessment of food habits of bird species was followed by as suggested in the study of Roberts (1991, 1992). The preference for the food by different groups of birds was documented. Photographs were taken by using field binocular and birds' species were subjected to identification by using keys (Ali and Ripley, 1987; Roberts, 1991, 1992). The relative abundance was calculated for each bird so as to determine the

most abundant bird species by using the terms described by Bull (1964) and McCaskie (1970). Biodiversity Index was calculated (Shannon and Wiener, 1963).

The samples were collected from the five different sites of the Mangla Dam in the study period from October 2010 to September 2011. The sampling purpose was to determine the quality of water and variation within different months of the year. A total of 12 samples were collected by using plastic bottles in such a way that bottles were disinfected, washed, dried followed by soaking in 5% nitric acid for 24 hrs. To restrict adsorption of heavy metals onto wall of sampling bottles 5 ml nitric acid was immediately added after collecting the samples (Ademoroti, 1996) and then safely transferred to laboratory. For the digestion of samples, the procedure proposed by Kar et al., 2008 was followed with little modifications. Digested samples were placed in pre-washed polythene bottle. Analytical grade reagents were used throughout the processing period. Various standards of heavy metals were prepared from certified standard stock solution (ppm) by using double distilled water. These standards were used to obtain calibration curve on Atomic Absorption Spectrophotometer. Water samples were analyzed for heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn) in Shimadzu (AA-6300) Atomic Absorption Spectrophotometer. One way ANOVA was applied to summarize results and analyzes the variation (Steel and Torrie, 1980).

RESULTS AND DISCUSSIONS

Mangla dam consists of an area of 26,500 ha and is (33.12 N, 73.39 E) located in Mirpur, Azad Kashmir, Pakistan. In the current study, five points were marked for the observation of bird diversity named as Pounch, Khund, Jarrikus, Jarricus and Mangla. The counting of avifauna was carried out from 2010 and 2011. In the study period, number and diversity of bird species varied. A total of 37,082 birds were observed belong to 37 species in 2010 whereas declining trend in the number of species was observed in 2011 viz. 28 species and their total number were 21,302 (Table 1).

Twenty one (21) bird species i.e. great cormorant, great egret, common teal, bar-headed goose, mallard, common coot, northern pintail, grey heron, red-wattled lapwing, little cormorant, indian pound heron, black-winged stilt, black-headed gull, eurasian wigeon, ruddy shelduck, northern shoveler, great crested grebe, common pochard, common sandpiper, slender billed gill and red shank were observed both in 2010 and 2011 years (Figure 1).

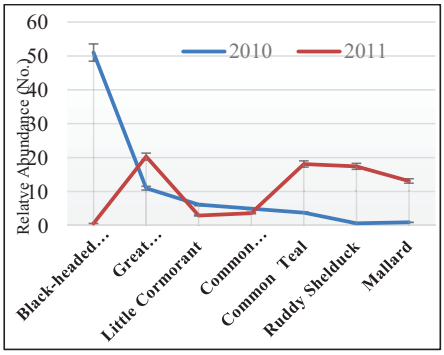

Figure 1. Comparison of dominant species in Mangla wetland, Azad Kashmir, Pakistan during 2010 and 2011

The relative abundance for each bird species was calculated in the current study (Tables 2 and 3). Black headed gull was the most abundant species in 2010 while great cormorant, in 2011. At the Mangla dam in 2010, the dominant and subdominant bird species were black headed gull (51.00 %) and great cormorant (10.99 %) (Table 3). However, the dominant and subdominant bird species in 2011 were great cormorant (20.31%) and common teal (18.11%) respectively (Table 2).

Table 1. Summary of different analysis in 2010 and 2011 years, in Mangla wetland, Azad Kashmir, Pakistan

Specifications	Year	
Parameters	2010	2011
Area Surveyed (ha)	26,500 ha	26,500 ha
Total Population	37,082	21,302
Number of Species	37	28
Census Index	139.93 /km²	80.38/km²
Dominant Species	Black Headed Gull (51.00; relative abundance)	Great Cormorant (20.31; relative abundance)
Sub-Dominant Species	Great Cormorant (10.99; relative abundance)	Common Teal (18.11; relative abundance)

Table 2. Dominant and sub-dominant index of avifauna observed during 2010 year in Mangla wetland, Azad Kashmir, Pakistan

S#	Common name	Khund	Mangla	Jarrikus	Jarrikus	Pounch	Total Population	Relative Abundance	Density hectare
1	Black-headed Gull	8772	7835	38	38	2230	18913	51.00	71.37
2	Great Cormorant	765	2265	238	104	702	4074	10.99	15.37
3	Little Cormorant	0	996	16	16	1242	2270	6.12	8.57
4	Common Pochard	307	33	698	698	94	1830	4.94	6.91
5	Common Teal	153	64	442	409	326	1394	3.76	5.26
6	Great Crested Grebe	85	66	232	237	264	884	2.38	3.34
7	Little Stint	0	0	5	0	862	867	2.34	3.27
8	Great Egret	23	575		0	193	791	2.13	2.98
9	Grey Heron	167	353	3	18	126	667	1.80	2.52
10	Tufted Duck	314	0	61	61	161	597	1.61	2.25
11	Whiskered Tern	11	6	0	0	554	571	1.53	2.15
13	Little Egret	19	153	146	24	223	565	1.60	2.13
14	Black Wing Stilt	0	221	0	0	322	543	1.46	2.04
15	Northern Pintail	132	81	17	24	231	485	1.30	1.83
16	Mallard	0	27	150	159	0	336	0.91	1.27
17	Greenshank	36	71	4	4	213	328	0.88	1.24
18	Common Coot	46	65	36	55	47	249	0.67	0.94
19	Ruddy Shelduck	178	0	0	0	43	221	0.60	0.83
20	Little Grebe	37	27	19	29	92	204	0.55	0.77
21	Slender Billed Gull	0	0	0	0	187	187	0.50	0.71
22	Bar-headed Goose	161	0	0	0	0	161	0.43	0.61
23	Ferruginous Duck	98	16	0	48	0	161	0.43	0.61
23	Indian River Tern	28	101	0	0	21	150	0.40	0.57
24	Redshunk	0	0	0	0	121	121	0.33	0.46
25	Northern Lapwing	21	0	17	17	62	117	0.32	0.44
26	Eurasion Wigeon	0	0	9	9	69	87	0.23	0.33
27	Black Stork	0	0	1	1	82	84	0.23	0.32
28	Indian Pond Heron	26	32	0	0	12	70	0.19	0.26
29	Common Sandpiper	13	6	25	25	0	69	0.19	0.26
30	Pied Kingfisher	12	32	0	4	18	66	0.18	0.25
31	Common Shelduck	0	0	0	0	4	4	0.01	0.02
32	Gosander	0	0	2	2	0	4	0.01	0.02
33	Red-wattled Lapwing	5	16	1	1	4	27	0.07	0.10
34	Pallasis Gull	3	3	0	0	4	10	0.03	0.04
35	Caspian Tern	2	0	0	0	0	2	0.005	0.01
36	White-breasted Kingfisher	2	0	0	0	0	2	0.005	0.01
37	Northren Shoveler	0	0	0	2	0	2	0.005	0.01

The least abundant bird species were Northern shoveler white-breasted kingfisher and Caspian tern, whose relative abundance was even less than 0.01. The total area of the lake was 26,500 ha and noted by using GIS techniques. It was done for the calculation of density of each bird species which were observed at the lake in 2010 and 2011. The density of birds at the site was higher for 2010 (139.93/km²) than to 2011 (80.38/km²). In the current study, 45 bird species were noted which include 27 winter visitors, 13 resident, 2 double passage migrants, 1 summer breeders and 2 irregular year round visitors (Figure 2).

Furthermore, among 37 bird species that were observed of which are include 14 abundant, 11 very common, 5 very abundant, 5 rare, 1 common and 1 fairly common in 2010. However, 27 bird species were recorded in 2011 which were 4 very abundant, 9 abundant, 9 very common and 5 common (Figure 3). Birds feeding preferences was also determined. The highest count of birds were carnivorous (68.88%; insects, molluscs, crustaceans, fish, and frog) followed by omnivores (24.44%; leaves, shoots, seeds, submerged vegetation, insects, insect larvae, small beetles and worms)

and herbivorous (6.66%; shoots of plants and vegetation in water) birds (Figure 5).

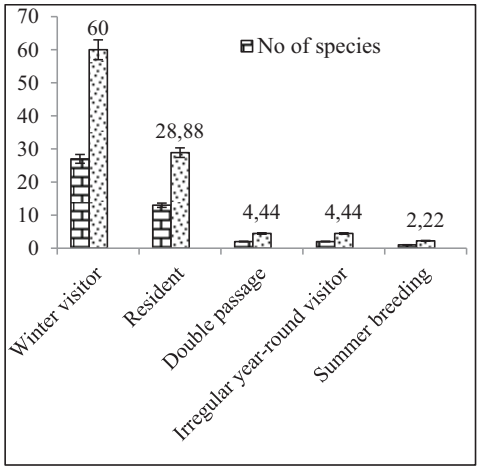

Figure 2. Relative abundance of migratory, breeding and resident avifauna in Mangla wetland, Azad Kashmir, Pakistan, during 2010 and 2011 years

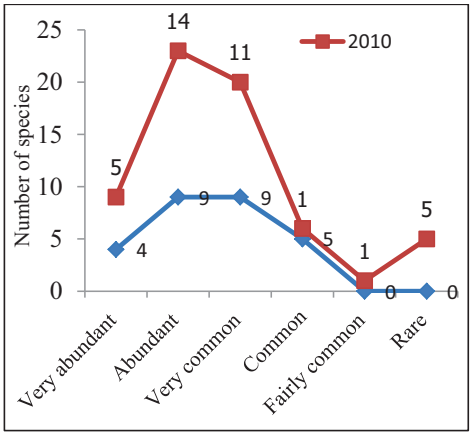

Figure 3. Species status and their relative abundance, during 2010 and 2011 years, in Mangla wetland, Azad Kashmir, Pakistan

In 2010, the number of bird species observed at selected sites were in the order of (30) Pounch, (26) Khund, Mangla (23), (23) Jarrikus (2) and (22) Jarrikus (1). However, a different pattern was observed in 2011 for these sites.

The concentration level of heavy metals in the water samples from Mangla Lake followed the decreasing trend: Cr>Fe>Co>Zn>Pb>Ni>Cu>Cd>Mn for both sites (Figure 4).

Minimum values for Cr, and Cd was recorded in September. However, maximum level for Co, Mn, Ni and Cu was found in August, Aril, October and June respectively. At both sampling sites, monthly variation for metals found to be significant at $p<0.05$ (Table 3).

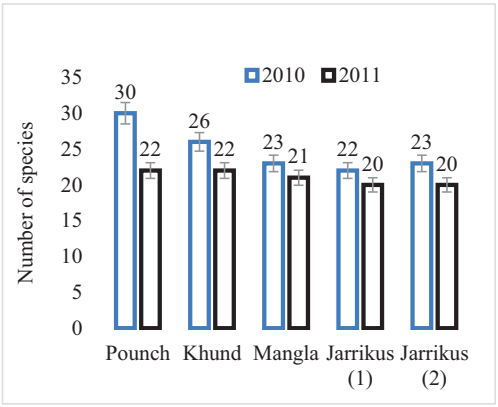

Figure 4. Number of bird species in different sites of Mangla wetland, Azad Kashmir, Pakistan during 2010 and 2011 years

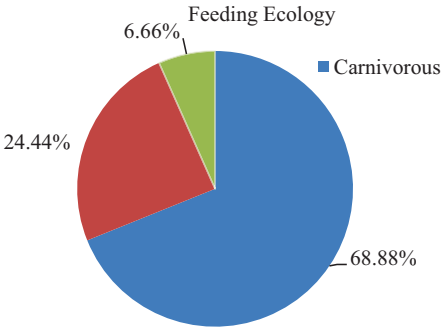

Figure 5. Feeding ecology of birds in Mangla wetland, Azad Kashmir, Pakistan

However, temporal variation between sampling stations was non-significant ($p>0.05$). Bird migration is a litmus test for environment quality and healthy bird population indicate healthy habitats; declining bird populations indicate ill or degraded habitats. One third of the bird endangered species choose their habitat near or in wetland areas. It is too much alarming situation that 42% populations of the migratory water birds are declining in the flyways (Ali et al., 2011). In 1992 has reported 347 bird species at the lake area out of which 176 were non passerines. 336 bird species were reported by Grimmett et al., (2001) whereas 141 species were observed at the reservoir in 2006 by Ali et al.

Mangla dam provides staging and wintering area for various water bird species (Robert, 1991). According to Chaudhary (2009), there were 102 bird species were present at the study site. However, in our study a total of 45 species of birds were recorded. The reason for such a difference in number of species visiting this wetland may be owed to the fact that lakes have suffered degradations (anthropogenic activities) secondly partial or local migration of birds. Khan (1992) studied that population trends of waterfowl species declined due to increase in human and livestock activities. Water birds used different types of habitats in different times of their lifecycle. But it is difficult to assess the factors responsible for the decrease trend of change of population (Chaudhary, 2009).

Table 3. Dominant and sub-dominant index of avifauna observed during 2011 year in Mangla wetland, Azad Kashmir, Pakistan

S #	Common Name	Khund	Mangla	Jarrikuss 1	Jarrikuss 2	Pounch	Total Population	Relative Abundance	Density/ Hectare
1	Great Cormorant	801	2432	248	117	729	4327	20.31	16.33
2	Common Teal	659	602	978	821	798	3858	18.11	14.56
3	Ruddy Shelduck	1332	542	439	455	942	3710	17.42	14.00
4	Mallard	159	570	910	987	162	2788	13.09	10.52
5	Common Pochard	207	129	287	383	164	1170	5.49	4.41
6	Northern Pintail	223	101	67	83	234	1108	5.20	4.18
7	Bar-headed Goose	495	0	0	0	589	1084	5.09	4.09
8	Little Cormorant	0	76	2	74	470	622	2.92	2.35
9	Gadwall	168	88	54	61	149	520	2.44	1.96
10	Slender Billed Gull	86	97	0	0	104	287	1.35	1.08
11	Northren Shoveler	0	0	142	128	0	270	1.27	1.02
12	Great Crested Grebe	30	26	55	60	63	234	1.10	0.88
13	Redshank	25	15	0	0	164	204	0.96	0.77
14	Eurasion Wigeon	0	0	80	100	0	180	0.84	0.68
15	Indian Pond Heron	52	64	0	0	24	140	0.66	0.53
16	Black-headed Gull	55	49	0	0	20	124	0.58	0.47
17	Common Tern	46	31	0	0	28	105	0.49	0.40
18	Common Coot	9	46	2	25	14	96	0.45	0.36
19	Grey Heron	17	33	6	9	13	78	0.37	0.29
20	Intermediate Egret	30	25	11	0	12	78	0.37	0.29
21	Tufted Duck	45	0	14	11	0	70	0.33	0.26
22	Red-wattled Lapwing	11	26	6	6	10	59	0.28	0.22
23	Common Sandiper	0	0	23	30	0	53	0.25	0.20
24	Great Egret	7	20	0	0	10	37	0.17	0.14
25	Black Wing Stilt	0	9	0	0	20	29	0.14	0.11
26	White Eyed Pochard	15	0	6	4	0	25	0.12	0.09
27	Red Crested Pochard	0	0	12	13	0	25	0.12	0.09
28	Gull Billed Tern	0	0	10	11	0	21	0.10	0.08

Due to fragile ecosystem and vulnerability to degradation, Lakes are the most affected ecosystem in the world in which contaminants are deposited and take a time to flush out than rivers. They need more careful management than streams and rivers (McCalla, 1995).

The food chain and food network have important relation with birds. As most of the birds feed on insects so, they also participate to control the insects population within the ecosystem. Previous study of the lake shows that 56.87% birds were insectivorous (Chaudhary, 2009). Similar results trend was observed in our study as 68.88% birds were carnivorous followed by 24.44% omnivorous and 6.66% herbivorous at the site. Black headed gull and common pochard have high relative abundance in our study in two consecutive years 2010 and 2011 respectively. However, low relative abundance was calculated for ruddy shelduck in 2010 and mallard in 2011. Similarly, little grebe, common teal and black headed gull have low relative abundance at Uchhali lake in 2010 as compare to 2011 by Mehmood (2011). The reason of decline at Mangla wetland in 2011 could be the restoration of actual habitat at Uchhali. As these birds were shifted their place

due to low water level from Uchhali to Mangla. In our study, birds such as common pochard, great cormorant, black headed gull, mallard, common teal, little cormorant, and ruddy shelduck have most frequent population.

However, least abundant bird species were Caspian tern, white breasted kingfisher and Northern shoveler. Similar trend was observed by Ali et al. (2006) where Northern shoveler was most frequent species but in our study its population decreased at the lake as 2 and 270 individuals observed in 2010 and 2011 respectively. The decrease population of this bird could be due to occupation of the habitat at the Mangla Lake by other bird species which may migrate from other wetlands.

The feeding habit of great cormorant was carnivorous and winter visitor in Pakistan (Robert, 1991). It was very abundant bird at Taunsa and Mangla lakes (Ali et al., 2006). Similarly, great cormorant also possess very abundant bird status in the current study at the site. Black headed gull is a winter visitor's bird with a population status of most common and abundant found at major water bodies in Pakistan (Roberts, 1991). There were 144 individuals of black headed gull present at Taunsa Barriage (Mahboob and Nisa, 2009). The population status of black headed gull was abundant in Jiwani and rare in Taunsa reservoir (Ali et al., 2011). However, it was very abundant and very common bird at Mangla dam in 2010 and 2011 respectively. Common teal (*Anas crecca*) is most common and abundant bird that is winter visitors at major wetlands of Pakistan.

It is also game bird widely hunted due to its abundance, wing versatility and palatability. It lives in small groups and flocks of about 200 birds. Its favorite habitat was the shallow pools along the sides of water bodies (Grimmett et al., 2008).

In our study, largest population consists of 3,858 birds of common teal were counted at Mangla dam in 2011 which was lower in 2010. However, a very small population as only 45 individuals of this bird was reported by Ali et al. (2006) at the same wetland. Common pochard is a diving duck and forms large flock on water bodies which is also winter visitor bird species (Grimmett et al., 2008). It was reported that common pochard was most dominated bird species at Mangla wetlands (Ali, 2005). Similarly, it is also noted to be most abundant bird species in current study.

Mallard (*Anas platyrhynchos*) is the most abundant species widely distributed from Canada to Alaska and Russia to Western Europe (Roberts, 1991). It is a winter visitor water bird least encountered in Sindh (Grimmett et al., 2008). The maximum number up to 600 individual of this bird was present at Drigh Lake (Gabol et al., 2005). Due to readily adaptable and high tolerance for disturbance, it adjusts in variety of breeding and wintering habitats (Ali, 2005). Its population range in between 336-2,788 individuals at the reservoir in current study. However, 729 individuals were reported at Uchhali complex by Ali (2005). Furthermore, it was noticed that the population of Mallard was also reduced in last three years as only few individuals of this bird were observed in Muzaffarabad (Awan and Saleem, 2007). Same decline trend was observed at Rasool Barrage where population number had reduced 200 from 1996 to 2005. The reason of population decline is uncontrolled hunting (Akbar et al., 2009). Northern shoveler (*Anas clypeata*) is also winter visitor bird at Mangla dam (Ali et al., 2006). It prefers the stagnant and muddy water. They are not liked by hunters because their bodies have musky smell (Roberts, 1991).

In our study, Northern shoveler was least abundant bird species at the site. Similar status of this bird was noted by Ali et al. (2006) at the Mangla dam. However, highest population of Northern shoveler up to 3,500 was reported at Drigh Lake in 2,000 (Gabol et al., 2005). In addition, it was also most abundant bird up to 546 individuals at Kallar Kahar Lake (Reis et al., 2010).

Kingfisher is the bird species linked to water bodies for their feeding (Roberts, 1991). It was noted that kingfisher was resident bird species in Muzaffarabad (Awan et al, 2004). White breasted kingfisher occupied the status of common bird at the Trimmu dam (Ali et al., 2011). Previous studies showed that this bird was fairly common at Mangla Dam (Ali et al., 2011). However, white breasted kingfisher was found to be rare in our study at the Lake. The trend of decline of avifauna at Jiwani wetland Pakistan was noted as 120 bird species by

Robert (1991, 1992), 101 bird species by Grimmett et al. (1998) and 84 bird species by Mirza and Wasiq (2007) respectively. Similar trend of decline of birds were observed at Taunsa Barrage by Robert (1991); Grimmett et al., (1998); Mirza and Wasiq (2007); Mehboob and Nisa (2009). Similarly, a trend of decline of avifauna was observed at Mangla reservoir. A total of 347 bird species were reported by Roberts (1991, 1992) while 336 bird species were noted by Grimmett et al., (2001). According to Ali et al. (2006), 141 bird species were present at the reservoir. Whereas, a total of 102 bird species were present at Mangla wetland (Chaudhary, 2009). However, in the current study period, the observed number of bird species was 37 and 28 in 2010 and 2011 respectively.

The decline of bird species could be due to the disturbance of water quality which occurs by the excessive use of fertilizers, agrochemical and ill planned management of municipal waste. The municipal waste contains heavy metals like Pb, Cr and Cd were in higher concentration than WHO permissible limit. The other cause of decline of avifauna may be due the degradation of habitat by anthropogenic activities, use of some species as game birds and illegal hunting. Physical, chemical and biological processes occur permanently in the water bodies and should be considered to explain the inorganic elements and heavy metals concentrations (Narayanan and Vijayan, 2007).

Anthropogenic activities also affect the behavior and concentration level of heavy metals and inorganic elements (Baeyens et al., 1998). The seasonal variation in the water modifies the population density of both animal and plant species (Odum, 1971). The productivity of fresh water ecosystem in the form of planktonic biomass is regulated by factors such as temperature, pH, transparency, total hardness, etc. (Mahboob and Sheri, 2001). Various metals are essential micronutrient at low concentration but their higher level is toxic for biodiversity. Higher level of these metal ions creates turbidity and discoloration of lake water (Trivedi and Gurdeep, 1992).

In the current study, the order of higher concentration of heavy and trace metals in the water of Mangla Lake were Cr>Fe>Co>Zn>Pb>Ni>Cu>Cd and Mn. Except Cr, Pb and Cd all other metal were within safe limit (Table 4).

Table 4. Analytical values of heavy metals in Mangla dam during 2010-2011

Specifications	Metals								
Sampling Period	Cr	Co	Mn	Ni	Cu	Zn	Pb	Fe	Cd
Oct	0.1100	0.0620	0.0032	0.0222	0.0112	0.0288	0.0199	0.0709	0.0103
Nov	0.1518	0.0510	0.0045	0.0210	0.0118	0.0320	0.0181	0.0953	0.0111
Dec	0.1629	0.0672	0.0048	0.0204	0.0101	0.0381	0.0186	0.1029	0.0119
Jan	0.1642	0.0560	0.0051	0.0124	0.0142	0.0260	0.0210	0.0910	0.0108
Feb	0.1272	0.0671	0.0048	0.0135	0.0155	0.0281	0.0221	0.1017	0.0113
March	0.1250	0.0512	0.0053	0.0133	0.0155	0.0406	0.0216	0.1081	0.0115
April	0.1315	0.0501	0.0056	0.0139	0.0154	0.0502	0.0223	0.1100	0.0129
May	0.1312	0.0274	0.0037	0.0141	0.0176	0.0601	0.0221	0.0599	0.0140
June	0.1283	0.0294	0.0028	0.0150	0.0183	0.0605	0.0201	0.0460	0.0176
July	0.1290	0.0940	0.0023	0.0139	0.0102	0.0583	0.0199	0.0440	0.0129
Aug	0.1209	0.0990	0.0022	0.0107	0.0038	0.0670	0.0212	0.0321	0.0112
Sep	0.1092	0.0419	0.0033	0.0101	0.0031	0.0681	0.0182	0.0652	0.0041
Mean	**0.1326**	**0.0580**	**0.0039**	**0.0150**	**0.0122**	**0.0464**	**0.0204**	**0.0772**	**0.0116**
SD	0.01809	0.0220	0.0011	0.0039	0.0049	0.0159	0.0015	0.0276	0.0030
Min	0.1092	0.0274	0.0022	0.0101	0.0031	0.0260	0.0181	0.0321	0.0041
Max	0.1642	0.0990	0.0056	0.0222	0.0183	0.0681	0.0223	0.1100	0.0176
WHO Limit	**0.05**	**1.0**	**0.05**	**0.05**	**0.05**	**0.10**	**0.01**	**1.0**	**0.005**

Similar trend of concentration was reported by Gulfraz et al. (2001) in which Cr, Cd and Pb were in higher concentration in Mangla Lake. It may be due to the addition of untreated municipal waste from poultry forms and geological weathering taking place in these areas. Chromium is used in manufacturing of paints, pigments, glass, ceramics industry, photographic and chromo metals products. So, it enters into municipal waste and reaches into Lake Water which are the main sources of chromium pollution (Lone et al., 2003). The highest level of Cr was observed in Jan in our study. Similar result was obtained in the same water body and Rawal Lake by Gulfraz et al. (2001). Cr with higher levels in the reservoir in the January may be attributed to the low water level and metal deposition. However, low metal level was noted in September as water inflow and outflow was higher which did not allow the metal deposition. Cr concentration in higher quantity in diet decreases the muscle fat in birds and increase protein deposition (Ward et al., 1995). Feed Conversion Ratio (FCR) and body mass of bird is disturbed (Hossain et al., 1998). Lead contamination occurs by snow, rain, waste water treatment, smelting works and by anthropogenic activities (Fardous et al., 2011). It is not necessary for the metabolic activities of the animals. It enters into water bodies from dyes, mining and petroleum industry and affects aquatic life adversely (Ciftci et al., 2008).

In our study, Lead concentration was higher than permissible limits in the month of January to May. Similarly, higher concentration level of Lead was reported by Iqbal et al., (2006) and Gulfraz et al., (2001) in the water of Mangla and Kollar Kohar. The higher level could be due to snow fall and rainy season by which atmospheric Lead comes to the soil and ultimately enter into this wetland. Lead is very toxic element for the biodiversity. It causes several diseases in birds such as anemia, weight loss and green watery faeces (Redig et al., 1980). It also affects circulatory system, nervous system, kidneys, biochemical and behavioral changes in animals (Scheuhammer, 1998). In our observation, the level of Cadmium was not within safe limits. The higher value was recorded in March to June. Similar trend of values were observed by Yaha (2008), Iqbal et al., (2006) and Gulfraz et al., (2001), and in the water of Mangla, Kallar Kohar and Manchar Lake respectively. This higher level could be due to emission of Cadmium from natural resources to environment by volcanic activities, weathering of rocks and sea spray or it may be by anthropogenic activities such as burning of fossil fuels, industrial processes, municipal effluents, metal extraction, and phosphate fertilizers and also from the uncontrolled open burning of wastes. This atmospheric Cadmium comes to ground with rain and snow in winter and ultimately into lake water. As Cadmium is metal toxicant in the environment naturally and industrially, that enters into biological system via food, soil and water. It causes the reproductive toxicity in birds (Toman et al., 2002) and decline in egg production (Rahman et al., 2007). The observed values of Mn, Co, Ni, Fe, Zn and Cu indicated that these metals lie under safe limits. Similar findings were recorded in Manchar Lake by Yaha (2008).

CONCLUSIONS

Mangla wetland avifauna population estimation and hydrological analysis of surface water recorded a total of 37,082 birds belonging to 37 species visited the wetland in 2010 whereas 28 with population of 21,302 birds in 2011. In surface water analysis, the level of heavy metals (Co^{+2}, Cu^{+2}, Fe^{+2}, Mg^{+2}, Mn^{+2} and Zn^{+2}) were found to be within the permissible limits whereas levels of Cr^{+2}, Pb^{+2} and Cd^{+2} were higher than safe limits.

The research work emphasize on extensive monitoring of this Ramsar site and catchment area in order to address the threats posed by heavy metals.

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RESEARCH ON THE EVOLUTION OF THE MEAT PRODUCTION CHARACTERS AND THE CORRELATIONS AMONG THEM IN INEU CRAP BREED

Carmen Georgeta NICOLAE, MirceaCătălin ROTAR, Monica Paula MARIN, Elena POGURSCHI, Gratziela BAHACIU, Alina UDROIU

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd., District 1, Bucharest, Romania

Corresponding author email: rotar.mircea.catalin@gmail.com

Abstract

The knowledge of fish characters for meat production and the correlations among them presents a major importance. Ineu carp breed can have a significant contribution by providing a high quality protein to Romanian and other consumers also. The increased human population requires a larger production of food and aquaculture production must meet this need. The study was based on the analysis of morphologic characters of descendants of five Ineu carp females. The studied characters were weight, body depth and standard length; each descendent being measured and weighed over a period of three years. The Fisher test was used to check variance homogeneity, and Pearson correlation coefficient was used to verify the interdependence of the three characters. Regarding the homogeneity of the variance, no significant differences were found ($P \geq 0.05$). According to the results, the correlation between the weight and the body depth was the highest, namely 0.99; 0.95 between weight and standard length and 0.96 between standard length and body depth. In conclusion, the three production characters are strongly correlated and have a major influence on meat production.

Key words*: carp, correlation, fish, productions, traits.*

INTRODUCTION

The body growth is the third major change that defines the complicated process of development, along with gene replication and cell differentiation. During development, the body undergoes an increasing of body mass and dimensions, a process that is even the phenomenon of growth.

Any fish population subjected to the genetic improvement process must be known in terms of performance for the studied characters (Oroian, 2007). The quantitative characters represent the phenotypic expression of genotype occurrence under certain environmental conditions, phenotypic expression so called performance.

Due to its qualities (high growth rate, good utilization of feed, precocity and good prolificacy), Ineu carp is registered from morphological, constitutional and productive point of view in the category of breeds with advanced degree of amelioration. Ineu carp is part of the category of high carp breeds, small headed, with short and tall caudal peduncle. The scales are low, and specimens without scales have been identified. All these features recommend it to both breeders and consumers.

The study of the evolution of characters for meat production has a great theoretical and practical importance (Ivancia, 2007).

Thus, knowing the age distribution of a fish population, as well as the growth rate of this population, it can easily tell if there is overcapacity in a fish pond or, on the contrary, an insufficient exploitation of the fishery resource. Considering these, a favourable moment for fishing and the choice of the earliest specimens and breeds can be identified which leads to the increase of the economic profitability of the fish farm.

When the goal is to increase meat production, it is recommended to consider the measurement of those characters that have the greatest influence on the amount of meat. Recording the information on weight, body depth, standard length and other characters translates as phenotypic information in statistics. If the analyze of data obtained by direct measurement on the live animal, shows that there are highly positive correlations between the characters that are to be traced, then we can say that the

intended aim was achieved: improving the meat production in a sufficient quantity and with an adequate quality to satisfy the increasing needs of consumers.

MATERIALS AND METHODS

For an accurate analysis of the three studied production characters, five Ineu carp families were evaluated. Thus, the descendants of five carp females were measured for 3 years and the three characters were registered each year.

The five females had 10 males of the Ineu breed carp as partners, each female had two partners. 5 descendants from each pair were kept, a total of 50 offspring obtained after the mating and they were observed and recorded for 3 summers. Every end of summer we measured: weight, body depth and standard length; for each of the measurements, the appropriate tools were used to prevent the measurement error from occurring. In the first step, a primary statistical analysis was performed and for each trait was calculated average, standard deviation and the coefficient of variability (Table 1). The calculation was based on the specific formulas (Grosu et al., 2005):

Average:

$$\bar{X}=\frac{\sum X}{n} \quad (1)$$

Standard Deviation:

$$S_X = \sqrt{S_X^2} \quad (2)$$

Average Error:

$$S_{\bar{X}}=\frac{S_X}{\sqrt{n}} \quad (3)$$

Coefficient of variability:

$$C.V.\%=\frac{S_X}{\bar{X}}\cdot 100 \quad (4)$$

Regarding the correlation between the analyzed characters, the Pearson Correlation was used in order to verify whether the three characters are dependent or not and whether they can influence the meat production.

Correlations among mother families and the actual total were also verified.

The correlation formula was proposed by Karl Pearson and named by him (Karl Pearson cited by Bolboacă and Jantschi, 2006):

$$r=\frac{\sum (X-\bar{X})\cdot(Y-\bar{Y})}{\sqrt{\sum (X-\bar{X})^2\cdot \sum (Y-\bar{Y})^2}} \quad (5)$$

The analyzed data were collected from the Fish Culture Research and Development Station of Nucet.

RESULTS AND DISCUSSIONS

Using the classical formulas for the primary statistics, the average with its error, the standard deviation and the coefficient of variability were calculated for the analyzed population.

Analyzing the results we can observe that the studied population is within the specific species limits.

The averages for each of the characters had different evolutions: for body weight the increase was significant from one year to the next, as it can be seen in Table 1.

Table 1. Phenotypic characterisation of the analyzed population

Parameter	Weight (W)			Body Depth (BD)			Standard Length (SL)		
	0+	1+	2+	0+	1+	2+	0+	1+	2+
n	50	50	50	50	50	50	50	50	50
Average	36.46 ± 0.67	594.48 ± 17.25	1462.8 ± 50.54	53.6 ± 1.4	114.56 ± 1.35	152.18 ± 1.84	130.78 ± 3.26	249.62 ± 2.82	330.84 ± 3.70
Standard Deviation	4.7	121.9	357.4	9.9	9.5	13.0	23.0	19.9	26.2
CV	12.9	20.5	24.4	18.5	8.3	8.6	17.6	8.0	7.9

The weight increased from 36 g to 1462 g, being 40 times higher in the third year of measurement compared to the first one. For the other two characters the evolution was less representative: the standard length increased from 130 cm to 330 cm, an increase of only 2.5

times higher. These evolutions show that the most fluctuating character is body weight. Considering the analysis of the variability coefficient, it can be observed that for all three analyzed characters and years of measurement we have a homogeneous population, the value of the coefficient is between 8 and 24%, no figure exceeded 30%.

The analysis of correlations shows that the three analyzed characters are positively correlated: when one increases, the other two automatically increase also. As it can be seen in Table 2, for the first year of measurement, the highest correlation was between body depth and standard length (0.989, respectively).

Table 2. Pearson Correlation for all population in first year of measurement

0+	Weight	Body Depth	Standard Length
Weight	1		
Body Depth	0.982	1	
Standard Length	0.976	0.989	1

In the second year of measurement, the correlation values dropped slightly compared to the previous year, but the same positive trend is maintained and all correlations are higher than 0.850 (Table 3). In this case, the highest correlation was obtained between weight and body depth (0.939).

Table 3. Pearson Correlation for all population in second year of measurement

1+	Weight	Body Depth	Standard Length
Weight	1		
Body Depth	0.939	1	
Standard Length	0.901	0.865	1

As expected, in the third year of measurement the correlations were positive and with very high values, the highest correlation was recorded between standard length and weight (0.960) (Table 4).

Tabel 4. Pearson Correlation for all population in third year of measurement

2+	Weight	Body Depth	Standard Length
Weight	1		
Body Depth	0.924	1	
Standard Length	0.960	0.921	1

The dynamic of productions parameters of Ineu carp breed is shoved in Figure 1.

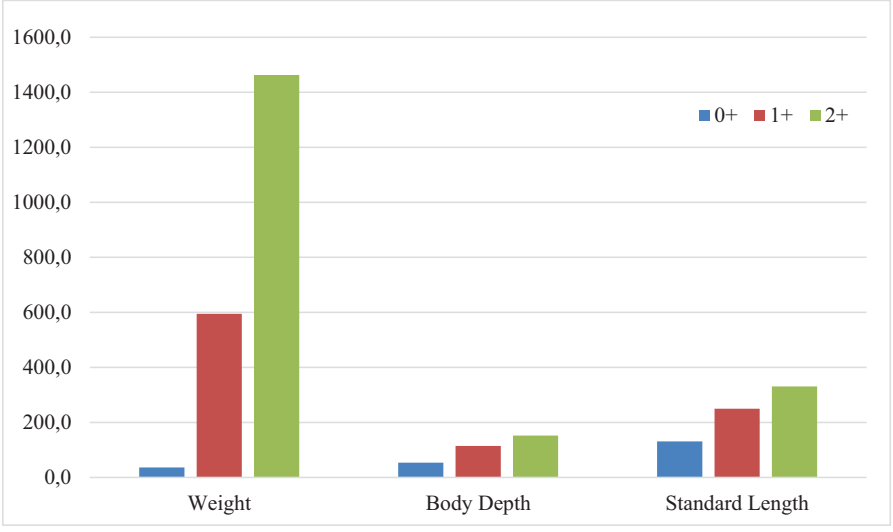

Figure 1. Evolution in time for the three characters

In Romania, the carp was, is and will remain the main fish species in aquaculture and human preferences (Nicolae et al., 2012). The improvement objectives of Ineu carp breed are reflected in the transformation of production characters (Simeanu, 2016).
The knowledge of fish production contributes to development of distribution chain of fish and fishery products which include the links between the production point and final consumer.

CONCLUSIONS

In conclusion, it can say that the three characters are highly positively correlated, and together they have a major influence on meat production.
The increasing competitiveness on the biological material market requires, in addition to fish farming, the obtaining and improvement of some fish breeds.
The values of the analysed meat production characters refer only to sample of the population and specific environmental conditions in which it has evolved.

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RESEARCHES REGARDING THE FORESTRY ARRANGEMENTS OF UPF IV CALARASI COUNTRY

Lucica NISTOR, Marius Laurian MAFTEI, Paula POSAN, Camelia HODOSAN, Sorin BARBUICA

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: lucia_mamina@yahoo.com

Abstract

The forestry measures applied consist in adjusting and decreasing of the forestry personnel for obtaining wooden material as required, with a good physiological state, without any specific flaw on its surface. The IV production Unit of Chirnogi belongs and develops in district Campiei Burnasului, it is administered by Mitreni SilvicDistrict, while being a public property of the state in the R.N.P. Romsilva. Thinning shave been done in UPF IV Chirnogi on a surface of 41.16 ha with a volume of 455 m^3 and a 46 m^3 annual average. The major modifications have been done by assignations in the parcel and underlake area in UP III Frasin Ulmeni.

Key words*: assignations, thinning, parcels.*

INTRODUCTION

Forestry deals with the problems of creating and cultivating forests, harvesting and benefit from forests, and organizing and managing the entire administration process (Nicolescu, 2003).

Like the major importance, the forests have presented the main vector in developing the human community, due to the quality of the wood disposed, the paraphernalia and the variety of utility that it holds within the construction and tool area and also the heating in homes (Daia, 2003).

Thus if the great exploitations during the antic times would have been avoided, the big citadels and historical vestiges that can be seen today as well, would have ceased to exist, proving again its role as a significant resource. That is why through forestry we understand the science that deals with studying, rationalizing and managing of all the wood resources of the Earth.

MATERIALS AND METHODS

The cleaning methods of the trees have the thinning as purpose from both point views (quantity and quality) and takes place only in the early stage of growth (underdeveloped sapling).

Those cleaning methods have taken place within UPF 4 Chirnogi on a surface of 41.87 ha with a total volume of 56 m cube and a 6 m cube annual volume. The hygiene works of the tress have as a main purpose taking out the dried or damaged trees in the wake of the weather phenomena outside the other established things. They are done to prevent an eventual disease infestation, such as mushrooms, insect pests and other destructive agents from within the forest that can affect the quality of the wooden material. Those hygiene works have taken place within UPF 4 Chirnogi on a surface of 138.23 ha with a total volume of 1,062 m cube and a 106 m cube annual volume.

RESULTS AND DISCUSSIONS

Methods of storing trees consist in adjusting and decreasing the forest effect with the purpose of obtaining wooden material developed according to our requests with a good physiological state without any flaw on its surface. There are three types of storing: selective, schematically, combined.

Execution technique: a first step would be the recognition of all the trees with a maximum economic value. Thus the numeric status of the trees, the distance, as well as the distribution and layout of all the tree crowns to decide

which measures are to be taken to act upon (Nistor, 2003).

Intensity and periodicity: Varies based on the physiological conditions of each tree and also the state of the technical forestry from before the thinning**.**

It is also in a decreasing order in reverse to the early developing stages of the young trees.

That's why a new intervention of this kind will take place only in case of multiple deficiencies found in the superior level of the stands. The appliance is not indicated after a certain cycle of thinning established without knowing the state of the land because it can disturb the quality development especially the ability to protect against disasters. Thus for a maximum efficiency of work there are consistent artificial adjustments made at level 0.8 or 0.7.

In Romania thinning at the state takes between 4 and 6 years and for the rest of the procedures the execution period is between 5 and 10 years.

In UPF IV Chirnogi thinning were done on a surface of 41.16 ha with a 455 m cube volume and a 46 m cube annual average.

The cleaning methods of the trees are similar to the thinning ones (quantitative and qualitative), but they only take place in the early growth stage. It is applied only where competition is excessive between the younglings and leads to keeping the useless species economically speaking which exclude the artificial species unable to cope. There are several steps.

The execution technique: takes in consideration the trees with a negative affect towards the remaining stock.

Thus the ratio between component species and the excess growth (individual and collective as well) is adjusted, the species that stimulate the growth getting excluded only when it come to the conclusion that they are harmful towards the dominating species or prevent circulation on forest roads. For the pure species for an efficient yield is recommended keeping the density of certain parameters, and for the mixed species is recommended favoring the natural seed grown trees. In the mountain area the leading methods are more reduced compared to the country area.

With these methods the internal forest roads get well shaped too. As a parallel between the two types of woods, the hardwoods compared with the softwoods show an inexistent interest for the woodworking industry in order to obtain the main products due to the poor plastic processing characteristics and the low quality aspect.

In the U.P.F. IV Chirnogi cleanings were carried out on an area of 41.87 ha with a total volume of 56 m cubic meters and an annual volume of 6 m cubic meters. Hygiene works of the trees - removing the dry or damaged trees after meteorological phenomena, other than the other established works. This is made in order to prevent a possible infestation with diseases, fungi, insect pests and other destructive factors inside the forest, not to damage the quality of the wood.

There are occurrences of any type of forest presenting periodicity, being required only in cases of necessity and having an independent character. The quantity of wood resulted from these works reflect the forest's state at the moment, with a margin of 1 m cube/year/ha.

They take place throughout the year without any inconvenience.

In U.P.F. 4 Chirnogi, hygienic works were carried out on an area of 138.23 ha with a total volume of 1,062 m cubic meters with an annual volume of 106 cubic meters.

Given the background, followed by the restitution and the assignations remembered, as internal structure and evolution we have the following situation (Table 1 and Table 2).

Table 1. Size of parcel and underparcel

Year		2004	2014
Parcels	Number	61	18
	Average	14.6	18.01
	Maximum	64.4	35.50
	Minimum	0.5	6.26
Underparcels	Number	402	136
	Average	2.2	2.38
	Maximum	17.2	19.11
	Minimum	0.1	0.14

Table 2. Density evolution of plots

Year	Subunit of production	Surface	Consistency			Average consistency
			0,1-0,3	0,4-0,6	> 0,7	
1994	Abies alba	219.9	2	0.4	4	0.8
	Quercus	114.4	0	0	0	0.7
2004	Abies alba	249	5	1	9.4	0.8
	Quercus	103.3	0	1	9.9	0.8
2014	Abies alba	213.61	3	1.1	8.6	0.7
	Quercus	109.98	0	3.1	6.9	0.8

According to the table the density of the stands rises up to 50% until 2004 followed by a decrease in 2014 by 30%.
Analyzing the results, we can observe that no matter the age, the trees show an ascent trend until year III and IV followed by a decrease down to 0 in year V and VI (Figure 1).

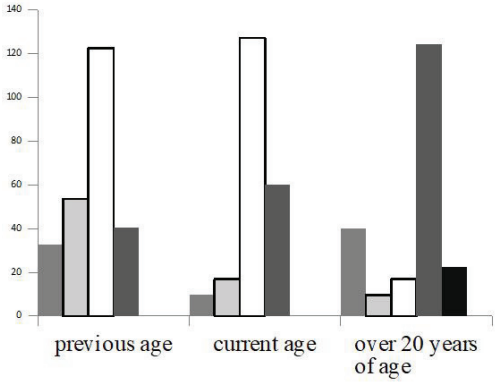

Figure 1. Dinamic of structure based on age

With an annual possibility of 300 m cube/year, there are only 3 possibilities that met a 70% increase, the fourth remaining null (Figure 2).

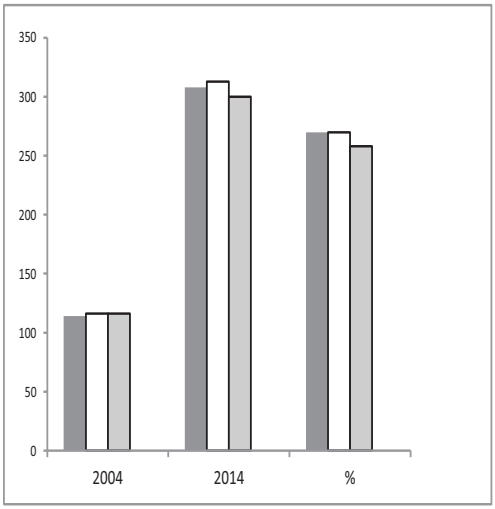

Figure 2. Possibility of plots

According to the calculated possibilities from 10 to 10 years it is concluded that in the next 10 years a small decrease of only 10 m cube will take place followed by an increase of 30 m cube after 20 years and another 10 m cube increase after 30 years (Table 3).

Table 3. Possibility laid out in years

Present arrangement		after 10 years		after 20 de years		after 30 de years	
Surface	Possibility	Surface	Possibility	Surface	Possibility	Surface	Possibility
36.49 ha	450 m^3	45.51 ha	440 m^3	27.98 ha	470 m^3	43.99 ha	480 m^3

CONCLUSIONS

As a result of the research made in UPF 4 Chirnogu, there are the following conclusions:
- the quantitative and wood indices underwent numerous changes over several years until now;
- specifically, the wood volume averaged 118 cube meters (while it currently averages 104 cube meters), the total increase got to 6,319 cube meters (in comparison with the current 1,034 cube meters), and the average increase went from the previous 7.2 cube meters to the actual 3.2 cube meters, only so that afterwards, the previous total increase to reach 693 cube meters in comparison to the current one, reaching 438 cube meters;
- similarly, it can be state the same thing about the average increase index (previously 2.8 cube meters) which is now 2 cube meters, followed by the possibility of main products of 4,541 cube meters compared to the current value of

750 cube meters, with a harvesting index (previously reaching 166 cube meters) currently of 98 cube meters;
- this is followed by a possibility of secondary products of 480 cube meters, now reaching a current 52 cube meters and a harvesting index of the secondary products (previously 17 cube meters), now reaching 6.2 cube meters.

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FRESHWATER MOLLUSCA FAUNA OF AKYAKA KADINAZMAGI STREAM (GOKOVA - MUGLA)

Hüseyin SASI

Mugla Sitki Kocman University, Fisheries Faculty, Department of Marine - Freshwater Sciences and Tecnology, 48100, Mugla, Turkey

Corresponding author email: hsasi@mu.edu.tr

Abstract

In order to determine the Mollusca fauna of species in Akyaka Azmak Stream, seasonal samplings were made from 2 stations between September 2008–May, 2009. After collecting benthic materials by using a hand-net with 500 µm mesh size and Ekman grab, after sifted, they fixed in 4% formalin solution. As a result of study, totally 10 taxa were determined; all of them belong to Gastropoda as Galba truncatula, Theodoxus anatolicus, Theodoxus fluviatilis, Theodoxus heldreichi, Gyraulus albus, Potamopyrgus antipodarum, Bithynia tentaculata, Bythinella byzanthina, Valvata cristata and Pseudamnicola roddail. The genera of Thedoxus is important for water quality. In the study, they are found very richness at the first station. Also, it has determined some physico-chemical parameter and discussed.

Key words*: Akyaka Stream, Bentic, Freshwater, Mollusca, Mugla.*

INTRODUCTION

Mollusks include large and most intelligent examples of invertebrates and they have occurred entry into nearly every aquatic habitat. Mollusca are probably the most recognizable invertebrate organisms for scientist.

Also their obvious importance in human history, mollusks have played more important role in metazoan evolution and have become one of the most successful animal groups in the earth (Smith, 2001).

Anatolia, with a highly variable relief and a diversified climate and vegetation, can support a similarly rich and diversified fauna of terrestrial and aquatic gastropods. Gastropoda and Bivalvia are common group of macro-benthic. Molluscs have shown to react to environmental changes (Armitage et. al., 1983; Rosenberg and Resh, 1993; Yilmaz, 1992; Smith, 2001; Yildirim et al., 2006).

This reason is important for studies of the relationship between organism and environment. These invertebrates also have economic uses, which some molluscs are used as feed for cattle. Also, Molluscs are well to the understanding of geologic historical developments (Bilgin, 1980; Oktener, 2004). In this group freshwater snails can be intermediate host to some trematoda, also they begin important for human health and economy (Zhadin, 1952).

The classis of Gastropoda is richness and biological as the widest groups with around 40.000 species. Generally, freshwater molluscs (Gastropoda and Bivalvia) were commonly determined from West Anatolia Region to South and East Anatolia Region (Balik et. al., 1994, 2002, 2003a, 2003b; Ustaoglu et. al, 2000; Bilgin 1967, 1973). The investigations freshwater molluscs concerned with in the Blach Sea are quite less (Oktener, 2004).

Akyaka Stream is located Southwestern part of Turkey, in West Mediterranean Basin (Figure 1).

Figure 1. Akyaka Stream and stations (Gokova)

Gokova Area includes Akyaka Azmak Stream is very important wetland area, which is

protected area with PEA (Private Environment Area) by the Ministry of Environment and Forestry. Akyaka is touristic city and near the stream has many restaurants.
There are many threats which are pollution such as tourism activities.
Akyaka Stream is connected in the sea approximately with 5 km canal, which it has a small boat trip for natural aquarium.
This study was carried out to determine mollusca fauna Akyaka Azmak Stream (Gokova, Mugla).

MATERIALS AND METHODS

The materials were taken from littoral zones. The mud (II. station) on these materials was cleaned off on varied mesh sieve with water.
Some species were taken out of aquatic plants. These molluscs found were preserved in 4% formalin or in 70% ethyl-alcohol stored in glass vials with plastic caps.
The data to relate their biotopes were recorded on these glass vials and the molluscs cards. For identification, the most important diagnostic characters of the molluscs as general appearance, apex, apertur, helozon were examined.
All collected samples were examined with a Stereo-microscope, then some photographs were taken and their species were determined. During identification we were used various keys (Zhadin, 1952; Bilgin, 1973, 1980; Schütt, 1991; Yildirim, 1999; Smith, 2001; Yazar and Yildirim, 2005).
In order to determine the Mollusc fauna of species in Akyaka Kadinazmagi Stream (Gokova), seasonal samplings were taken from 2 stations between September 2008 and May 2009.
Sampling was carried out with standard techniques montly (Zhadin, 1952).
After collecting bentics materials by using a hand-net with 500 µm mesh size scoops, sieves, pliers and Ekman grab, were preserved.

RESULTS AND DISCUSSIONS

In this study molluscs, which were collected from Akyaka Stream in Gokova area, have been examined. As a result of this study, 10 species belonging to Gastropoda were determined.
The finding gastropods follow as:
Phylum: Mollusca
Classis: Gastropoda
Subclassis: Pulmonata
Ordo: Basommatophora
Familia: Lymnaeidae
- *Galba truncatula* (Müller, 1774)

Subclassis: Prosobranchia
Ordo: Archeogastropoda (=Diatocardia)
Familia: Neritidae
- *Theodoxus anatolicus* (Récluz, 1844)
- *Theodoxus fluviatilis* (Linnaeus, 1758)
- *Theodoxus heldreichi* (Mertens, 1879)

Ordo: Mesogastropoda (=Monotocardia)
Familia: Planorbidae
- *Gyraulus albus* (Müller, 1774)

Familia: Hydrobiidae
- *Potamopyrgus antipodarum* (Gray, 1843)
- *Bithynia tentaculata* (Linnaeus, 1758)
- *Bythinella byzanthina* (Küsler, 1852)
- *Pseudamnicola roddail* (Paulicci, 1878)

Familia: Valvatidae
- *Valvata cristata* (Müller, 1774)

The species of *Galba truncatula Theodoxus anatolicus*, *Theodoxus fluviatilis*, *Theodoxus heldreichi,Gyraulus albus*, *Potamopyrgus antipodarum, Bithynia tentaculata, Bythinella byzanthina, Valvata cristata*and *Pseudamnicola roddail* are recorded from new area in Akyaka Azmak Stream.

Table 1. Frequency of Mollusca species in Akyaka Stream

Mollusca	I. Station	II. Station
Galba truncatula	++	+++
Valvata cristata	++	+
Gyraulus albus	+++	-
Pseudamnicola roddail	++	-
Potamopyrgus antipodarum	+++	+ +
Bithynia tentaculata	++	+
Bythinella byzanthina	+++	+ +
Theodoxus anatolicus	+++	+
Theodoxus fluviatilis	+ ++	-
Theodoxus heldreichi	+ +	+

***(+++)** Affluent; **(++)** Richness; **(+)** Rarely; **(-)** None

Theodoxus anatolicus, Theodoxus fluviatilis, Gyraulus albus, Bythinella byzanthina and *Potamopyrgus antipodarum* are dominant species in I station (Table 2).

Table 2. The Mean of Some Pyhsico-Chemical Parameters of Akyaka Stream (September, December, 2008 and April, 2009)

Parameter	I. St	II. St
Temperature (^oC)	15.20	15.80
pH	7.32	7.15
DO (mg/l)	7.40	6.50
Saturation (%)	67	62
Conductivity (µS)	4646	5680
Salinity (‰)	2,31	2,32
$(CO_3)^{=}$	13.20	12.60
$HCO3^-$ (mg/l)	286.70	274.50

All the species our finding of Gastropoda have commonly determined Aegean and Mediterranean areas, except for *Pseudamnicola roddail*, which species the first time reported for this study.

Among these species, *Theodoxus anatolicus, Theodoxus fluviatilis* and *Theodoxus heldreichi* are bio-indicator species and important ecological. *Thedoxus* species have lived affluently in clean area. *Galba truncatula* is the richness on the mudy bottom in littoral zone, and we found dominant species at station II. *Gyralus albus*, *Pseudamnicola roddail* and *Theodoxus fluviatilis* is found only in station I. The others studies in Mediterrenean and Aegean Region have shown to the distribution and richness of our finding species which determined in Akyak Stream (Gokova). The stations with the highest species numbers were the 1st.

Defined by several researchers in Turkey, there are 11 species of the genus Theodoxus (Bilgin, 1967; Geldiay and Bilgin, 1969; Bilgin, 1980; Schutt and Sesen, 1993).

Gokova Region has a warm climate. Seasonably variations (September, December and April) of the some physico-chemical parameters are presented in Table 2. In Akyaka Azmak Stream, the water temperatures averaged 15.20 and 15.80 ^oC in the I. and II. station respectively. The mean dissolved oxygen levels were 7.4-6.5 mg/l and the average pH levels were 7.32-7.15.

Most of the systematic studies carried out so far in our country are typological studies. In studies based on populations, there is some statistical analysis of some features (Yildirim et. al., 2006; Sereflisan et al., 2009).

CONCLUSIONS

Gokova Area includes Akyaka Azmak Stream is very important wetland and unique area, which is protected area with PEA statue. The conservation of native species and biodiversity of the region is important for the ecosystem. This important and sensitive area protection status must be maintained and monitoring studies should be carried out. It is necessary that precautions should be taken against intensive tourism pressure.

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ASSESSING THE INTER-RELATIONS BETWEEN FISH HEALTH AND STOCK STATUS ON HUMAN HEALTH AND CONSUMER PERCEPTION

Aurelia TOTOIU[1], Magda-Ioana NENCIU[1], Carmen Georgeta NICOLAE[2]

[1]National Institute for Marine Research and Development "Grigore Antipa" Constanta, 300 Mamaia Blvd, 900581, Constanta, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd., District 1, 011464, Bucharest, Romania

Corresponding author e-mail: atotoiu@alpha.rmri.ro, atotoiu@yahoo.com

Abstract

This paper aims to discuss the influence of infectious-contagious and parasitic diseases that can be transmitted to humans and their impact on human health. Many fish bacteria can infect humans, mostly part of the genera Vibrio and Aeromonas. Another bacterium that has a very high resistance and can be transmitted is Mycobacterium marinum. Parasitic diseases, as well as infectious diseases can be transmitted to humans, in some cases the accidental host that interrupts the life cycle of the parasite. These can be transmitted by eating infected fish that are consumed in raw state, not cooked enough, or subjected to treatments that do not guarantee the destruction of larvae (marinated, vinegar, salted, cold-smoked, dry). These illnesses may occur to fishermen, people who manipulate fish, but also to consumers. Consumers expect safe and healthy fish and fishery products, which is why parasite infestation may also have economic repercussions by decreasing the commercial values.

Key words*: fish, human health, parasitic diseases.*

INTRODUCTION

Fish is a healthy food for humans. Fish is one of the most beneficial protein sources for human diet. Fish and seafood, in general, are filled with essential nutrients, like Omega-3 fatty acids, and are an excellent source of protein to keep the body lean and muscles strong. Fish has also impacts on other functions of the body, including liver, brain and heart (Ene et al., 2016).

However, research has shown over the years that fish, besides its food qualities, can cause serious illnesses to human communities.

Under certain conditions, there is a risk of human illness through fishing, handling, inappropriate storage and fish consumption. These risks are closely related to the way fish is cooked, the degree of industrialization, and the traditional habits of each geographical area (Durborow, 1999).

There are some fish diseases and infections that can be transmitted from fish, and the water in which they are cultured, to humans. Although the infection of humans with fish pathogens is a relatively unusual event, it is a health risk that needs to be recognised by fish farmers and other people who handle and/or consume farmed seafood (Lehane and Rawlin, 2000).

The incidence of transmission of diseases from fish to humans is dependent upon several factors including the type of organism (viral, parasitic or bacterial), the susceptibility of the host (immuno-compromised individuals, presence of open wounds) and environmental factors (quality of the water, depth of penetration of fish spines) (Durborow, 1999).

Optimum farm design, appropriate husbandry and handling, water quality management and regular fish health monitoring will reduce the risk of disease transfer from fish and their environs to workers in the aquaculture industry (Haenan et al., 2013).

The biological information about fish growth indicates the general well-being and characteristics of the specific environment (Stavrescu-Bedivan, 2016).

In the case of fishery and animals harvested from the natural environment affected by pathogens, only appropriate cooking and severe sanitary-veterinary regulations can prevent the spreading of illnesses to humans.

Moreover, the presence of visible parasites, for instance, in the flesh of marine fish has long

been known and is perceived to be both an aesthetic and human health problem.
A recent study (Bao et al., 2017) revealed that people tend to avoid eating parasitized fish, and are willing to pay above market price to avoid adverse effects on health and food quality. Overall, the results suggest that the presence of parasites in fish is an important health and aesthetic issue for consumers, and this is relevant for the fishing and food industries as well as for food safety authorities.

MATERIALS AND METHODS

The present study is a review of the most significant and potentially dangerous illnesses caused by either bacteria or parasites affecting fish.
The methodology for the diagnosis of ichthyozoonoses is made by performing sets of analyzes in specialized laboratories at persons suspected of these diseases that have come into contact or have consumed fish or fish products suspected of being infected with these pathogens or showing the symptoms of these bacterial or parasitic diseases.

RESULTS AND DISCUSSIONS

Diseases transmitted to humans through fish and other fishery products are called ichthyozoonoses.
These diseases cause problems for both fishery producers, by having their products rejected from the market, and consumers, by affecting their health.
By eating fish and fishery products, humans can be infected with or become hosts of pathogenic bacteria and parasites, biotoxins and chemicals.
The most common and known ichthyozoonoses are those caused by bacteria and parasites.

Bacterial ichthyozoonoses

The most common ichthyozoonoses are those produced by the bacteria: *Vibrio parahaemolyticus* and *Clostridium botulinum*.
Infection with *Vibrio parahaemolyticus* is specific to human communities (Figure 1).
V. parahaemolyticus is a marine bacterium that multiplies in fish and fish products at temperatures above 8^{o}C.

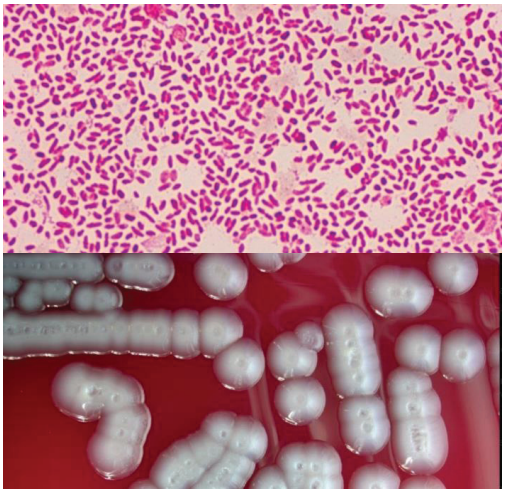

Figure 1. *Vibrio parahaemolyticus*
(Source: http://microbe-canvas.com)

This bacterium is able to cause food intoxication within hours of contamination. Symptoms of contamination can occur after 4 - 48 hours after the fish has been consumed in a raw or insufficiently fried, boiled state.
Its survival in various marine fishery products varies depending on temperature and shelf life.
The way to prevent this disease is to educate human communities on the risk of contamination with this bacterium if the fish have not been sufficiently heat-treated, but also to be aware of the importance of cooking hygiene (Huss, 1988).
There are other bacteria of the genus *Vibrio* that can be transmitted to humans and can cause major illnesses such as cholera, which in many countries is transmitted to humans by consuming marine fishery products. The population should be constantly informed of the risk of this disease and how it can be prevented.

Botulism is a food poisoning caused by botulinic bacillus toxin - *Clostridium botulinum*, as a result of human consumption of semi-conservers of fish. Research has shown that semi-conserved fish (salted, marinated, fermented and smoked) can become dangerous for humans if they contain pre-formed toxins in the raw product.
Clostridium botulinum is an anaerobic, spore-forming and spore-producing bacterium (Figure 2). Fresh fish is a potent botulinum toxin

producer. It is widely consumed without causing incidents, because, if it is cooked at 60-80 ^{o}C it is destroyed in 5 minutes.

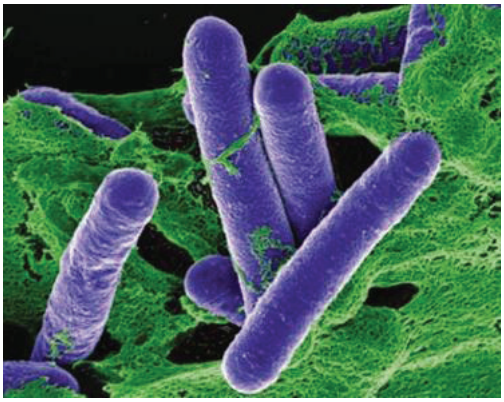

Figure 2. *Clostridium botulinum*
(Source: www.medical-actu.com)

In the event of contamination with this botulinic toxin and the occurrence of food poisoning, death may occur. Preventing this type of intoxication requires strict control of the raw fish before processing (Huss, 1988).

Mycobacteriosis or "fish tuberculosis" caused by *Mycobacterium marinum* is an infectious disease reported in many fish species.
Mycobacteria are potentially infectious in humans, they penetrate the surface of the skin due to the existence of cuts or injuries at its level, causing serious granulomas infectious (Figure 3).
This bacterium can be transmitted to fishermen, personnel handling fish and fishermen, consumers.

Salmonellosis can be caused by all *Salmonella* serotypes. The source of human infection can be fish in the waters contaminated with wastewater.

Erysipelosis is an infectious-contagious disease of the skin and subcutaneous tissue caused by the bacterial *Erysipelothrix insidiosa*, following contact with infected fish and fishery products. It is a professional disease that is recorded in fish farming workers, sports fishermen, workers in fish processing plants.

Other pathogenic bacteria present in fish that can cause infections in humans are: *Aeromonas hydrophila* and *Edwardsiella tarda*. These can cause skin lesions and gastroenteritis (Munteanu and Bogatu, 2003).

Fish-handler's disease is a nonspecific term that is in the medical and lay literature that describes a disease or syndrome of humans that may occur after handling fish or, in some instances, other aquatic organisms. It has been associated with occupations (fishermen or lobstermen), hobbies (tropical fish tanks, pet shop workers), or water sports (boating, swimming pool use). Researchers also discovered that at least two different genera of bacteria (*Mycobacterium* and *Erysipelothrix*) were the main causative infective agents of the disease (Durborow, 1999).

Figure 3. Skin lesion caused by *Mycobacterium marinum*
(Source: NYC Dept. of Health and Mental Hygiene, www. nymag.com)

Fish-handler's disease occurs when cuts or scrapes in the skin become infected with the bacteria *Erysipelothrix* sp. and/or *Mycobacterium* sp. (Figure 3). Handling and preparing fish and shellfish and many other similar activities can create small cuts and scrapes in the skin, where bacteria may enter. Developing fish-handler's disease requires deliberate contact with fish. Fish-handler's disease occurs worldwide wherever fish and shellfish are handled (Lehane and Rawlin, 2000).

Parasitic ichthyozoonoses
Parasites are a common presence in fish. Most are not transmissible to humans, but raise questions about fish quality. However, there are

species of parasites with zoonotic features that infest some species of fish in the larvae stage, which can be transmitted to humans by eating raw fish or insufficiently heat treated.

The most frequent parasites of the fish belong to the groups: protozoa, worms and crustaceans.

Parasitic protozoa do not pose a danger to the health of the consumer, but there are several species that cause the degradation of fish meat and reduce its food value.

The most destructive mixosporidia belong to the genus *Kudoa*, which are capable of intense proteolytic activity even in frozen fish, producing the softening and gelling of their meat.

Infested fish with mixosporids and microsporids have a much diminished food value and a repellent aspect, which is why they cannot be marketed. Infested fish is only used for the preparation of fish flour and fish oil (Sindermann, 1970).

The parasitic worms transmissible through fish to humans belong to the groups: Trematoda, Cestoda, Nematoda and Acanthocephala.

Researchers have shown that there are a fairly large number of worms that can spread from fish, where larvae are found, to humans.

In the human body, through fish digestion, the larvae develop by transforming themselves into adult worms, which, through their coalescing, toxic, inoculating action, cause the hosts serious illnesses.

Some species of trematod worms can cause inflammation and degeneration of the gallbladder and liver, asthma, headaches. These worms are resistant to salting and freezing and, in order to prevent human infestation, it is advisable to treat the fish well before consumption. Severe infestations can lead to death (Bauer, 1987).

Anisakidosis is one of the most important nematodoses of humans transmitted by fish consumption. It is caused by infection with larval stages of fish nematodes of the genera *Anisakis, Contracaecum* etc. Anisakidosis is a serious zoonotic disease caused by the consumption of raw or undercooked fish dishes containing the larvae of parasite with clinical entity of acute or chronic infection.

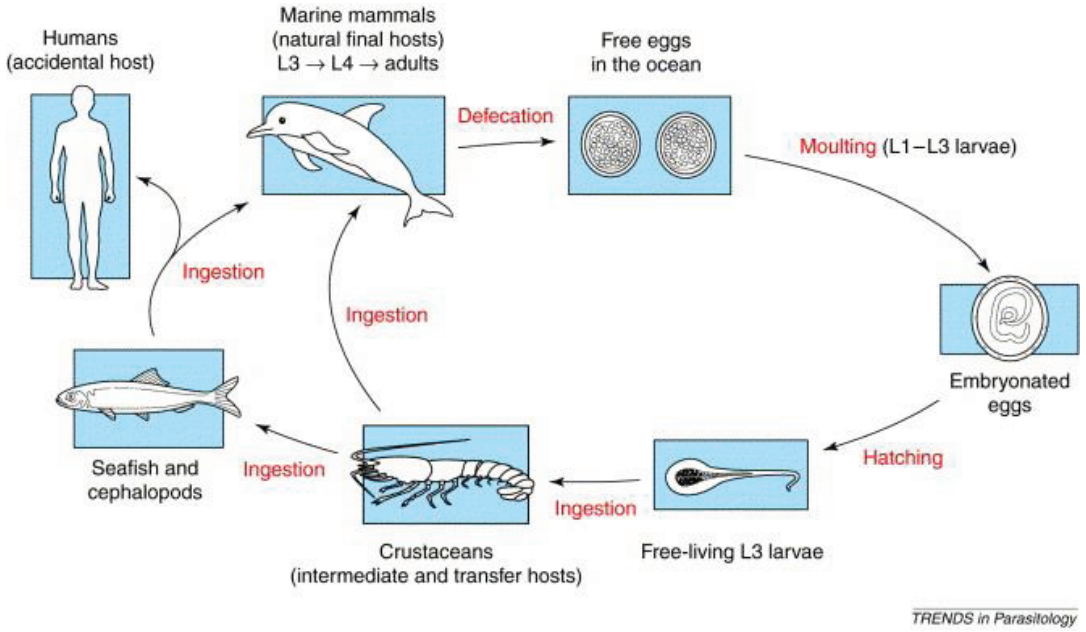

Figure 4. Life cycle of anasakids (after Audicana et al., 2002)

Anisakids are parasites widely distributed in marine mammals, particularly in colder temperate and polar waters. This parasite causes eosinophilic granuloma in the alimentary tract of man when raw or inadequately cooked fish with live larvae are ingested (Oshima, 1972).

Anisakis species have complex lifecycles which pass through a number of hosts through the course of their lives. Eggs hatch in seawater, and larvae are eaten by crustaceans. The infected crustaceans are subsequently eaten by fish or squid, and the nematodes burrow into the wall of the gut and encysted in a protective coat, usually on the outside of the visceral organs, but occasionally in the muscle or beneath the skin. The lifecycle is completed when an infected fish is eaten by a marine mammal, such as a whale, seal, sea lion, dolphin and other animals like seabirds and sharks. The nematode encysted in the intestine, feeds, grows, mates, and releases eggs into the seawater in the host's feces. As the gut of a marine mammal is functionally very similar to that of a human, Anisakis species are able to infect humans who eat raw or undercooked fish (Figure 4).

Anisakids pose a risk to human health through intestinal infection with worms from the eating of under processed fish, and through allergic reactions to chemicals left by the worms in fish flesh (Amato et al., 2007).

The application of HACCP (Hazard Analysis and Critical Control Points) principles and traceability in the production system, where each stage is evaluated for the existence of hazards and risks for public health, is a tool that has the potential to assume safety of cultivated and/or caught fish at risk of infection with zoonotic fish borne pathogens (Nicolae et al., 2015).

CONCLUSIONS

Ichthyozoonoses can be very dangerous for human communities, causing serious illness, sometimes even death. In some cases, their transmission can be done very easily, just by simply touching the fish and the presence of a hand injury can be the gateway of contamination. Their treatment can only be done by accurately diagnosing them and then treating them with the appropriate medication.

Fish borne pathogens are derived from the consumption of raw fish, or fish dishes or products that have not been cooked or have not been processed sufficiently to kill the parasites in farm. Apparently, the control is easy with consumer education campaigns, mass education, besides the consumption of well cooked/processed fish products.

Ichthyozoonoses can affect human communities along the Romanian Black Sea coast, as many of the bacteria and parasites that cause them have been identified in fish caught locally.

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Rapana venosa - NEW EXPLOITABLE RESOURCE AT THE ROMANIAN BLACK SEA COAST

Cristian-Sorin DANILOV[1], George TIGANOV[1], Eugen ANTON[1], Magda-Ioana NENCIU[1], Victor Nicolae NITA[1], Victor CRISTEA[2]

[1]National Institute for Marine Research and Development "Grigore Antipa",
300 Mamaia Blvd., 900581, Constanta, Romania
[2]"Lower Danube" University of Galați, Faculty of Food Science and Engineering,
47 Domneasca Street, Galați, Romania

Corresponding author email: cdanilov@alpha.rmri.ro

Abstract

Starting with 2009, Romanian Black Sea fishery catches have recorded an increasing trend. Yet, this is not the consequence of a massive restoration of fish stocks or an increase of fishing effort, but the result of shifting the target species. The invasive gastropod Rapana venosa (Valenciennes, 1846) has raised the interest of economic operators due to its low exploitation costs compared to other valuable species (turbot, for instance). At the Romanian coast, fishing for R. venosa was first performed only using divers, because this is a method which provides high selectivity of the catches and protection of habitats. Starting with 2013, beam trawls were legalized and started to be used (Order no. 1696 of 11.07.2013, Order no. 400 of 2013). After the legalization of the beam trawl, the catch increased 2.27 times compared to 2012 (from 588 t in 2012 to 1,338 tonnes in 2013), the TAC being carried out at a rate of 23.5%. Many commercial companies in the field have shifted their business towards purchasing or manufacturing this type of gear, corresponding to their vessel capacity. From the selectivity point of view, the gear used for rapa whelk fishing (beam trawl) does not retain immature specimens of R. venosa and no juvenile fish belonging to certain demersal fish species (gobies, red mullet, whiting). Yet, there is some concern on the potential effects of beam trawl on the seabed, which should be investigated in the future. Also, as a consequence of exploitation, the drop of R. venosa populations was acknowledged, which requires future research meant to determine the actual stock size and total allowable catch (TAC), aiming at underpinning the rapa whelk fisheries on a scientific background and to reconcile these economically valuable activities for coastal communities with nature conservation.

Key words*: beam trawl, catches, invasive species, rapa whelk, resource.*

INTRODUCTION

The gastropod *Rapana venosa* originates in the Far East (Sea of Japan, South China Sea) and it is documented that it was first introduced in the Pontic basin around 1930-1940 (Grossu, 1986, 1993). It was first sighted and reported around the Novorossysky Port in 1947. Being a predator, with no natural enemies and competitors for food, *R. venosa* spread quickly eastwards, on the coasts of the Caucasus, and later to the south and west, decimating native oyster banks (*Ostrea edulis*, Linnaeus, 1758). In 1949, the rapa whelk was reported in Gudautsk, in 1954 in Yalta and Sevastopol, on the Crimean coasts (Golikov and Starobogatov, 1972), and in 1963 it was first sighted at the Romanian coast (Gomoiu, 1972; Skolka and Gomoiu, 2004) (Figure 1).

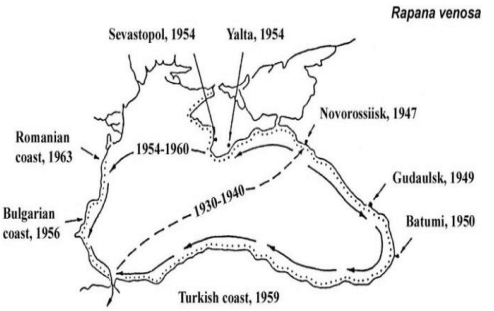

Figure 1. Expansion routes of *R. venosa* in the Black Sea (after Gomoiu and Skolka, 1996)

After its acclimation in the Pontic basin, *R. venosa* entered the Bosphorus Strait and spread into the Mediterranean Sea (Grossu, 1986; Goulletquer, 2002).

A major contribution to the rapid expansion of this gastropod in the Black Sea was held by the fact that it virtually exterminated oyster and mussel banks from the areas initially covered by these bivalves and, developing huge populations, it was necessary for it to seek other areas with unaltered food resources.
As such, during the 1950s, *R. venosa* recorded an exponential development, the population effecttives reaching considerable values. Subsequenly, as a follow-up of decreasing food resources, the rapa whelk population effectives dropped significantly.
The species tolerates well a wide range of temperatures, salinities and oxygen concentrations (Zolotarev, 1996; Mann and Harding 2003).
The rapa whelk has separate sexes. Its spawn has the appearance of white filaments, minimum 7 mm and maximum 30 mm high (Harding et al., 2007). It breeds yearly and starts laying the eggs at an 18^0C water temperature. Spawning continues for 11-15 weeks. The average female lays between 150-200 eggs/week (Harding et al., 2008).
After hatching, veliger larvae remain in the water column for 14-80 days, before fixating on the seabed, thus insuring a great dispersion potential for this species. A study performed by Harding (2006) showed that larvae fixate at a shell length of 1.2 mm, after a planktonic larval period which ranges between 24-42 days.
The great plasticity of the planktonic period increases the survival and dispersion probability. Larvae tolerate a wide range of salinities, up to 10‰, the survival threshold being 7‰ six days after hatching (Mann and Harding, 2000).
Imposex individuals (a pathologic state which causes the development of male gonads in females) have been reported in the rapa whelk (Mann et al. 2006).
This paper is an outline of the current state of the invasive *R. venosa* fisheries at the Romanian Black Sea coast.
The state-of-the-art knowledge on *R. venosa*'s biology is due to the research of Romanian and Russian experts during 1960-1990. However, during the past 20 years, various changes in the environmental conditions and the exploitation manner of this gastropod have occurred. The species was studied at the Romanian coast by Gomoiu (1972), in collaboration with other researchers.
Some studies on the rapa whelk stock dynamics in relation to the recent commercial exploitation have been performed at the Romanian Black Sea coast.
The biology, ecology and state of alien species in the Black Sea have been studied, starting with the early 20^{th} century, by several authors: Antipa (1941), Bacescu and Carausu (1964), Golikov and Starobogatov (1972), Gomoiu (1972), Grossu, (1986), Teaca et al. (2006), Zaitsev and Ozturk (2001) etc.
The knowledge of the breeding, growth, development and distribution conditions of this species in territorial waters can be useful in elaborating the harvesting and stock management measures (Tiganov et al., 2017).
Currently, due to its gastronomic properties, it has become object of industrial scale exploitations in Romania, as well. Only a small share of this biological resource remains in the domestic market, the rest being exported.
In recent years, the drop of *R. venosa* stocks has steepened in Black Sea countries, as a consequence of commercial exploitation of the species (food source, also known for aphrodisiac properties) and export of large amounts to Far East countries (Turkey exports yearly 800 tons to Japanese markets).

MATERIALS AND METHODS

This paper is based on the results obtained by research performed during 2009-2016 with industrial fishing vessels, equipped with beam trawl fishing gear (Figure 2), as well as with boats for specialized manual harvesting (with divers) (Figure 3).

Figure 2. Beam trawl fishing (Photo: Danilov)

Figure 3. Manual harvesting by diver (Photo: Danilov)

A total of 10 surveys with vessels (152 hauls, 76 trawling hours) and 10 hand harvesting surveys with divers were performed (around 50 hours).

The work areas were the following (Figure 4):

- Northern sector - between Cape Midia and Chituc, on the 18-25 m isolines;
- Southern sector - between Mangalia - Vama Veche, at 3-12 m depths.

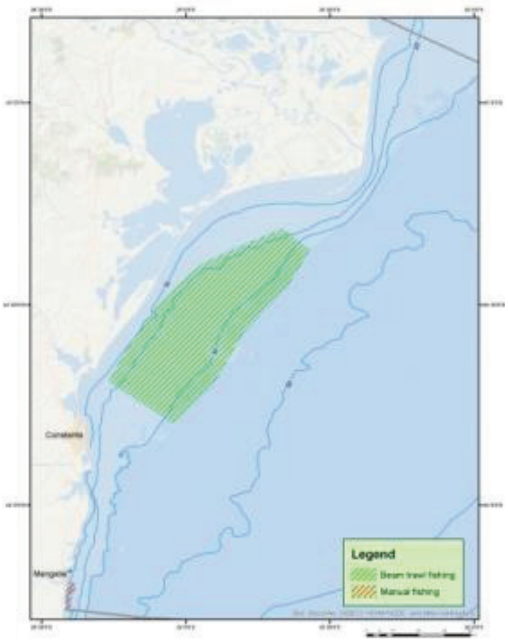

Figure 4. Map of *R. venosa* fishing areas

RESULTS AND DISCUSSIONS

During 2009-2012, rapa whelk fishing was made only by hand, by divers, being the only harvesting technique allowed by legislation. Starting with 2013, beam trawls were legalized and started to be used (Order no. 1696 of 11.07.2013, Order no. 400 of 2013, setting-up the fishing prohibition periods and areas, as well aquatic resources protection zones in 2013).

Catches have increased from one year to the other, from approximately 1.7 t (2009) up to 6,504 t (2016) (Figure 6).

Once the beam trawl use has become legal, many commercial companies in the field have shifted their business towards purchasing or manufacturing this type of gear, corresponding to their vessel capacity.

As such, the number of vessels equipped with this type of gear has increased yearly, and many of them have two beam trawls towed simultaneously (Figure 5).

During the analyzed time frame (2016), 6,435 *R. venosa* individuals were measured.

The length ranged between 4 and 10 cm, individuals with 6-8 cm were dominant. Individual weight ranged between 20 and 177 g, with a mean weight of 76.87 g and mean length 7.38 cm (Figure 7).

The length-weight relationship was calculated according to the equation $W(i) = q.L^b(i)$, where:

W(i) - body weight **i;**
L (i) - total height **i**;
q and **b** - parameters

The correlation between height - weight resulted in a $R^2 = 0.704$ coefficient, which indicated a good correlation of biometric parameters and the studied sample was homogeneous (Figure 8).

Figure 5. Various fishing vessels equipped with beam trawl (Photo: Danilov)

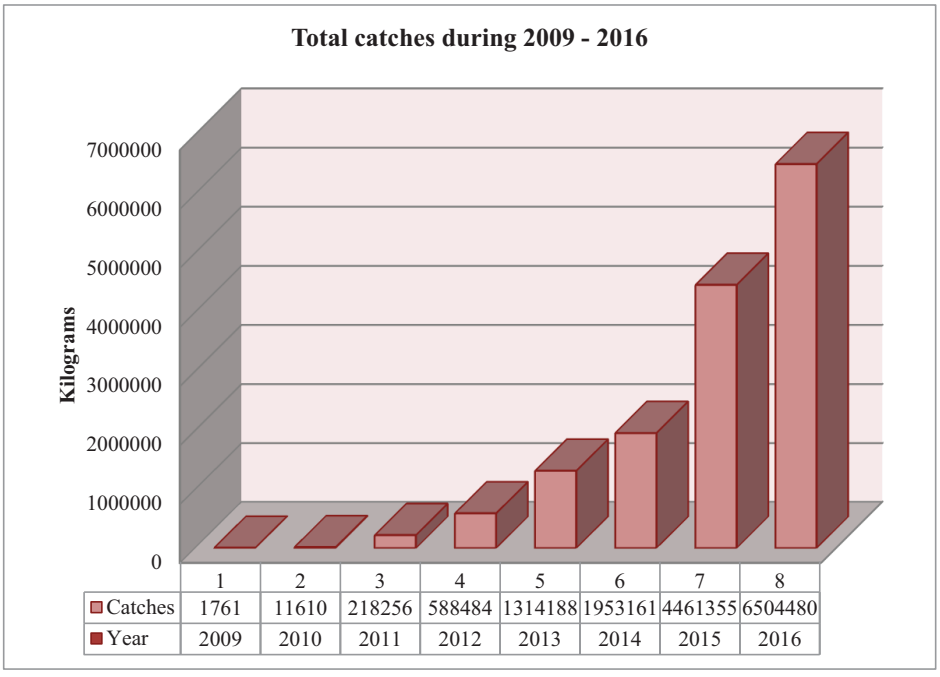

Figure 6. Total *R. venosa* catches during 2009 - 2016

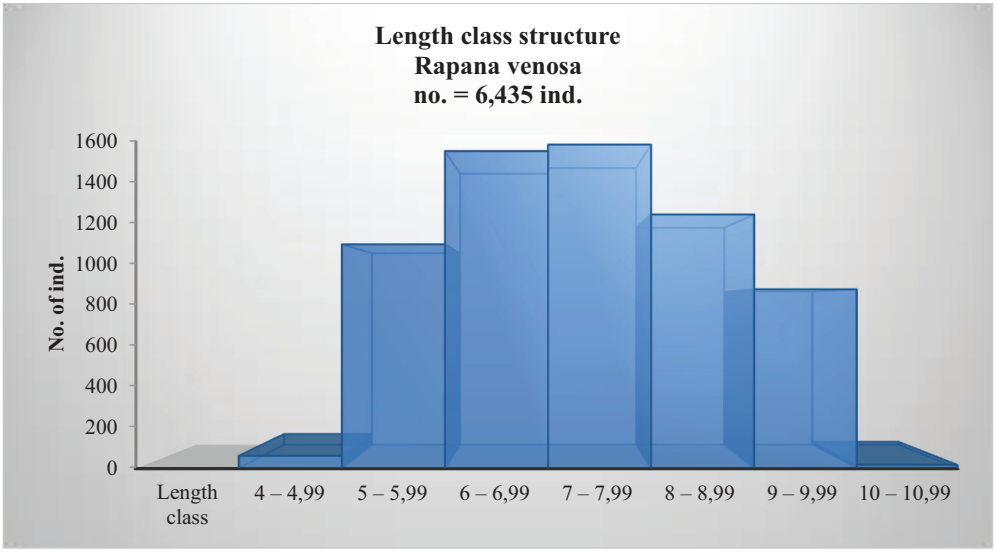

Figure 6. Length class structure of *R. Venosa* in 2016

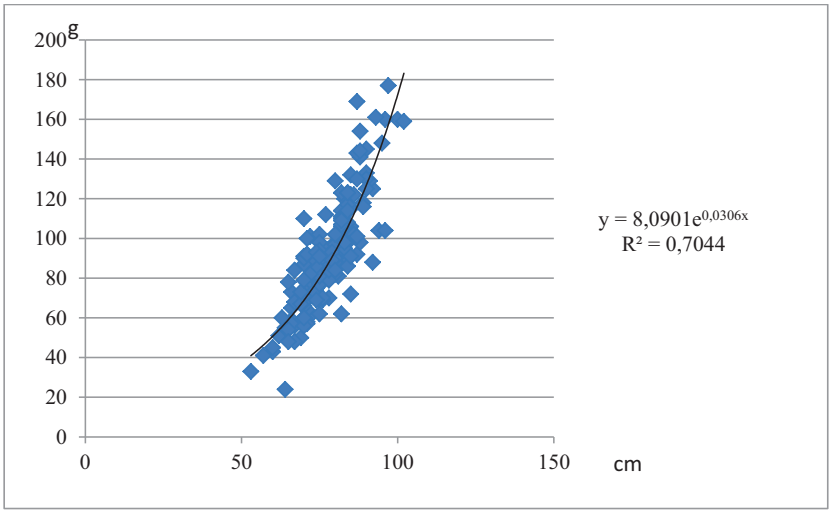

Figure 8. Length - weight correlation in *R. venosa* harvested in 2016

CONCLUSIONS

As a follow-up of investigations made by NIMRD "Grigore Antipa" during 2009-2016, the gastropod *R. venosa* was identified in almost all perimeters at depths ranging between 3-25 m, located along the Romania coast, especially in 18-26 water depth, in areas covered by beam trawl fishing on sandy substrate. In rocky areas, where rapa whelk is harvested by divers, it is encountered at depths between 3 and 10 m.

Rapa whelk catches at the Romanian Black Sea coast increased from year to year, ranging between 1 t in 2009 and approximately 6,500 t in 2016. In 2016, *R. venosa* catches counted for 87.5% of the total marine living resources catches along the Romanian Black Sea coast.

As a consequence of exploitation, the drop of *R. venosa* populations was acknowledged, which requires future research meant to determine the stock size and total allowable catch (TAC), aiming at underpinning the rapa whelk fisheries on a scientific background.

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